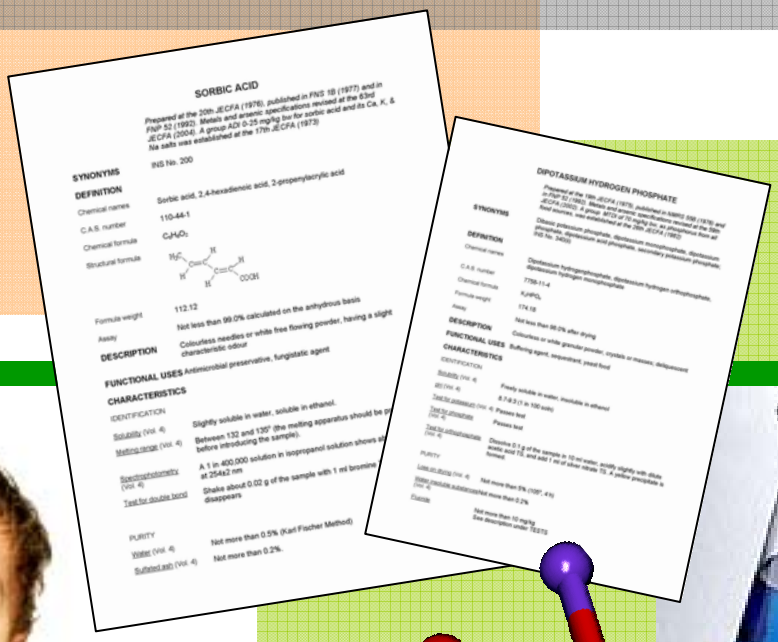
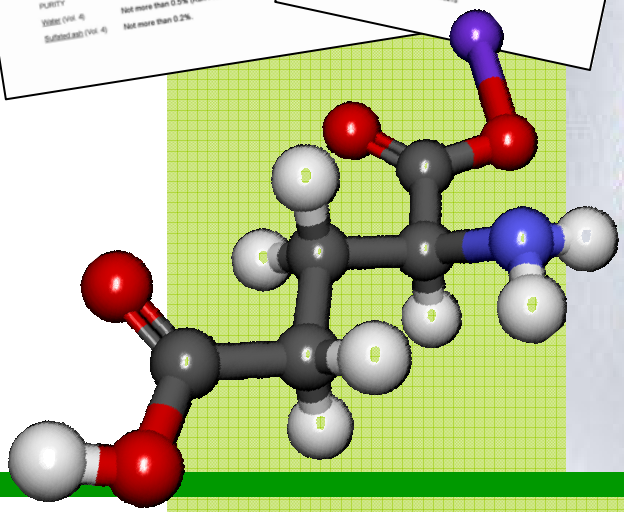
ข้อกำหนดคุณภาพหรือมาตรฐานวัตถุเจือปนอาหารตามประกาศสำนักงานคณะกรรมการอาหารและยาและมาตรฐานโคเด็กซ์ (Codex Advisory Specification for the Identity and Purity of Food Additives 2012)

เล่ม:1





สำนักอาหาร สำนักงานคณะกรรมการอาหารและยา

คำนำ

กระทรวงสาธารณสุข โดยสำนักงานคณะกรรมการอาหารและยา (อย.) ได้ออกประกาศกระทรวงสาธารณสุข (ฉบับที่ 281) พ.ศ.2547 เรื่องวัตถุเจือปนอาหาร ซึ่งมีข้อกำหนดของวัตถุเจือปนอาหารที่อนุญาตให้ใช้ต้องมีคุณภาพหรือมาตรฐานตามเงื่อนไขใดเงื่อนไขหนึ่ง ดังนี้ (1) ตามที่กำหนดไว้ใน Codex Advisory Specification for the Identity and Purity of Food Additives หรือ (2) ตามประกาศสำนักงานคณะกรรมการอาหารและยา โดยความเห็นชอบของคณะกรรมการอาหาร หรือ (3) ตามที่ได้รับความเห็นชอบจากคณะอนุกรรมการเพื่อศึกษาวิเคราะห์ปัญหาและวินิจฉัยในเชิงวิชาการเกี่ยวกับอาหาร ดังนั้นเพื่อให้เจ้าหน้าที่และผู้ที่เกี่ยวข้องสามารถนำไปใช้งานได้สะดวกขึ้น อย.จึงได้รวบรวมข้อกำหนดของวัตถุเจือปนอาหารที่อนุญาตให้ใช้ตามเงื่อนไขดังกล่าวมาจัดทำเป็นเอกสารฉบับเดียวกัน ซึ่งแบ่งออกเป็นเอกสารข้อกำหนดคุณภาพหรือมาตรฐานวัตถุเจือปนอาหารตามประกาศสำนักงานคณะกรรมการอาหารและยาและมาตรฐานโคเด็กซ์ (Codex Advisory Specification for the Identity and Purity of Food Additives) เล่ม1 และเล่ม2 เพื่อให้สามารถสืบค้นและใช้งานควบคู่กันไปกับประกาศกระทรวงสาธารณสุข (ฉบับที่ 281) พ.ศ.2547 เรื่องวัตถุเจือปนอาหาร

การจัดทำเอกสารฉบับนี้จะไม่สามารถสำเร็จลงได้ หากไม่ได้รับการสนับสนุนจากผู้เกี่ยวข้อง ดังนั้นในโอกาสนี้จึงขอแสดงความขอบคุณ นพ.พิพัฒน์ ยิ่งเสรี เลขาธิการคณะกรรมการอาหารและยา, ภญ.ศรีนวล กรกชกร รองเลขาธิการคณะกรรมการอาหารและยา, ดร.ทิพย์วรรณ ปริญญาศิริ ผู้อำนวยการสำนักอาหาร ที่ให้การสนับสนุนในการจัดทำเอกสารฉบับนี้ และดร.ชนินทร์ เจริญพงศ์ ที่ปรึกษาของสำนักอาหารที่ให้คำปรึกษาในการจัดทำเอกสารฉบับนี้จนสำเร็จ เพื่อใช้ประโยชน์สำหรับการปฏิบัติงานของผู้เกี่ยวข้องต่อไป

คณะผู้จัดทำ
สำนักอาหาร
สิงหาคม 2555

สารบัญ

บทนำ

วัตถุเจือปนอาหาร หมายถึง วัตถุที่ตามปกติไม่ได้ใช้เป็นอาหารหรือส่วนประกอบที่สำคัญของอาหาร แต่ใช้เจือปนในอาหารเพื่อประโยชน์ทางเทคโนโลยีการผลิต การแต่งสี การปรุงแต่งกลิ่นรสอาหาร การบรรจุ การเก็บรักษา หรือการขนส่ง ซึ่งมีผลต่อคุณภาพหรือมาตรฐานหรือลักษณะของอาหาร รวมถึงวัตถุที่ไม่ได้เจือปนในอาหาร แต่มีภาชนะบรรจุไว้เฉพาะใส่รวมกับอาหารเพื่อประโยชน์ดังกล่าวข้างต้นด้วย ทั้งนี้ให้หมายความรวมถึงวัตถุที่มิได้เจือปนในอาหาร แต่มีภาชนะบรรจุไว้เฉพาะแล้วใส่รวมกับอาหารเพื่อประโยชน์ดังกล่าวข้างต้นด้วย เช่น วัตถุกันชื้น, วัตถุดูดออกซิเจน เป็นต้น แต่ไม่รวมสารอาหารที่เติมเพื่อเพิ่มหรือปรับให้คงคุณค่าทางโภชนาการของอาหาร

วัตถุเจือปนอาหารที่สามารถใช้ได้ในผลิตภัณฑ์อาหารจะต้องมีข้อกำหนดคุณภาพหรือมาตรฐานตามข้อ 4 ของประกาศกระทรวงสาธารณสุข (ฉบับที่ 281) พ.ศ. 2547 เรื่อง วัตถุเจือปนอาหาร กำหนดให้วัตถุเจือปนอาหาร ต้องมีคุณภาพหรือมาตรฐานตามเงื่อนไขใดเงื่อนไขหนึ่ง ดังต่อไปนี้

(1) ตามที่กำหนดไว้ใน Codex Advisory Specification for the Identity and Purity of Food Additives (เข้าถึงได้จาก http//:www.codexalimentarius.org/ codex-home/en/)

(2) ตามประกาศสำนักงานคณะกรรมการอาหารและยา โดยความเห็นชอบของคณะกรรมการอาหาร (เข้าถึงได้จาก http://newsser.fda.moph.go.th/food/Law.php)

ประกาศสำนักงานคณะกรรมการอาหารและยา เรื่อง การกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารชนิดเดี่ยว (11 ชนิด)	กำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหาร ดังนี้ • กรดซักซินิก (Succinic Acid) • ไกลซีน (Glycine) • ไดโซเดียมซักซิเนต (Disodium Succinate) • ดีแอลแอละนีน (DL-Alanine) • เชื้อราที่ใช้ในกระบวนการหมัก (Starter Culture) • มอลโทเดกซ์ทริน (Maltodextrin) • ยีสต์ผงจากยีสต์ในกลุ่มแซ็กคาโรไมซีส (Saccharomyces) หรือทอรูลา (Torula) • ยีสต์ชนิดอื่น • แล็กติกแอซิดแบคทีเรีย (Lactic Acid Bacteria) • ออกซิไดส์พอลิเอทิลีน (Oxidised Polyethylene) • เอนไซม์แทรนส์กลูทามิเนสจากเชื้อจุลินทรีย์สายพันธุ์สเตรปโทเวอร์ทิซิลเลียม มอบารีนส์ วาร์ (Enzyme Transglutaminase from *Streptoverticillium mobaraense var.*)
ประกาศสำนักงานคณะกรรมการอาหารและยา เรื่อง การกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารชนิดเดี่ยว (ฉบับที่ 2) (เพิ่มเติม 2 ชนิด)	กำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหาร ดังนี้ • โซเดียมไฮโดรเจนซัลเฟต (Sodium Hydrogen Sulfate) • โซเดียมไตรเมตาฟอสเฟต (Sodium Trimetaphosphate)

ประกาศสำนักงานคณะกรรมการอาหารและยา เรื่อง การกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารชนิดเดี่ยว (ฉบับที่ 3) (เพิ่มเติม 1 ชนิด)	กำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหาร ดังนี้ • โปรตีนจับโครงสร้างน้ำแข็งผลิตจากยีสต์ดัดแปรพันธุกรรม (Ice Structuring Protein produced from genetically modified yeast)
ประกาศสำนักงานคณะกรรมการอาหารและยา เรื่อง การกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารชนิดเดี่ยว (ฉบับที่ 4) (เพิ่มเติม 2 ชนิด)	กำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหาร ดังนี้ • โมโนโพแทสเซียมทาร์เทรต (Monopotassium Tartrate) • แอล-ซีสเตอีนไฮโดรคลอไรด์ (L-Cysteine hydrochloride)
ประกาศสำนักงานคณะกรรมการอาหารและยา เรื่อง การกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารลักษณะผสม	กำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารของวัตถุเจือปนอาหารลักษณะผสม *“วัตถุเจือปนอาหารลักษณะผสม หมายความว่า วัตถุเจือปนอาหารที่ได้จากการผสมวัตถุกันตั้งแต่ ๒ ชนิดขึ้นไปเข้าด้วยกันหรือผสมกับวัตถุอื่น”*
ประกาศสำนักงานคณะกรรมการอาหารและยา เรื่อง การกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารประเภทวัตถุที่ใช้รักษาคุณภาพหรือมาตรฐานอาหาร	กำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารของประเภทวัตถุที่ใช้รักษาคุณภาพหรือมาตรฐานอาหาร *“วัตถุเจือปนอาหารประเภทวัตถุที่ใช้รักษาคุณภาพหรือมาตรฐานของอาหาร (Food additives which are used to prolong or maintain quality of food) หมายความว่า วัตถุเจือปนอาหารที่ได้จากวัตถุเจือปนอาหารชนิดเดี่ยว หรือ วัตถุเจือปนอาหารลักษณะผสมที่มิได้ใช้เจือปนโดยตรงในอาหาร แต่บรรจุอยู่ในภาชนะบรรจุแยกต่างหากและรวมอยู่ในภาชนะบรรจุอาหาร โดยมีวัตถุประสงค์เพื่อรักษาคุณภาพหรือมาตรฐานของอาหาร เช่น วัตถุดูดออกซิเจน (Oxygen absorber) วัตถุกันชื้น (Desiccator หรือ Desiccant) เป็นต้น”*

(3) ตามที่ได้รับความเห็นชอบจากคณะอนุกรรมการเพื่อศึกษาวิเคราะห์ปัญหาและวินิจฉัยในเชิงวิชาการเกี่ยวกับอาหาร โดยผู้ผลิตหรือผู้นำเข้าจะต้องส่งมอบผลการประเมินความปลอดภัยของวัตถุเจือปนอาหารชนิดนั้น พร้อมรายละเอียดข้อมูลประกอบการยื่นขอ ตามข้อ 4(3) ของประกาศฯ มาให้สำนักงานคณะกรรมการอาหารและยา พิจารณากำหนดคุณภาพหรือมาตรฐานและประกาศไว้ในประกาศสำนักงานคณะกรรมการอาหารและยาเพื่อบังคับใช้ต่อไป

ข้อมูลประกอบการพิจารณากรณีวัตถุเจือปนอาหารที่ยังไม่มีข้อกำหนดคุณภาพหรือมาตรฐานหรือเงื่อนไขการใช้ตามประกาศกระทรวงสาธารณสุข (ฉบับที่281) พ.ศ. 2547 เรื่อง วัตถุเจือปนอาหาร

ผู้ขออนุญาตต้องส่งข้อมูล ผลการประเมินความปลอดภัยวัตถุเจือปนอาหาร กรณีวัตถุเจือปนอาหารชนิดใหม่ ตามข้อ 4(3) ของประกาศกระทรวงสาธารณสุขฉบับที่ 281 พ.ศ. 2547 เรื่อง วัตถุเจือปนอาหาร

(1) การระบุส่วนประกอบและลักษณะทางเคมีของวัตถุเจือปนอาหารที่นำ มาประเมินความปลอดภัยโดยมีรายละเอียด ดังนี้

(1.1) เอกลักษณ์และความบริสุทธิ์ของวัตถุเจือปนอาหารที่ใช้ในการทดสอบความเป็นพิษเพื่อประเมินความปลอดภัย (Identity and Purity)

(1.2) ปฏิกิริยาที่เกิดขึ้นและวิถีของวัตถุเจือปนอาหารนั้นๆในอาหาร (Reactions and Fate of Food Additives in Food)

(1.3) ข้อกำหนดคุณลักษณะเฉพาะของวัตถุเจือปนอาหาร (Specifications)

(2) กระบวนการทดสอบและประเมินความปลอดภัย โดยแสดงรายละเอียดดังนี้

(2.1) ระบุตัวชี้วัดในการทดลองและการศึกษาข้อมูลเรื่องการเกิดพิษ ดังต่อไปนี้

ก) ผลกระทบต่อหน้าที่การทำงานของร่างกาย (Functional Manifestations)

ข) การก่อให้เกิดการเปลี่ยนแปลงรูปร่างลักษณะ) (Morphological Manifestations)

ค) การก่อมะเร็ง (Neoplasms)

ง) ความเป็นพิษต่อระบบสืบพันธุ์และการพัฒนาการของร่างกาย (Reproduction and Developmental Toxicity)

จ) ผลการศึกษานอกสัตว์ทดลอง (In Vitro Studies)

(2.2) การนำข้อมูลด้านการเปลี่ยนแปลงในร่างกายและเภสัชจลนศาสตร์ของวัตถุเจือปนอาหารนั้นๆ มาใช้ในการประเมินความปลอดภัย (The use of Metabolic and Pharmacokinetic Studies in Safety Assessment) โดยกล่าวถึงในประเด็น ดังต่อไปนี้

ก) ชนิดของสัตว์ที่นำมาใช้ในการศึกษาทดลองว่ามีความเทียบเคียงกับมนุษย์ได้หรือไม่ มากน้อยเพียงใด (Identifying Relevant Animal Species)

ข) กลไกการเกิดพิษของวัตถุเจือปนอาหารที่ประเมิน (Determining the Mechanisms of Toxicity)

ค) การเปลี่ยนแปลงของวัตถุเจือปนอาหารนั้นๆในร่างกาย (Metabolism into Normal Body Constituents)

ง) ผลกระทบของจุลินทรีย์ที่อยู่ในทางเดินอาหารต่อวัตถุเจือปนอาหารนั้นๆและผลกระทบของวัตถุเจือปนอาหารนั้นๆ ต่อจุลินทรีย์ที่อยู่ในทางเดินอาหาร (Effects of the Gut Microflora on the Chemical and Effects of the Chemical on the Gut Microflora)

(2.3) อิทธิพลของอายุ ภาวะโภชนาการ และภาวะสุขภาพของกลุ่มตัวอย่างที่ใช้ศึกษาทดลองต่อการแปลผลการศึกษา และลักษณะของการออกแบบการศึกษาทดลอง (Influence of Age, Nutritional Status, and Health Status in the Design and Interpretation of Studies)

(2.4) ข้อมูลการศึกษาในมนุษย์ที่นำมาใช้ในการประเมินความปลอดภัย ดังต่อไปนี้

ก) การศึกษาทางระบาดวิทยา (Epidemiological Studies)

ข) อาการไม่พึงประสงค์ที่เกิดขึ้นจากการรับประทานอาหารที่มีวัตถุเจือปนอาหารนั้นๆ เป็นส่วนประกอบอยู่ (Food Intolerance)

(2.5) การกำหนดค่าที่ปลอดภัยสำหรับมนุษย์ในการรับสัมผัสโดยการรับประทานต่อวัน (Acceptable Daily Intake: ADI) โดยกล่าวถึงข้อมูลที่นำมาใช้ในการกำหนดค่า ดังต่อไปนี้

ก) ค่าของขนาดสูงสุดที่ให้แก่สัตว์ทดลองแล้วไม่สังเกตเห็นความผิดปกติ (No observed effect level: NOEL)

ข) การใช้องค์ประกอบความปลอดภัย (Safety factor) ในการคำนวณ

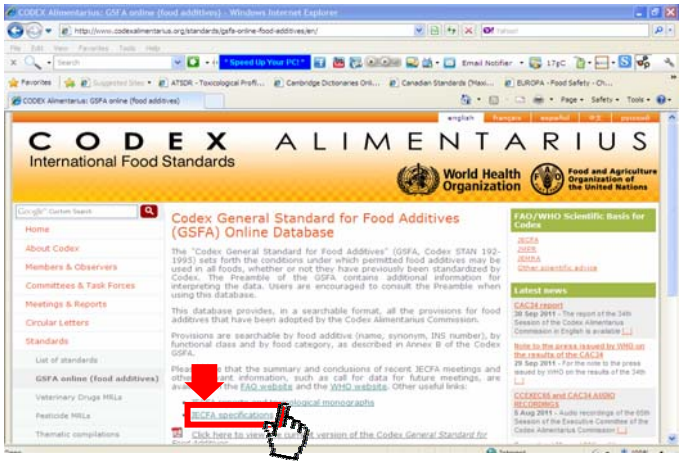
ค) การพิจารณาถึงความเป็นพิษและปฏิกิริยาการตอบสนองของร่างกาย (Toxicological verse physiological responses)

ง) การเปรียบเทียบค่าที่ปลอดภัยสำหรับมนุษย์ในการได้รับสัมผัสโดยการรับประทานต่อวัน (ADI) ที่กำหนดขึ้นกับแนวโน้มที่มนุษย์จะมีโอกาสได้รับสัมผัสวัตถุเจือปนอาหารนั้นๆ จริง

การเข้าถึงฐานข้อมูลข้อกำหนดคุณภาพหรือมาตรฐานตามโคเด็กซ์) Specification)

1. http//:www.codexalimentarius.org/codex-home/en/
2. เลือก “Standards”

3.เลือก “food additives”



4. เลือก “JECFA specifications”

ตัวอย่างคุณภาพหรือมาตรฐานวัตถุเจือปนอาหารที่อนุญาตให้ใช้ตาม

Codex Advisory Specification for the Identity and Purity of Food Additives

SODIUM NITRITE

Prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995) superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-0.06 mg/kg bw was established at 44th JECFA (1995). Nitrite should not be used for infants below 3 months

SYNONYMS	INS No. 250
DEFINITION	
Chemical names	Sodium nitrite
C.A.S. number	7632-00-0
Chemical formula	$NaNO_2$
Formula weight	69.00
Assay	Not less than 97.0% on the dried basis
DESCRIPTION	White or slightly yellow, hygroscopic and deliquescent granules, powder, or opaque, fused masses of sticks
FUNCTIONAL USES	Antimicrobial preservative, colour fixative
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Freely soluble in water, sparingly soluble in ethanol
Test for sodium (Vol. 4)	Passes test
Test for nitrite (Vol. 4)	Passes test
PURITY	
Loss on drying (Vol. 4)	Not more than 0.25% (over silica gel, 4 h)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."
METHOD OF ASSAY	Weigh, to the nearest mg, 1 g of the dried sample. Transfer to a 100 ml volumetric flask and dissolve in water diluting to the mark. Pipette 10.0 ml of this solution into a mixture of 50.0 ml of 0.1N potassium permanganate, 100 ml of water and 5 ml of sulfuric acid, keeping the tip of the pipette well below the surface of the liquid. Warm the solution to 40°, allow it to stand for 5 min, and add 25.0 ml of 0.1N oxalic acid. Heat the mixture to about 80° and titrate with 0.1N potassium permanganate.

$$\% NaNO_2 = \frac{(25+X)}{W} \times 1.450$$

where
X = ml of 0.1N potassium permanganate used for titration
W = weight (in grams) of the sample

รูปแบบของข้อกำหนดคุณภาพหรือมาตรฐาน (Specification)

1. อารัมภบท (Title)

ในส่วนอารัมภบทของคุณภาพหรือมาตรฐานตามมาตรฐานโคเด็กซ์จะมีการกล่าวถึง

(1) ชื่อของวัตถุเจือปนอาหาร

เป็นการระบุชนิดของวัตถุเจือปนอาหาร โดยชื่อของวัตถุเจือปนอาหารดังกล่าวจะเป็นชื่อทางเคมี ซึ่งคณะผู้เชี่ยวชาญด้านวัตถุเจือปนอาหารของโคเด็กซ์ (Joint WHO/FAO Expert of Committee on Food Additives; JECFA) จะมีการพิจารณากำหนดขึ้นอย่างเหมาะสมในการจำแนกหรือตามความจำเพาะของสารนั้น โดยทั่วไปแล้วชื่อของวัตถุเจือปนอาหารที่กำหนดจะสอดคล้องกับการประเมินความปลอดภัย แต่ในบางกรณีอาจมีการกำหนดเป็นกลุ่มสารหากมีการประเมินความปลอดภัยเป็นกลุ่มโดยรวม เช่น ฟอสเฟต

กรณีที่ข้อกำหนดคุณภาพหรือมาตรฐานระบุว่าเป็น 'Tentative' การกำหนดเช่นนี้จะใช้เมื่อคณะผู้เชี่ยวชาญเห็นว่าข้อมูลเกี่ยวกับวัตถุเจือปนอาหารดังกล่าวไม่เพียงพอที่จะกำหนดข้อกำหนดคุณภาพหรือมาตรฐานที่ยอมรับได้อย่างเต็มที่ดังนั้นในกรณีนี้จึงจำเป็นที่จะต้องมีข้อมูลเพิ่มเติมเพื่อพิจารณาต่อไป

(2) รายละเอียดที่มาของการกำหนดข้อกำหนดคุณภาพหรือมาตรฐาน (Specification)

มีการระบุรายละเอียดที่มาของการกำหนดข้อคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารชนิดนั้นๆโดยมีการอ้างอิงมติของการประชุม JECFA ในแต่ละครั้งที่มีการกำหนดหรือเปลี่ยนแปลงข้อกำหนดคุณภาพหรือมาตรฐาน นอกจากนั้นในส่วนนี้ยังมีการระบุค่าความปลอดภัยของวัตถุเจือปนอาหารชนิดนั้นๆ หรือปริมาณวัตถุเจือปนอาหารที่ร่างกายรับได้ต่อวันหรือต่อสัปดาห์โดยไม่ก่อให้เกิดอันตราย โดยมีการกำหนดค่าดังกล่าวไว้ 4 ลักษณะแตกต่างกันไปขึ้นกับชนิดของวัตถุเจือปนอาหารและการประเมินความปลอดภัย ได้แก่

ADI	**Acceptable Daily Intake**
PTWI	**Provisional Tolerable Weekly Intake**
PMTDI	**Provisional Maximum Tolerable Daily Intake**
MTDI	**Maximum Tolerable Daily Intake**

2. ชื่ออื่น (SYNONYMS)

ระบุชื่อสามัญ ตัวย่อ คำย่อ ของวัตถุเจือปนอาหารที่เป็นที่รู้จักกันโดยทั่วไปอย่างกว้างขวางนอกเหนือจากชื่อทางเคมี นอกจากนั้นยังมีการกำหนดระบบเลขรหัสสากล (International Numbering System: INS) และระบบ USA FD&C number ในกรณีของสีผสมอาหาร แต่ทั้งนี้ไม่รวมถึงชื่อที่มีการจดทะเบียนทางการค้า

3. คุณลักษณะ (DEFINITION)

โดยทั่วไปข้อมูลในส่วนนี้จะกล่าวถึงข้อมูลเกี่ยวกับวัตถุดิบ กรรมวิธีการผลิตที่ใช้ในการผลิตวัตถุเจือปนอาหารด้วยคำอธิบายสั้นๆ และสำหรับในกรณีของสารจากธรรมชาติหรือสารที่มีองค์ประกอบที่แตกต่างกันจะมีการระบุข้อมูลรายละเอียดเพิ่มเติมเกี่ยวกับกรรมวิธีการผลิตและการทำให้บริสุทธิ์ รวมทั้งหากมีการใช้วัตถุเจือปนอาหารอื่นที่จำเป็นสำหรับวัตถุเจือปนอาหารนี้ก็จะระบุรายละเอียดไว้

3.1 ชื่อทางเคมี ระบุชื่อทางเคมีของวัตถุเจือปนอาหารตามระบบ IUPAC หรือ IUBMB โดยในหัวข้อนี้อาจมีกำหนดชื่อสามัญที่เป็นที่รู้จักกันโดยทั่วไปเพิ่มเติมด้วย

3.2 CAS number ระบบเลข The Chemical Abstract Service registry number (CAS number)

3.3 Chemical formula สูตรทางเคมีของวัตถุเจือปนอาหาร

3.4 Structural formula สูตรโครงสร้างทางเคมีของวัตถุเจือปนอาหาร

3.5 Formula weight มวลโมเลกุลของวัตถุเจือปนอาหาร

3.6 Assay ปริมาณของสารนั้น ซึ่งจะแสดงเป็นปริมาณที่ไม่น้อยกว่าหรือแสดงค่าที่เป็นปริมาณของสารที่เป็นองค์ประกอบที่สำคัญของวัตถุเจือปนอาหารนั้น

5. คำจำกัดความ (DESCRIPTION)

ข้อมูลส่วนนี้จะกล่าวถึงคุณสมบัติทางกายภาพและคุณสมบัติจำเพาะอื่นๆ เช่น ความคงตัว กลิ่น รวมทั้งในกรณีที่ต้องมีเงื่อนไขในการเก็บรักษาหรือการใช้วัตถุเจือปนอาหารไว้เป็นการเฉพาะ

6. วัตถุประสงค์ในการนำไปใช้ (FUNCTIONAL USES)

ข้อมูลในส่วนนี้แสดงถึงหน้าที่ทางด้านเทคโนโลยีการผลิตของวัตถุเจือปนอาหารชนิดนั้นๆในอาหารหรือกระบวนการผลิตอาหาร โดยวัตถุประสงค์ในการนำไปใช้ (FUNCTIONAL USES) ที่แสดงไว้ต้องสอดคล้องกับ Codex Alimentarius International Numbering System (CAC/GL 36-1989)

7. คุณลักษณะพิเศษจำเพาะ (CHARACTERISTICS)

ข้อมูลในส่วนนี้จะเป็นการแสดงข้อกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหาร โดยสามารถจำแนกเป็นรายละเอียดตามหัวข้อ ดังนี้

7.1 <u>การระบุเอกลักษณ์ (Identification)</u> จะเป็นการระบุคุณสมบัติในเชิงคุณภาพของวัตถุเจือปนอาหารชนิดนั้นๆ ได้แก่ การละลายน้ำ (solubility in water), การละลายในตัวทำละลายอินทรีย์ (solubility in organic solvents), ปฏิกิริยาของสี (colour reactions), การดูดกลืนแสง (absorption spectra) และ ค่าความเป็นกรด-ด่าง (pH values) โดยวิธีทดสอบที่มีการระบุไว้จะอ้างอิงขั้นตอนและรายละเอียดของวิธีวิเคราะห์ไว้ตาม Volume 4 ซึ่งในข้อกำหนดคุณภาพหรือมาตรฐานจะมีการอธิบายวิธีการไว้อย่างสั้นๆ หากต้องการรายละเอียดเพิ่มเติมให้พิจารณาจากหัวข้อ IDENTIFICATION TESTS

7.2 <u>ความบริสุทธิ์ (Purity)</u> จะเป็นการกำหนดข้อจำกัดของการปนเปื้อน ได้แก่ การปนเปื้อนทางด้านจุลินทรีย์ การปนเปื้อนโลหะหนัก รวมทั้งข้อกำหนดอื่นๆที่จำเป็นในการควบคุมความบริสุทธิ์ของวัตถุเจือปนอาหารนั้น โดยค่าที่กำหนดจะสอดคล้องกับหลักเกณฑ์และวิธีการที่ดีในการผลิตอาหาร (Good Manufacturing Practice; GMP) โดยวิธีทดสอบที่มีการระบุไว้จะอ้างอิงขั้นตอนรายละเอียดการวิเคราะห์ไว้ตาม Volume 4 ซึ่งในข้อกำหนดคุณภาพหรือมาตรฐานจะมีการอธิบายวิธีการไว้อย่างสั้นๆ หากต้องการรายละเอียดเพิ่มเติมให้พิจารณาในหัวข้อ PURITY TESTS

8. การทดสอบ (TEST)

8.1 <u>การทดสอบเพื่อระบุเอกลักษณ์ (IDENTIFICATION TESTS)</u> อธิบายเกี่ยวกับระเบียบวิธีในการทดสอบโดยอ้างอิงในส่วนของคุณลักษณะพิเศษจำเพาะ (CHARACTERISTICS) ภายใต้หัวข้อ *IDENTIFICATION* โดยมีการอธิบายเกี่ยวกับทฤษฎีพื้นฐาน วิธีการวิเคราะห์ ซึ่งโดยทั่วไปจะมีการกำหนดรายชื่อของอุปกรณ์และสารเคมีที่จำเป็น รายละเอียดของขั้นตอนในการวิเคราะห์และวิธีการสำหรับการคำนวณผลการวิเคราะห์

8.2 <u>การทดสอบเพื่อทดสอบความบริสุทธิ์ (PURITY TESTS)</u> อธิบายเกี่ยวกับระเบียบวิธีในการทดสอบโดยอ้างอิงในส่วนของคุณลักษณะพิเศษจำเพาะ (CHARACTERISTICS) ภายใต้หัวข้อ *PURITY* โดยมีการอธิบายเกี่ยวกับทฤษฎีพื้นฐาน วิธีการวิเคราะห์ ซึ่งโดยทั่วไปจะมีการกำหนดรายชื่อของอุปกรณ์และสารเคมีที่จำเป็น รายละเอียดของขั้นตอนในการวิเคราะห์และวิธีการสำหรับการคำนวณผลการวิเคราะห์

9. วิธีวิเคราะห์ (METHOD OF ASSAY)

กำหนดหลักการและรายละเอียดของวิธีการวิเคราะห์ โดยมีการอธิบายเกี่ยวกับทฤษฎี รายชื่อของอุปกรณ์และสารเคมีที่จำเป็น รายละเอียดของขั้นตอนในการวิเคราะห์และวิธีการสำหรับการคำนวณผลการวิเคราะห์

การตรวจสอบข้อกำหนดคุณภาพหรือมาตรฐานของวัตถุเจือปนอาหารทั้งเจ้าหน้าที่และผู้ประกอบการจะต้องตรวจสอบข้อมูลเกี่ยวกับข้อกำหนดคุณภาพหรือมาตรฐานดังกล่าวข้างต้นให้เป็นไปตามที่ Codex Advisory Specification for the Identity and Purity of Food Additives กำหนดไว้

การแบ่งกลุ่มวัตถุเจือปนอาหารตามหน้าที่ด้านเทคโนโลยี (Function of Food Additives)

โคเด็กซ์ได้จัดทำเอกสาร Codex Class Names and the International Numbering System for Food Additives CAC/GL 36-1989 เพื่อรวบรวมและแบ่งกลุ่มหน้าที่ทางด้านเทคโนโลยีการผลิตของวัตถุเจือปนอาหารออกเป็น 27 กลุ่ม ดังนี้

กลุ่มตามหน้าที่	นิยามความหมาย	หน้าที่ด้านเทคโนโลยี
1. สารควบคุมความเป็นกรด (Acidity regulator)	เปลี่ยนหรือควบคุมความเป็นกรดหรือด่างของอาหาร	acidity regulator, acid, acidifier, alkali, base, buffer, buffering agent, pH adjusting agent
2. สารป้องกันการจับเป็นก้อน (Anticaking agent)	ลดการจับตัวเป็นก้อนของส่วนผสมของอาหาร	anticaking agent, anti-stick agent, drying agent, dusting agent
3. สารป้องกันการเกิดฟอง (Antifoaming agent)	ป้องกันหรือลดการเกิดฟอง	antifoaming agent, defoaming agent
4. สารป้องกันการเกิดออกซิเดชั่น (Antioxidant)	ยืดอายุของอาหาร โดยป้องกันการเสื่อมเสียจากการออกซิเดชั่น เช่น การหืนของไขมันและการเปลี่ยนสีของอาหาร	antioxidant, antioxidant synergist, antibrowning agent
5. สารฟอกสี (Bleaching agent)	วัตถุเจือปนอาหารที่ใช้สำหรับฟอกสีอาหารชนิดอื่นๆยกเว้นแป้ง	bleaching agent
6. สารเพิ่มปริมาณ (Bulking agent)	สารซึ่งนอกเหนือจากอากาศหรือน้ำ ซึ่งช่วยเพิ่มปริมาณของอาหารโดยไม่มีผลต่อค่าพลังงานของอาหารอย่างมีนัยสำคัญ	bulking agent, filler
7. สารให้ก๊าซคาร์บอนไดออกไซด์ (Carbonating agent)	วัตถุเจือปนอาหารที่เติมลงไปเพื่อให้ก๊าซคาร์บอนไดออกไซด์	carbonating agent
8. สารช่วยทำละลาย หรือช่วยพา (Carrier)	วัตถุเจือปนอาหารที่ใช้สำหรับช่วยในการทำละลาย เจือจาง หรือทำให้เกิดการกระจายตัว มีผลต่อการปรับสภาพทางกายภาพของวัตถุเจือปนอาหารหรือสารอาหารอื่น โดยไม่ก่อให้เกิดผลทางเทคโนโลยี แต่ใช้เพื่อช่วยในการเก็บรักษาหรือเสริมการใช้วัตถุเจือปนอาหารหรือสารอาหารอื่นๆ	carrier, carrier solvent, nutrient carrier, diluent for other food additives, encapsulating agent
9. สี (Colour)	เพิ่มหรือรักษาสีของอาหาร	colour, decorative pigment, surface colourant

กลุ่มตามหน้าที่	นิยามความหมาย	หน้าที่ด้านเทคโนโลยี
10. สารคงสภาพของสี (Colour retention agent)	คงสภาพหรือรักษาหรือเพิ่มสีของอาหาร	colour retention agent, colour fixative, colour stabilizer, colour adjunct
11. อิมัลซิไฟเออร์ (Emulsifier)	รักษาส่วนผสมของสารที่รวมตัวกัน เช่น น้ำและน้ำมันในอาหาร ให้มีความสม่ำเสมอ	emulsifier, plasticizer, dispersing agent, surface active agent, crystallization inhibitor, density adjustment (flavouring oils in beverages), suspension agent, clouding agent
12. เกลืออิมัลซิไฟอิ้งค์ (Emulsifying salt)	เพื่อป้องกันการแยกตัวของไขมัน โดยจัดโปรตีนในเนยแข็งในการผลิตเนยแข็งเสียใหม่	emulsifying salt, melding salt
13. สารทำให้แน่น (Firming agent)	รักษาหรือทำให้เนื้อเยื่อของผลไม้หรือผักแน่นและกรอบ หรือทำปฏิกิริยากับสารให้เจล เพื่อทำให้เกิดเจลหรือให้เจลแข็ง	firming agent
14. สารเพิ่มรสชาติ (Flavour enhancer)	เพิ่มรสชาติหรือกลิ่นของอาหาร	flavour enhancer, flavour synergist
15. สารปรับปรุงคุณภาพแป้ง (Flour treatment agent)	สารที่เติมไปในแป้งเพื่อเพิ่มคุณสมบัติในการอบและการเกิดสี	flour treatment agent, flour bleaching agent, flour improver, dough conditioner, dough strengthening agent
16. สารทำให้เกิดฟอง (Foaming agent)	ทำให้เกิดฟองหรือคงการกระจายตัวของอากาศในอาหารที่เป็นของเหลวหรือของแข็ง	foaming agent, whipping agent, aerating agent
17. สารทำให้เกิดเจล (Gelling agent)	ให้เนื้อสัมผัสของอาหารโดยการสร้างเจล	gelling agent
18. สารเคลือบผิว (Glazing agent)	สารซึ่งเมื่อใช้กับผิวภายนอกของอาหารแล้ว จะมีลักษณะปรากฏที่เป็นผิวมัน หรือช่วยเคลือบผิวเพื่อป้องกัน	glazing agent, sealing agent, coating agent, surface-finishing agent, polishing agent, filmforming agent
19. สารทำให้เกิดความชุ่มชื้น (Humectant)	ป้องกันการแห้งของอาหาร เนื่องจากบรรยากาศที่มีความชื้นต่ำ	humectant, moisture-retention agent, wetting agent
20. ก๊าซที่ช่วยในการเก็บรักษาอาหาร (Packaging gas)	วัตถุเจือปนอาหารที่เป็นก๊าซใช้เติมลงไปในภาชนะบรรจุทั้งก่อน ระหว่างการบรรจุ และหลังการบรรจุ เพื่อยืดอายุการเก็บรักษาอาหาร โดยลดการเกิดปฏิกิริยาออกซิเดชั่น	packaging gas

กลุ่มตามหน้าที่	นิยามความหมาย	หน้าที่ด้านเทคโนโลยี
21. สารกันเสีย (Preservative)	ยืดอายุของอาหารโดยการป้องกันการเสื่อมเสียจากจุลินทรีย์	preservative, antimicrobial preservative, antimycotic agent, bacteriophage control agent, fungistatic agent, antimould and antirope agent, antimicrobial synergist
22. ก๊าซที่ใช้ขับดัน (Propellant)	ก๊าซที่นอกเหนือจากอากาศ ซึ่งช่วยขับดันอาหารออกจากภาชนะบรรจุ	propellant
23. สารช่วยให้ฟู (Raising agent)	สารหรือของผสมของอาหารที่ทำให้อากาศแยกตัวเป็นอิสระและเพิ่มปริมาณของแป้งทำขนมปัง	raising agent
24. สารช่วยจับอนุมูลโลหะ (Sequestrant)	วัตถุเจือปนอาหารที่ใช้จับกับสารที่มีประจุบวก เช่น โลหะหนัก เพื่อป้องกันการเกิดปฏิกิริยาของโลหะหนัก	sequestrant
25. สารทำให้คงตัว (Stabilizer)	ทำให้การแขวนลอยของสารตั้งแต่ 2 ชนิด ที่ไม่รวมกันในอาหารมีความสม่ำเสมอ	stabilizer, foam stabilizer, colloidal stabilizer, emulsion stabilizer
26. สารให้ความหวาน (Sweetener)	สารที่ไม่ใช่น้ำตาลแต่ให้รสหวานแก่อาหาร	sweetener, intense sweetener, bulk sweetener
27. สารให้ความข้นเหนียว (Thickener)	ให้ความหนืดแก่อาหาร	thickener, bodying agent, binder, texturizing agent

รายชื่อหน่วยงานราชการที่ให้บริการตรวจวิเคราะห์ คุณภาพหรือมาตรฐาน เพื่อประกอบการขอขึ้นทะเบียน ตำรับอาหารที่คณะกรรมการอาหารให้การยอมรับ

กำหนดส่วนราชการหรือสถาบันที่คณะกรรมการอาหารให้การยอมรับผลการตรวจวิเคราะห์อาหารเพื่อประกอบการขอขึ้นทะเบียนตำรับอาหาร ดังนี้

ข้อ 1. หน่วยงานของรัฐทั้งในประเทศและต่างประเทศ

ข้อ 2. หน่วยงานหรือองค์กรที่ได้รับมอบหมายหรือได้รับการรับรองจากหน่วยงานของรัฐของประเทศนั้นๆ

ข้อ 3. หน่วยงานหรือองค์กรทั้งในประเทศและต่างประเทศที่ได้รับการรับรองโดยหน่วยงานรับรองห้องปฏิบัติการตามมาตรฐานสากล

- **ตัวอย่างรายชื่อหน่วยงานราชการที่ให้บริการตรวจวิเคราะห์ คุณภาพหรือมาตรฐาน**

หน่วยงานวิเคราะห์	โทรศัพท์	โทรสาร
1. กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข 88/7 หมู่ 4 ซอยโรงพยาบาลบำราศนราดูร ถนนติวานนท์ ตำบลตลาดขวัญ อำเภอเมือง จังหวัดนนทบุรี 11000 **http://www.dmsc.moph.go.th/ossc/**	0-2589-9850-2 ต่อ 9561 หรือ 9562 (ในประเทศ) ต่อ 9503 (เพื่อส่งออก)	0-2951-1023
2. กรมวิทยาศาสตร์บริการ กระทรวงวิทยาศาสตร์และเทคโนโลยี ถนนพระรามที่ 6 เขตราชเทวี กรุงเทพฯ 10400 **http://www.dss.go.th**	0-2201-7183-4 (โครงการวิทยาศาสตร์ชีวภาพ)	0-2201-7181
3. ห้องปฏิบัติการกองโภชนาการ กรมอนามัย กระทรวงสาธารณสุข ถนนติวานนท์ อำเภอเมือง จังหวัดนนทบุรี 11000 (วิเคราะห์เฉพาะชนิดและปริมาณสารอาหารทางโภชนาการ) **http://nutrition.anamai.moph.go.th**	0-2968-7619	0-2968-7616
4. ศูนย์วิทยาศาสตร์การแพทย์		
4.1 ศูนย์วิทยาศาสตร์การแพทย์สงขลา	0-7444-7024-8	0-7433-3809
4.2 ศูนย์วิทยาศาสตร์การแพทย์ชลบุรี	0-3878-4006-7	0-3878-3767
4.3 ศูนย์วิทยาศาสตร์การแพทย์นครราชสีมา	0-4424-1522	0-4424-5860
4.4 ศูนย์วิทยาศาสตร์การแพทยขอนแก่น	0-4324-2871-2	0-4324-2845

- **ตัวอย่างรายชื่อหน่วยงานราชการที่ให้บริการตรวจวิเคราะห์ คุณภาพหรือมาตรฐาน (ต่อ)**

หน่วยงานวิเคราะห์	โทรศัพท์	โทรสาร
4.5 ศูนย์วิทยาศาสตร์การแพทย์เชียงใหม่	0-5321-1065-6	0-5321-9223
4.6 ศูนย์วิทยาศาสตร์การแพทย์พิษณุโลก	0-5524-7579-80	0-5525-8859
4.7 ศูนย์วิทยาศาสตร์การแพทย์อุบลราชธานี	0-4531-2231-4	0-4531-2231
4.8 ศูนย์วิทยาศาสตร์การแพทย์ตรัง	0-7521-3105-7	0-7521-3104
4.9 ศูนย์วิทยาศาสตร์การแพทย์เชียงราย	0-5379-3148-50	0-5379-3148
4.10 ศูนย์วิทยาศาสตร์การแพทย์สมุทรสงคราม	0-3472-0543	0-3472-0540
4.11 ศูนย์วิทยาศาสตร์การแพทย์สุราษฎร์ธานี	0-7728-2670	0-7728-2672
4.12 ศูนย์วิทยาศาสตร์การแพทย์อุดรธานี	0-4220-7364-9	0-4220-7367
4.13 ศูนย์วิทยาศาสตร์การแพทย์ภูเก็ต	0-5626-7423	0-5626-7433
4.14 ศูนย์วิทยาศาสตร์การแพทย์นครสวรรค์	0-7635-4611	0-7635-4612
5. สถาบันค้นคว้าและพัฒนาผลิตภัณฑ์อาหาร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตบางเขน ตู้ ปณ.1043 ปณฝ.เกษตรศาสตร์ จตุจักร กรุงเทพฯ 10903 **http://www.ifrpd.ku.ac.th**	0-2942-8629-35 ต่อ 800,811	0-2942-7601-2
6. สถาบันวิจัยโภชนาการ มหาวิทยาลัยมหิดล ถนนพุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170 **http://www.nu.mahidol.ac.th**	0-2441-9346 (สายตรง) หรือ 0-2800-2380 ต่อ 406, 418 (ประสานงานรับตัวอย่าง)	0-2441-9344
7. สถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย 196 ถนนพหลโยธิน แขวงลาดยาว เขตจตุจักร กรุงเทพฯ 10900 **http://www.tistr.or.th/**	0-2579-5515 หรือ 0-2579-1121-30 ต่อ 5219, 5226	0-2579-8592
8. คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา จังหวัดชลบุรี 169 ถนนลงหาดบางแสน ตำบลแสนสุข อำเภอเมือง จังหวัดชลบุรี 20131	0-3874-5815 0-3874-5266	0-3839-3497 0-3839-3492

รายชื่อวัตถุเจือปนอาหารที่มีการกำหนดข้อกำหนดคุณภาพหรือมาตรฐาน (Specification)
ตามประกาศสำนักงานคณะกรรมการอาหารและยา

รายชื่อวัตถุเจือปนอาหารที่มีการกำหนดข้อกำหนดคุณภาพหรือมาตรฐาน (Specification)
โดย The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
950	ACESULFAME POTASSIUM	อะซีซัลเฟม โพแทสเซียม	0-15	JECFA 1990	22
260	ACETIC ACID, GLACIAL	กรดอะซีติก	Not limited	JECFA 1997	24
472a	ACETIC AND FATTY ACID OF GLYCEROL	กลีเซอรอลเอสเตอร์ของกรดแอซีติกและกรดมัน	Not limited	JECFA 1973	26
406	AGAR	อะการ์	Not limited	JECFA 1973	27
400	ALGINIC ACID	กรดอัลจินิก	Not specified	JECFA 1992	29
956	ALITAME	อลิแตม	0-1	JECFA 1996	31
129	ALLURA RED AC	อะลูราเรด เอซี	0-7	JECFA 1981	35
523	ALUMINIUM AMMONIUM SULFATE	อลูมิเนียม แอมโมเนียม ซัลเฟต	2[b]	JECFA 2011	37
559	ALUMINIUM SILICATE	อลูมิเนียมซิลิเคต	2[b]	JECFA 2011	39
403	AMMONIUM ALGINATE	อลูมิเนียมอัลจิเนต	Not specified	JECFA 1992	41
503(i)	AMMONIUM CARBONATE	อลูมิเนียมคาร์บอเนต	Not specified	JECFA 1982	43
510	AMMONIUM CHLORIDE	อลูมิเนียมคลอไรด์	Not limited	JECFA 1979	45
503(ii)	AMMONIUM HYDROGEN CARBONATE	อลูมิเนียมไฮโดรเจนคาร์บอเนต	Not specified	JECFA 1982	47
527	AMMONIUM HYDROXIDE	อลูมิเนียมไฮดรอกไซด์	Not specified	JECFA 1999	49
442	AMMONIUM SALTS OF PHOSPHATIDIC ACID	เกลือแอมโมเนียมของกรดฟอสฟาติดิก	0-30	JECFA 1974	51
1100	AMYLASE FROM ASPERGILLUS ORYZAE VAR., ALPHA-	เอนไซม์อัลฟ่า-อมัยเลสจากเชื้อแอสเพอจิลลัสโอไรเซ วาร์	Acceptable	JECFA 1985	55
1100	AMYLASE FROM BACILLUS LICHENIFORMIS (CARBOHYDRASE), ALPHA-	อัลฟา-อะไมเลสจาก BACILLUS LICHENIFORMIS (CARBOHYDRASE)	Not specified	JECFA 1990	56
1100	AMYLASE FROM BACILLUS MEGATERIUM EXPRESSED IN BACILLUS SUBTILIS, ALPHA-	อัลฟา-อะไมเลสจาก BACILLUS MEGATERIUM แสดงออกใน BACILLUS SUBTILIS	Not specified	JECFA 1990	59
1100	AMYLASE FROM BACILLUS STEAROTHERMOPHILUS, ALPHA-	อัลฟา-อะไมเลสจาก BACILLUS STEAROTHERMOPHILUS	Not specified	JECFA 1990	60
1100	AMYLASE FROM BACILLUS STEAROTHERMOPHILUS EXPRESSED IN BACILLUS SUBTILIS, ALPHA-	อัลฟา-อะไมเลสจาก BACILLUS STEAROTHERMOPHILUS แสดงออกใน BACILLUS SUBTILIS	Not specified	JECFA 1990	61
1100	AMYLASE FROM BACILLUS SUBTILIS, ALPHA-	อัลฟา-อะไมเลสจาก BACILLUS SUBTILIS	Not specified	JECFA 1999	62

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160b(i)	ANNATTO EXTRACTS, BIXIN-BASED	สีคำแสด	0-12 (bixin)	JECFA 2006	63
			0-0.6 (norbixin)	JECFA 2006	
300	ASCORBIC ACID, L-	กรดแอล-แอสคอร์บิก	Not specified	JECFA 1981	65
	ASCORBYL ESTERS	แอสคอร์บิลเอสเทอร์			
304	Ascorbyl palmitate	แอสคอร์บิล ปาล์มิเตต	0-1.25	JECFA 1973	67
305	Ascorbyl stearate	แอสคอร์บิล สเตียเรต	0-1.25	JECFA 1973	69
951	ASPARTAME	แอสปาแตม	0-40	JECFA 1981	71
962	ASPARTAME-ACESULFAME SALT	เกลือของแอสปาแตม-อะซีซัลเฟม	0-40 (ASPARTAME)	JECFA 1981	76
			0-15 (ACESULFAME K)	JECFA 1990	
927a	AZODICARBONAMIDE	เอโซไดคาร์โบนาไมด์	ไม่กำหนด		80
901	BEESWAX	บีแวกซ์	ไม่กำหนด		82
162	BEET RED	บีตเรด	Not specified	JECFA 1987	85
	BENZOATES	เบนโซเอต			
210	Benzoic acid	กรดเบนโซอิก	0-5	JECFA 1996	89
211	Sodium benzoate	โซเดียม เบนโซเอต	0-5	JECFA 2001	91
212	Potassium benzoate	โพแทสเซียม เบนโซเอต	0-5	JECFA 1996	93
213	Calcium benzoate	แคลเซียม เบนโซเอต	0-5	JECFA 1996	95
928	BENZOYL PEROXIDE	เบนโซอิลเปอร์ออกไซด์	ไม่กำหนด		97
133	BRILLIANT BLUE FCF	บริลเลียนท์บลู เอ็ฟซีเอ็ฟ	0-12.5	JECFA 1969	99
1101(iii)	BROMELAIN	โบรมีเลน	Not limited	JECFA 1971	102
320	BUTYLATED HYDROXYANISOLE	บิวทิเลตเตตไฮดรอกซีอะนีโซน	0-0.5	JECFA 1988	103
321	BUTYLATED HYDROXYTOLUENE	บิวทิเลตเตตไฮดรอกซีโทลูอีน	0-0.3	JECFA 1995	107
629	CALCIUM 5'-GUANYLATE	แคลเซียม 5'-กัวนิเลต	Not specified	JECFA 1974	110
633	CALCIUM 5' INOSINATE	แคลเซียม 5'-อิโนซิเนต	Not specified	JECFA 1985	112
634	CALCIUM 5'-RIBONUCLEOTIDES	แคลเซียม 5'-ไรโบนิวคลีโอไทด์	Not specified	JECFA 1974	114
263	CALCIUM ACETATE	แคลเซียมอะซีเตต	Not limited	JECFA 1973	118
404	CALCIUM ALGINATE	แคลเซียมอัลจิเนต	Not specified	JECFA 1992	120
556	CALCIUM ALUMINIUM SILICATE	แคลเซียมอลูมิเนียมซิลิเคต	2[b]	JECFA 2011	122
302	CALCIUM ASCORBATE	แคลเซียมแอสคอร์เบต	Not specified	JECFA 1981	125
170(i)	CALCIUM CARBONATE	แคลเซียมคาร์บอเนต	Not limited	JECFA 1965	127
509	CALCIUM CHLORIDE	แคลเซียมคลอไรด์	Not limited	JECFA 1973	130
623	CALCIUM DI-L-GLUTAMATE	แคลเซียมได-แอล-กลูตาเมต	Not specified	JECFA 1987	132
578	CALCIUM GLUCONATE	แคลเซียมกลูโคเนต	Not specified	JECFA 1998	134

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526	CALCIUM HYDROXIDE	แคลเซียมไฮดรอกไซด์	Not limited	JECFA 1965	136
327	CALCIUM LACTATE	แคลเซียมแลกเตต	Not limited	JECFA 1973	138
352(ii)	CALCIUM MALATE,DL-	แคลเซียมมาเลต, ดีแอล-	Not specified	JECFA 1999	140
529	CALCIUM OXIDE	แคลเซียมออกไซด์	Not limited	JECFA 1965	142
282	CALCIUM PROPIONATE	แคลเซียมโปรปิโอเนต	Not limited	JECFA 1973	144
552	CALCIUM SILICATE	แคลเซียมซิลิเคต	Not specified	JECFA 1985	146
516	CALCIUM SULFATE	แคลเซียมซัลเฟต	Not limited	JECFA 1973	148
902	CANDELILLA WAX	แคนเดลลิลล่าแวกซ์	ไม่กำหนด		150
161g	CANTHAXANTHIN	แคนธาแซนธีน	0-0.03	JECFA 1995	152
	CARAMEL COLOURS				155
150a	CARAMEL I - PLAIN CARAMEL	คาราเมล I	Not specified	JECFA 1985	
150c	CARAMEL III - AMMONIA CARAMEL	คาราเมล III (แอมโมเนีย)	0-200	JECFA 1985	
			0-150 (on solids basis)	JECFA 1985	
150d	CARAMEL IV - SULFITE AMMONIA CARAMEL	คาราเมล IV (ซัลไฟต์แอมโมเนีย)	0-200	JECFA 1985	
			0-150 (on solids basis)	JECFA 1985	
290	CARBON DIOXIDE	คาร์บอนไดออกไซด์	Not specified	JECFA 1985	166
120	CARMINES	คาร์มีน	0-5	JECFA 2000	170
903	CARNAUBA WAX	คาร์นูบาแวกซ์	0-7	JECFA 1992	173
410	CAROB BEAN GUM	คาร็อบบีนกัม	Not specified	JECFA 1981	175
	CAROTENOIDS	คาโรทีนอยด์			
160a(i)	beta-Carotenes, synthetic	เบต้า-คาโรทีน, สังเคราะห์)	0-5	JECFA 2001	183
160a(iii)	beta-Carotenes, Blakeslea trispora	เบต้า-คาโรทีน, บลาเคสเซีย ไตรสปอรา	0-5	JECFA 2001	187
160e	beta-apo-8'-Carotenal	เบต้า-อะโป-8’-คาโรทีนาล	0-5	JECFA 2001	190
160f	Carotenoic acid, ethyl ester, betaapo-8'-	กรดคาโรทีนิก, เอทิลเอสเทอร์, เบต้า-อะโป-8’-	0-5	JECFA 2001	194
407	CARRAGEENAN	คาราจีแนน	Not specified	JECFA 2001	198
1503	CASTOR OIL	น้ำมันละหุ่ง	0-0.7	JECFA 1979	204
925	CHLORINE	คลอรีน	ไม่กำหนด		205
140	CHLOROPHYLLS	คลอโรฟิลล์	Not limited	JECFA 1969	207
	CHLOROPHYLLS AND CHLOROPHYLLINS, COPPER COMPLEXES	คลอโรฟิลล์และคลอโรฟิลิน, คลอโรฟิลล์และคลอโรฟิลินคอปเปอร์คอมเปลกซ์			
141(i)	Chlorophylls, copper complexes	คลอโรฟิลล์และคลอโรฟิลล์ คอปเปอร์คอมเปลกซ์	0-15	JECFA 1969	211

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141(ii)	Chlorophyllin copper complexes, potassium and sodium salts	คลอโรฟิลินคอปเปอร์คอมเปลกซ์, เกลือโพแทสเซียมและโซเดียม	0-15	JECFA 1978	214
330	CITRIC ACID	กรดซิตริก	Not limited	JECFA 1973	217
472c	CITRIC AND FATTY ACID ESTERS OF GLYCEROL	กลีเซอรอลเอสเตอร์ของกรดซิตริกและกรดไขมัน	Not limited	JECFA 1973	219
468	CROSS-LINK SODIUM CARBOXYMETHYL CELLULOSE (CROSS-LINK-CELLULOSE GUM)	ครอสลิงค์โซเดียมคาร์บอกซีเมทิลเซลลูโลส	Not specified	JECFA 1989	223
424	CURDLAN	เคอร์ดแลน	Not specified	JECFA 2001	227
952	Cyclamic acid	กรดซัยคลามิก	0-11	JECFA 1982	230
952(ii)	Calcium cyclamate	แคลเซียม ซัยคลาเมต	0-11	JECFA 1982	232
952(iv)	Sodium cyclamate	โซเดียม ซัยคลาเมต	0-11	JECFA 1982	234
459	CYCLODEXTRIN, BETA-	ไซโคลเดกซ์ตริน, เบต้า	0-5	JECFA 1995	236
472e	DIACETYLTARTARIC AND FATTY ACID ESTERS OF GLYCEROL	ไดกลีเซอไรด์เอสเตอร์ของกรดไดอะซีติลตาร์ตาริก	0-50	JECFA 2003	241
242	DIMETHYL DICARBONATE	ไดเมทิล ไดคาร์บอเนต	ไม่กำหนด		246
628	DIPOTASSIUM 5'-GUANYLATE	ไดโพแทสเซียม 5'-กัวนิเลต	Not specified	JECFA 1985	250
627	DISODIUM-5'-GUANYLATE	ไดโซเดียม 5'-กัวนิเลต	Not specified	JECFA 1974	252
631	DISODIUM-5'-INOSINATE	ไดโซเดียม 5'-อิโนซิเนต	Not specified	JECFA 1985	254
635	DISODIUM-5'-RIBONUCLEOTIDES	ไดโซเดียม 5'-ไรโบนิวคลีโอไทด์	Not specified	JECFA 1974	256
315	ERYTHORBIC ACID	กรดอีริทอร์บิก	Not specified	JECFA 1990	260
968	ERYTHRITOL	อีริทรีทอล	Not specified	JECFA 1999	262
127	ERYTHROSINE	เออริโธรซิน	0-0.1	JECFA 1991	265
	ETHYLENE DIAMINE TETRA ACETATES	เอทิลีนไดอามีนเตตระอะซีเตต			
385	Calcium disodium ethylenediaminetetraacetate	แคลเซียม ไดโซเดียม เอทิลีนไดอามีน เตตระอะซีเตต	0-2.5	JECFA 1973	269
386	Disodium ethylenediaminetetraacetate	ไดโซเดียม เอทิลีนไดอามีนเตตระอะซีเตต	0-2.5	JECFA 1973	271
462	ETHYL CELLULOSE	เอทิลเซลลูโลส	Not specified	JECFA 1989	274
467	ETHYL HYDROXYETHYL CELLULOSE	เอทิลไฮดรอกซีเอทิลเซลลูโลส	Not specified	JECFA 1989	276
143	FAST GREEN FCF	ฟาสต์กรีน เอฟซีเอฟ	0-2.5	JECFA 1986	284
381	FERRIC AMMONIUM CITRATE	เฟอร์ริคแอมโมเนียมซิเตรต	0.8[c]	JECFA 1985	287

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	FERROCYANIDES	เฟอร์โรไซยาไนด์			289
535	Sodium ferrocyanide	โซเดียม เฟอร์โรไซยาไนด์	0-0.025	JECFA 1974	
536	Potassium ferrocyanide	โพแทสเซียม เฟอร์โรไซยาไนด์	0-0.025	JECFA 1974	
538	Calcium ferrocyanide	แคลเซียม เฟอร์โรไซยาไนด์	0-0.025	JECFA 1974	
579	FERROUS GLUCONATE	เฟอร์รัสกลูโคเนต	0.8[c]	JECFA 1983	291
585	FERROUS LACTATE	เฟอร์รัสแลคเตต	0.8[c]	JECFA 1989	293
297	FUMARIC ACID	กรดฟูมาริก	Not specified	JECFA 1989	295
418	GELLAN GUM	เจลแลนกัม	Not specified	JECFA 1990	297
575	GLUCONO DELTA-LACTONE	กลูโคโนเดลต้าแลกโตน	Not specified	JECFA 1998	301
1102	GLUCOSE OXIDASE	กลูโคสออกซิเดส	Not specified	JECFA 1974	303
620	GLUTAMIC ACID, L(+)-	กรดแอล-กลูตามิก	Not specified	JECFA 1987	304
422	GLYCEROL	กลีเซอรอล	Not specified	JECFA 1976	306
445	GLYCEROL ESTERS OF WOOD ROSIN	เอสเตอร์กัม	0-12.5 (temporary ADI)	JECFA 2011 (TENTATIVE)	310
163(ii)	GRAPE SKIN EXTRACT	สีจากสารสกัดเปลือกองุ่น	0-2.5	JECFA 2011	312
314	GUAIAC RESIN	กัวไอแอคเรซิน	0-2.5	JECFA 1973	315
626	GUANYLIC ACID, 5'-	กรด5'-กัวนิลิก	Not specified	JECFA 1974	317
412	GUAR GUM	กัวร์กัม	Not specified	JECFA 1975	319
414	GUM ARABIC	กัมอาราบิก	Not specified	JECFA 1989	327
239	HEXAMETHYLENE TETRAMINE	เฮกซะเมทิลีนเตตระมีน	0-0.15	JECFA 1973	329
507	HYDROCHLORIC ACID	กรดไฮโดรคลอริก	Not limited	JECFA 1965	331
	HYDROXYBENZOATES, PARA-	พารา-ไฮดรอกซีเบนโซเอต			
214	Ethyl para-hydroxybenzoate	เอทิล พารา-ไฮดรอกซีเบนโซเอต	0-10	JECFA 1973	337
218	Methyl para-hydroxybenzoate	เมทิล พารา-ไฮดรอกซีเบนโซเอต	0-10	JECFA 1973	339
463	HYDROXYPROPYL CELLULOSE	ไฮดรอกซีโพรพิลเซลลูโลส	Not specified	JECFA 1989	341
464	HYDROXYPROPYL METHYL CELLULOSE	ไฮดรอกซีโพรพิลเมทิลเซลลูโลส	Not specified	JECFA 1989	346
132	INDIGOTINE (INDIGO CARMINE)	อินดิโกตินหรืออินดิโกคาร์มิน	0-5	JECFA 1974	354
630	INOSINIC ACID, 5'-	กรด5'-อิโนซินิก	Not specified	JECFA 1985	359
	IRON OXIDES	ไอรอนออกไซด์			361
172(i)	Iron oxide, black	ไอรอนออกไซด์, สีดำ	0-0.5	JECFA 1999	
172(ii)	Iron oxide, red	ไอรอนออกไซด์, สีแดง	0-0.5	JECFA 1999	
172(iii)	Iron oxide, yellow	ไอรอนออกไซด์, สีเหลือง	0-0.5	JECFA 1999	

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953	IISOMALT	ไอโซมอลต์	Not specified	JECFA 1985	363
384	ISOPROPYL CITRATES	ไอโซโปรปิลซิเตรต	0-14	JECFA 1973	368
416	KARAYA GUM	คารายากัม	Not specified	JECFA 1988	370
425	KONJAC FLOUR	คอนยักฟลาว	Not specified	JECFA 1996	373
270	LACTIC ACID, L-, D- and DL-	กรดแลกติก	Not limited	JECFA 1979	375
472b	LACTIC AND FATTY ACID ESTERS OF GLYCEROL	กลีเซอรอลเอสเตอร์ของกรดแลกติกและกรดไขมัน	Not limited	JECFA 1973	378
966	LACTITOL	แลกติทอล	Not specified	JECFA 1983	379
243	LAURIC ARGINATE ETHYL ESTER	ลอริกอาร์จิเนตเอทิลเอสเตอร์	0-4	JECFA 2008	382
322(i)	LECITHIN	เลซิทิน	Not limited	JECFA 1973	387
1104	LIPASE	ไลเปส	Not limited	JECFA 1971	391
1105	LYSOZYME	ไลโซไซม์	ไม่กำหนด		395

หมายเหตุ

[a] **Acceptable Daily Intake (ADI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

[b] **Provisional Tolerable Weekly Intake (PTWI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

[c] **Provisional Maximum Tolerable Daily Intake (PMTDI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

[d] **Maximum Tolerable Daily Intake (MTDI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

ACESULFAME POTASSIUM

Prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001), superseding specifications prepared at the 46th JECFA (1996) and published in FNP 52 Add 4 (1996). An ADI of 0-15 mg/kg body weight was established at the 37th JECFA (1990).

SYNONYMS Acesulfame K; INS No. 950

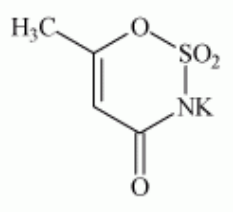
DEFINITION

Chemical names: Potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide; potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide

C.A.S. number: 55589-62-3

Chemical formula: $C_4H_4KNO_4S$

Structural formula:



Formula weight: 201.24

Assay: Not less than 99.0% and not more than 101.0% on the dried basis

DESCRIPTION Odourless, white crystalline powder

FUNCTIONAL USES Sweetener, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, very slightly soluble in ethanol

Spectrophotometry: Dissolve 10 mg of the sample in 1,000 ml of water. The solution shows an absorbance maximum at 227±2 nm

Test for potassium (Vol.4): Passes test
Test the residue obtained by igniting 2 g of the sample

Precipitation test: Add a few drops of a 10% solution of sodium cobaltinitrite to a solution of 0.2 g of the sample in 2 ml of acetic acid TS and 2 ml of water. A yellow precipitate is produced.

PURITY

Loss on drying (Vol. 4)	Not more than 1.0% (105°, 2 h)
pH (Vol. 4)	5.5 - 7.5 (1% soln)
Organic impurities	Passes test for 20 mg/kg of UV active components See description under TESTS
Fluoride (Vol. 4)	Not more than 3 mg/kg Method III; using an appropriate sample size and appropriate volumes of the standard solution for construction of the calibration curve.
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Organic impurities

Proceed as directed under the method for Chromatography (High Performance Liquid Chromatography, FNP 5) using the following conditions and using 4-hydroxybenzoic acid ethyl ester as the reference substance:

Column: 25 cm x 4.6 mm stainless steel
Stationary phase: Reversed phase (C18 silica gel, 3 - 5 µm)
Elution: Isocratic
Mobile phase: Acetonitrile/0.01 mol/l tetrabutyl ammonium hydrogen sulfate (TBAHS) in water; 40/60 v/v
Flow: About 1 ml/min
Detector type: UV or Diode array, 227 nm
Sample size: 20 µl of a 10 g/l solution of the sample in deionized water

The chromatographic system must be capable of separating acesulfame K and 4-hydroxybenzoic acid ethyl ester with a resolution of 2.
If peaks other than that due to acesulfame K appear within three times the elution time of acesulfame K, carry out a second run using 20 µl of a 0.2 mg/l solution of the sample.

The sum of the areas of all peaks eluted in the first run within 3 times the elution time of acesulfame K elution time, except for the acesulfame K peak, does not exceed the peak area of acesulfame K in the second run.

METHOD OF ASSAY

Dissolve about 0.15 g of the dried sample (dissolution may be slow), accurately weighed, in 50.0 ml glacial acetic acid and titrate potentiometrically with 0.1 N perchloric acid, or add two drops of crystal violet TS and titrate with 0.1 N perchloric acid, to a blue-green end-point which persists for at least 30 sec. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 20.12 mg of $C_4H_4KNO_4S$.

ACETIC ACID, GLACIAL

Prepared at 63rd JECFA (2004) and published in FNP 52 ADD 12 (2004) superseding specifications prepared at the 19th JECFA (1975), and published in FNP 52 (1992). Metal contaminants specifications amended at the 59th JECFA (2002). A group ADI 'not limited' for acetic acid and its potassium and sodium salts was established at the 17th JECFA (1973) and maintained at the 49th JECFA (1997).

SYNONYMS INS No. 260

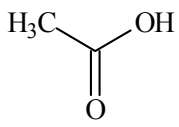
DEFINITION Acetic acid is manufactured by aerial oxidation of C5-C6 fractions of aliphatic hydrocarbons, and separation of the various acids by distillation. Also by oxidation of acetaldehyde, methanol and of butane or as the reaction product of methanol and carbon dioxide.

Chemical name: Acetic acid, ethanoic acid

C.A.S. number: 64-19-7

Chemical formula: $C_2H_4O_2$

Structural formula:



Formula weight: 60.05

Assay: Not less than 99.5%

DESCRIPTION Colourless liquid, having a pungent characteristic odour

FUNCTIONAL USES Acid, flavouring agent (see Flavouring agent specification, JECFA No. 81)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Miscible with water, ethanol, glycerol and diethyl ether

Test for acid: 1 in 3 aqueous solution is acidic

Test for acetate (Vol. 4): Apply to a 1 in 3 solution of the sample
Passes test

PURITY

Solidification point (Vol. 4): Not lower than 15.6º

Non-volatile residue (Vol. 4): Not more than 0.01% after evaporation of 20 g of the sample and holding at 100° for 2 h.

Readily oxidizable substances: Dilute 2 ml of the sample in a glass-stoppered container with 10 ml of water and add 0.1 ml of 0.1 N potassium permanganate. The pink colour does not change to brown within 30 min.

Lead (Vol. 4): Not more than 0.5 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental methods.”

METHOD OF ASSAY

Measure about 2 ml of the sample into a tared, glass-stoppered flask, and weigh accurately. Add 40 ml of water, then add phenolphthalein TS and titrate with 1 N sodium hydroxide. Each ml of 1 N sodium hydroxide is equivalent to 60.05 mg of $C_2H_4O_2$.

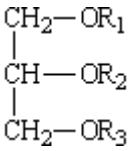
ACETIC and FATTY ACID ESTERS of GLYCEROL

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992) Metals specifications revised at the 55th JECFA (2000).
An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS Acetic acid esters of mono- and diglycerides, acetoglycerides, acetylated mono- and diglycerides, INS No.472a

DEFINITION Mixed glycerol esters of acetic acid and fatty acids of food fats. Contains mono- and diesters of fatty acids with glycerol which is itself partially acetylated; may also contain free glycerol and free fatty acids.

Structural formula



where R_1, R_2 and R_3 each may be a fatty acid moiety, - $COCH_3$ or H

DESCRIPTION From liquid to solid in consistency; white to pale yellow, may have the odour of acetic acid

The article of commerce may be further specified as the saponification value, acid value, free fatty acid content, solidification point of the free fatty acids, Reichert-Meissl value, iodine value and free glycerol content.

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in ethanol

Test for fatty acid (Vol. 4) Passes test

Test for acetic acid (Vol. 4) Passes test

Test for glycerol (Vol. 4) Passes test

PURITY

Acids Acids other than acetic and fatty acids shall not be detectable

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

AGAR

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS Agar-agar; gelose; Japan agar; Bengal, Ceylon, Chinese or Japanese isinglass; Layor Carang; INS No. 406

DEFINITION Agar is the dried hydrophilic, colloidal substance extracted from certain marine algae of the class Rhodophyceae. It is a polysaccharide, consisting primarily of D- and L-galactose units. About every tenth D-galactopyranose unit contains a sulfate ester group. Calcium, magnesium, potassium or sodium cations are also associated with the polysaccharide.

C.A.S. number 9002-18-0

Assay Not higher than 0.25% for threshold gel concentration

DESCRIPTION Odourless or has a slight characteristic odour. Unground agar usually occurs in bundles consisting of thin, membranous, agglutinated strips, or in cut, flaked, granulated or powdered forms. It may be light yellowish orange, yellowish grey to pale yellow, or colourless. It is tough when damp, brittle when dry. Powdered agar is white to yellowish white or pale yellow.

FUNCTIONAL USES Thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility Insoluble in cold water; soluble in boiling water

Gel formation with water Prepare a 1.0% solution of the sample in boiling water in a flask and place the flask in water at 30^{o} for 15 min. A firm, resistant gel is formed. Place the flask in water at 70^{o} for 1 h, the gel is not molten. When heating the flask at higher temperature than 95^{o}, gel is liquefied to form a clear solution.

Precipitate formation with ammonium sulfate solution A warm (40^{o}) 0.5% solution of the sample gives a precipitate with half its volume of a warm (40^{o}) 40% ammonium sulfate solution. This test distinguishes agar from alginates, gum arabic, gum ghatti, karaya gum, pectin and tragacanth.

Precipitate formation with lead acetate solution A warm 0.5% solution of the sample gives a precipitate with one fifth its volume of basic lead acetate TS. This test distinguishes agar from methyl cellulose.

Microscopy Place a few fragments of unground agar or some powder on a slide and add some drops of water or chloral hydrate TS. When examined under a microscope, agar in water appears granular and somewhat filamentous. In chloral hydrate TS, the powdered agar appears more transparent than in water.

PURITY

Water absorption — Place 5 g of the sample in a 100-ml graduated cylinder, fill to the mark with water, mix, and allow to stand at 25° for 24 h. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into a second 100-ml graduated cylinder. Not more than 75 ml of water should be obtained.

Loss on drying (Vol. 4) — Not more than 22% after drying at 105° until the difference between two weighings is less than 1 mg (about 5 h). Unground agar should be cut into pieces from 2 to 5 mm^2 before drying.

Total ash (Vol. 4) — Not more than 6.5% on the dried basis

Acid-insoluble ash (Vol. 4) — Not more than 0.5% on the dried basis

Foreign insoluble matter — Not more than 1%
Boil 5 g of the sample with 500 ml of water and 12 ml of sulfuric acid under a reflux condenser for 2 h. Allow to cool and filter through a tared, fine, sintered glass crucible. Wash flask and filter with 50 ml of water, dry at 105° to constant weight and weigh. Calculate as percentage.

Starch and dextrins — Not detectable
To a warm (40°) 0.5% solution of the sample, add 2 drops of iodine TS. Where the drops fall, a red-violet colour appears. After mixing, the solution should be golden brown and not blue or reddish.

Gelatin and other proteins — Not detectable
To a warm (40°) 0.5% solution of the sample add 1 volume of warm (40°) picric acid TS. No turbidity should appear within 10 min.

Microbiological criteria (Vol. 4) — Total plate count: Not more than 5,000 colonies per gram.
Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate buffered dilution water and homogenizing in a high speed blender.
Yeasts and moulds: Not more than 500 colonies per gram
Coliforms: Negative by test
Salmonella: Negative by test

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY — Threshold gel concentration: Prepare serial dilutions of the sample with known solids content (0.15%, 0.20%, 0.25%, etc.) and place in tubes, 150 mm long by 16 mm internal diameter, stoppered at both ends. Cool for 1 h at 20-25°. Allow cylinders of gel to slide from the tubes to a level surface. The lowest concentration of gel that resists gravity without rupture for 5-30 sec is the threshold concentration of the sample.

ALGINIC ACID

Prepared at the 49th JECFA (1997) ,published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). Metals and arsenic specifications revised at the 49th JECFA (1997). An ADI 'not specified' was established at the 39th JECFA (1992)

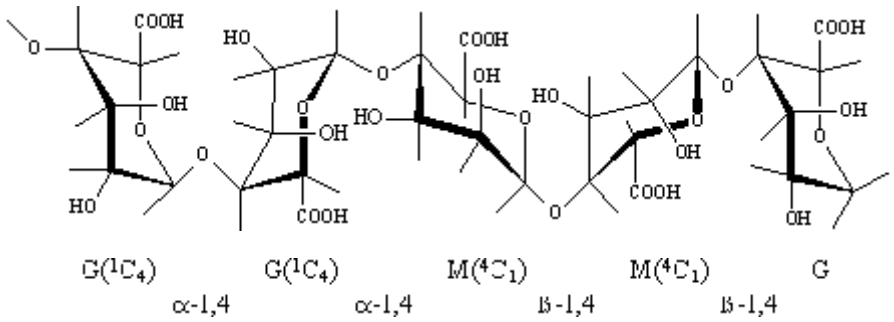
SYNONYMS INS No. 400

DEFINITION Alginic acid is a naturally occurring hydrophilic colloidal polysaccharide obtained from the various species of brown seaweed (*Phaeophyceae*). It is a linear copolymer consisting mainly of residues of ß-1,4-linked D-mannuronic acid and a-1,4-linked L-glucuronic acid. These monomers are often arranged in homopolymeric blocks separated by regions approximating an alternating sequence of the two acid monomers.

C.A.S. number 9005-32-7

Chemical formula $(C_6H_8O_6)_n$

Structural formula Structural formula from Phillips, Wedlock and Williams: Gums and Stabilizers for the Food Industry 5 (1990) by permission of Oxford University Press.



The number and sequence of the Mannuronate and Glucuronate residues shown above vary in the naturally occurring alginate. The associated water molecules are not shown.

Formula weight Structural unit: 176.13 (theoretical), 200 (actual average)
Macromolecule : 10,000 - 600,000 (typical average)

Assay Yields, on the dried basis not less than 20.0% and not more than 23.0% of carbon dioxide (CO_2), equivalent to not less than 91.0% and not more than 104.5% of alginic acid $(C_6H_8O_6)_n$.

DESCRIPTION White to yellowish brown filamentous, grainy, granular or powdered forms

FUNCTIONAL USES Stabilizer, thickener, gelling agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

pH (Vol. 4)	2.0-3.5 (0.3 in 10 suspension)
Precipitate formation with ammonium sulfate	To a 0.5% solution of the sample in sodium hydroxide TS add one-half of its volume of a saturated solution of ammonium sulfate. No precipitate is formed. This test distinguishes alginic acid from agar, sodium carboxymethyl cellulose, carrageenan, de-esterified pectin, gelatin, carob bean gum, methyl cellulose and starch.
Test for alginate	Passes test Dissolve as completely as possible 0.1 g of sample by shaking with 0.15 ml of 0.1 N sodium hydroxide and add 1 ml of acid ferric sulfate TS. Within 5 min, a cherry-red colour develops that finally becomes deep purple.
PURITY	
Loss on drying (Vol. 4)	Not more than 15% (105°, 4 h)
Sulfated ash (Vol. 4)	Not more than 8% on the dried basis
Sodium hydroxide insoluble matter (Vol. 4)	Not more than 2% on the dried basis Weigh accurately about 1 g of the sample and dissolve in 100 ml of sodium hydroxide TS, centrifuge and decant. Wash the residue five times with water by mixing, centrifuging and decanting. Transfer the residue by means of water to a tared fine glass filter, dry for 1 h at 105°, cool and weigh. Calculate as percentage of the dry weight.
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Microbiological criteria (Vol. 4)	Total plate count: Not more than 5,000 colonies per gram. Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield’s phosphate buffered dilution water and homogenizing in a high speed blender. Yeasts and moulds: Not more than 500 colonies per gram Coliforms: Negative by test Salmonella: Negative by test
METHOD OF ASSAY	Proceed as directed under Carbon Dioxide Determination by Decarboxylation in the *General Methods*, Volume 4. Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2) or 25 mg of alginic acid (equivalent weight 200).

ALITAME

Prepared at the 59th JECFA (2002), published in FNP 52 Add 10 (2002) superseding specifications prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996), and incorporating heavy metal limits established by the 57th JECFA (2001), published in FNP 52 Add 9 (2001). An ADI of 0-1 mg/kg bw was established at the 46th JECFA (1996).

SYNONYMS INS No. 956

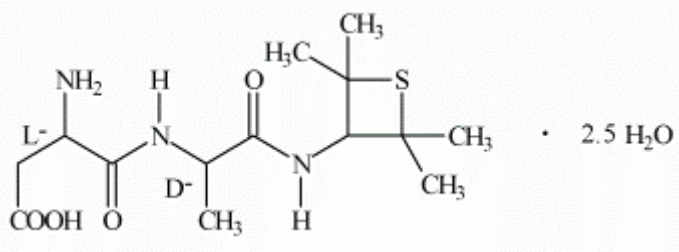
DEFINITION Alitame is prepared by a multistep synthesis involving the reaction between two intermediates, (S)-[2,5-dioxo-(4-thiazolidine)] acetic acid and (R)-2-amino-N-(2,2,4,4-tetramethyl-3-thietanyl)propanamide. The final product is isolated and purified through crystallization of an alitame / 4-methylbenzenesulfonic acid adduct followed by additional purification steps, and finally recrystallization from water as the 2.5 hydrate.

Chemical names L-α-Aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide, hydrated

C.A.S. number 99016-42-9 (hydrated form)
80863-62-3 (anhydrous form)

Chemical formula $C_{14}H_{25}N_3O_4S \cdot 2.5\ H_2O$

Structural formula



Formula weight 376.5 (hydrated form)

Assay Not less than 98.0% and not more than 101.0% on the anhydrous basis

DESCRIPTION White, crystalline powder, odourless or having a slight characteristic odour. Approximately 2000 times sweeter than sucrose.

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water and in ethanol

Infrared spectrum (Vol. 4) The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum below

pH (Vol. 4) Between 5.0 and 6.0 (5 in 100 soln)

Colour reactions

1. To 5 ml of a solution of 300 mg of ninhydrin in 100 ml of n-butanol and 2 ml of glacial acetic acid, add 10 mg of the sample, and heat to gentle reflux. An intense blue-violet colour is formed.

2. To 5 ml of a freshly prepared 0.001 mol/l potassium permanganate solution add 10 mg of the sample and mix thoroughly. The purple solution changes to brown.

PURITY

Beta isomer

Not more than 0.3%, calculated on the anhydrous basis
See description under METHOD OF ASSAY

Alanine amide

Not more than 0.2%, calculated on the anhydrous basis
See description under METHOD OF ASSAY

Water (Vol. 4)

Between 11 and 13% (Karl Fischer method)

Specific rotation (Vol. 4)

[α] 25, D: between +40º and +50º, 1% (w/v) in water

Sulfated ash (Vol. 4)

Not more than 1.0%

Lead

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Principle

Alitame and its constituents, the beta isomer and alanine amide, are measured by reverse-phase ion-pair HPLC.

Chromatographic System

Fit a high pressure liquid chromatograph, operated at room temperature, with a constant flow pump and a 15 x 0.4 cm NovaPak C_{18} reverse phase ion-pair column (Waters, or equivalent). The mobile phase is maintained at a pressure and flow rate (typically 1.0 ml/min) capable of giving the required elution time (see System Suitability Test). An ultraviolet detector that monitors absorption at 217 nm is used.

Mobile Phase

To make the buffer solution, add 0.69 g of sodium phosphate, monobasic, monohydrate and 4.32 g sodium 1-octanesulfonate, reagent grade (obtainable from Regis Chemical Co.) to a 1000-ml volumetric flask. Add 200 ml of water, stir to dissolve the salts and adjust the pH to 2.5 with phosphoric acid (85%, reagent grade). Add water to volume. Filter through 0.22 µm Millipore filter or equivalent. Accurately measure one part by volume of acetonitrile (LC grade, transmittance more than 90% at 210 nm) and three parts by volume of buffer solution, and combine. De-gas under vacuum.

Standard Solution A1

Weigh accurately about 25 mg each of the beta isomer and alanine amide

(both obtainable from Quality Control Department, Danisco Sweeteners, PO Box 8266, Terre Haute, Indiana 47808, USA), and transfer quantitatively to a 500 ml volumetric flask. Add 50 ml methanol to aid dissolution, and dilute with water to volume. Store in a refrigerator.

Standard Solution A2
Transfer 15.0 ml of Standard Solution A1 into a 50 ml volumetric flask and dilute with water to volume.

Working Standard W1
Weigh accurately about 50 mg Alitame Reference Standard (obtainable from Quality Control Department, Danisco Sweeteners, PO Box 8266, Terre Haute, Indiana 47808, USA), transfer quantitatively to a 10 ml volumetric flask, add 5 ml of Standard Solution A2, and dilute with water to volume.

Working Standard W2
Transfer 5.0 ml of Working Standard W1 to a 50 ml volumetric flask and dilute with water to volume.

Test Solution S1
Weigh accurately about 50 mg of the sample, transfer quantitatively to a 10 ml volumetric flask and dilute with water to volume.

Test Solution S2
Transfer 5.0 ml of Test Solution S1 to a 50 ml volumetric flask and dilute with water to volume.

System Suitability Test
Inject triplicate 100 µl portions of Working Standards W1 and W2 into the chromatograph. The retention times for the beta isomer, alitame, and alanine amide should be approximately 6, 10, and 15 min respectively. (Note: The retention time quoted is appropriate for a 15 x 0.4 cm NovaPak column. If a column of a different make or length is used, it may be necessary to adjust the proportion of acetonitrile in the eluent to obtain the required retention time). The coefficient of variation (100 x standard deviation divided by mean peak area) for the peak areas should not exceed 2%.

Procedure
Equilibrate the column by pumping mobile phase through it until a drift-free baseline is obtained. Analyze the Standard Solutions and Test Solutions under the conditions described above. Inject three replicate samples of Working Standard W1, and calculate the average peak areas for the beta isomer and for alanine amide. Inject three replicate samples of Working Standard W2 and calculate the average peak area for alitame. Inject three replicate samples of Test Solution S1 and calculate the average peak areas for the beta isomer and alanine amide. Inject three replicate samples of Test Solution S2 and calculate the average peak area for alitame.
Calculate the purity of alitame by the formula:

$$Wt\,\% = \frac{R_A \, x \, W_S \, x \, P_S}{R_S \, x \, W_A}$$

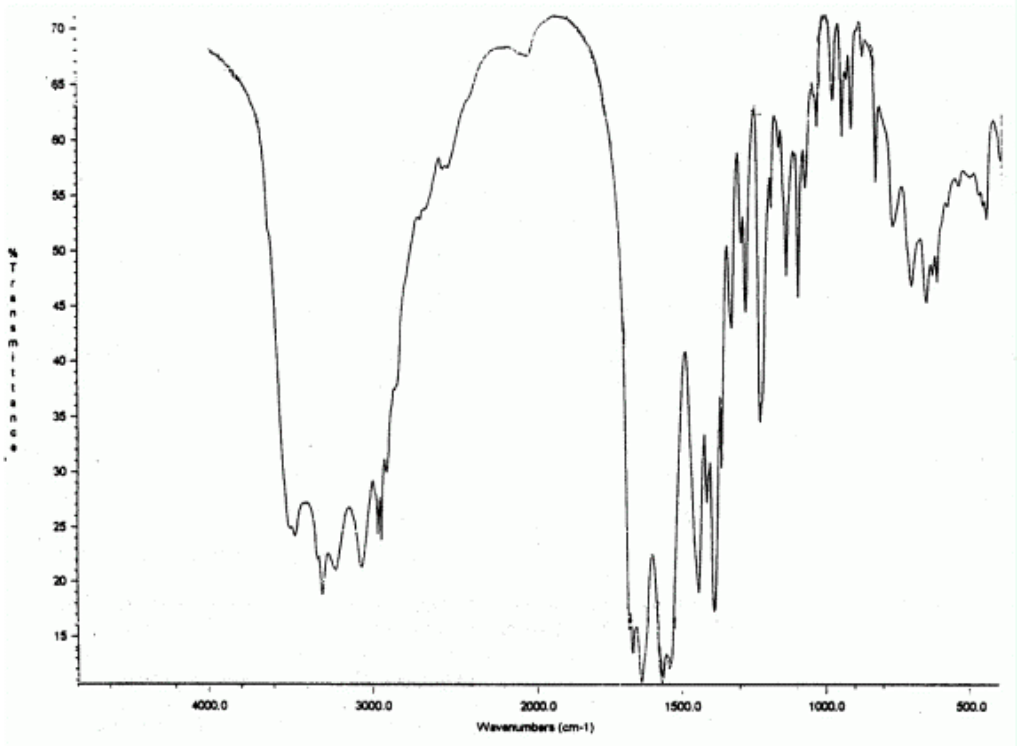
where
R_A = the response of the analyte peak in Test Solution S2

W_S = the weight of the alitame Reference Standard corrected for water content, in g
P_S = the percent purity of the Reference Standard, i.e., 100.00 - sum of impurities
R_S = the response of the analyte peak in Working Standard W2
W_A = the weight of the sample corrected for the water content, in g.
Calculate the percentage of the beta isomer and alanine amide by the following formula:

$$Wt\,\% = \frac{R_A \times W_S \times P_S \times DF}{R_S \times W_A}$$

where
R_A = the response of the analyte peak in Test Solution S1
W_S = the weight of the beta isomer or alanine amide standards, uncorrected for water content, in g
P_S = the percentage purity of the beta isomer or alanine amide standard, i.e. 100.00 - sum of impurities
R_S = the response of the analyte peak in Working Standard W1
W_A = the weight of the sample, uncorrected for water content, in g
DF = the dilution factor, i.e. 0.003.

Infrared spectra of Alitame



ALLURA RED AC

Prepared at the 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-7 mg/kg bw was established at the 25th JECFA (1981)

SYNONYMS CI Food Red 17, FD&C Red No.40, CI (1975) No.16035, INS No. 129

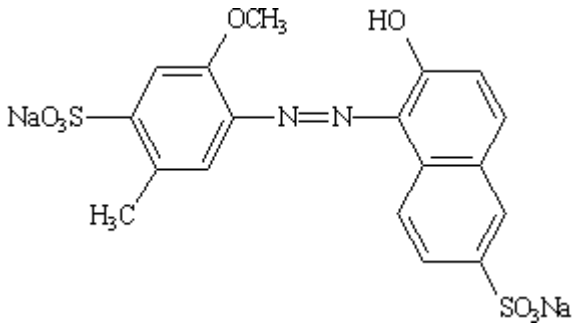
DEFINITION Consists essentially of disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalene-sulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* shall apply.

Chemical names: Disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalenesulfonate

C.A.S number: 25956-17-6

Chemical formula: $C_{18}H_{14}N_2Na_2O_8S_2$

Structural formula:



Formula weight: 496.43

Assay: Not less than 85% total colouring matters

DESCRIPTION Dark red powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility: Soluble in water, insoluble in ethanol
Identification of colouring matters (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 15% at 135º together with chloride and sulfate calculated as sodium salts
Water insoluble matter (Vol. 4)	Not more than 0.2%
Lead	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."
Subsidiary colouring matters (Vol. 4)	Not more than 3% Use the following conditions: Developing solvent: No.4 Height of ascent of solvent front: approximately 17 cm
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.3% of sodium 6-hydroxy-2-naphtalene sulfonate Not more than 0.2% of 4-amino-5-methoxy-2-methyl-benzene-sulfonic acid Not more than 1.0% of disodium 6,6'-oxybis(2-naphthalene-sulfonate) Use *liquid chromatography* under the following conditions: HPLC elution gradient: O to 18% at 1% per min (linear) then 18% to 62% at 7% per min (linear) followed by elution at 100% Flow rate: 0.6 ml per min
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether extractable matter (Vol. 4)	Not more than 0.2%
METHOD OF ASSAY	Proceed as directed under *Total Content by Titration with Titanous Chloride* in Volume 4, using the following: Amount to weigh: 0.5 - 0.6 g Buffer: 15 g sodium hydrogen tartrate Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N $TiCl_3$: 12.41 mg

ALUMINIUM AMMONIUM SULFATE

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group PTWI of 1 mg/kg bw for aluminium and its salts was established at the 67th JECFA (2006).

SYNONYMS Ammonium alum; INS No. 523

DEFINITION

Chemical names: Aluminium ammonium sulphate

C.A.S. number: 7784-25-0

Chemical formula: $AlNH_4(SO_4)_2 \cdot 12H_2O$

Formula weight: 453.32

Assay: Not less than 99.5% of $AlNH_4(SO_4)_2 \cdot 12H_2O$

DESCRIPTION Large, colourless crystals, white granules, or a powder; odourless

FUNCTIONAL USES Buffer, neutralizing agent, colour fixative

CHARACTERISTICS

IDENTIFICATION

Solubility: Freely soluble in water; insoluble in ethanol

Test for aluminium (Vol. 4): Passes test

Test for ammonium (Vol. 4): Passes test

Test for sulfate (Vol. 4): Passes test

PURITY

Alkalis and alkaline earths (Vol. 4 for TS solutions): Completely precipitate the aluminium from a boiling solution of 1 g of the sample in 100 ml of water by the addition of enough ammonia TS to render the solution distinctly alkaline to methyl red TS, and filter. Evaporate the filtrate to dryness, and ignite. The weight of the residue does not exceed 5 mg.

Fluoride: Not more than 30 mg/kg
See description under TESTS

Selenium (Vol. 4): Not more than 30 mg/kg
Test 0.2 g of the sample as directed in the Limit Test (Method II)

Lead

Not more than 3 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

Fluoride

Lime suspension
Carefully slake about 56 g of low fluoride calcium oxide (about 2 mg/kg F) with 250 ml of water, and add 250 ml of 60% perchloric acid slowly and with stirring. Add a few glass beads and boil until copious fumes of perchloric acid are evolved. Cool, add 200 ml of water, and boil again. Repeat the dilution and boiling once more. Cool, dilute considerably, and filter through a fritted-glass filter if precipitated silicon dioxide appears. Pour the clear solution, with stirring into 1000 ml of sodium hydroxide solution (1 in 10), allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing five times in large centrifuge bottles, shaking the mass thoroughly each time. Finally shake the precipitate into a suspension and dilute to 2000 ml. Store in paraffin-lined bottles and shake well before use. (Note: 100 ml of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed in the Limit Test, Method I *Thorium Nitrate Colorimetric Method*).

Procedure
Assemble the distilling apparatus as directed in the Limit Test (Method I*)*, and add to the distilling flask about 1.67 g of the sample, accurately weighed, and 25 ml of dilute sulfuric acid (1 in 2). Distil until the temperature reaches 160^{o}, then maintain at 160^{o} to 165^{o} by adding water from the funnel, collecting 300 ml of distillate. Oxidize the distillate by the cautious addition of 2 or 3 ml of fluorine-free 30% hydrogen peroxide (to remove sulfites), allow to stand for a few min, and evaporate in a platinum dish with an excess of Lime suspension. Ignite briefly at 600^{o}, then cool and wet the ash with about 10 ml of water. Cover the dish with a watch glass, and cautiously introduce under the cover just sufficient 60% perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 ml of the perchloric acid for dissolving the ash and transferring the solution. Add 10 ml of water and a few drops of silver perchlorate solution (1 in 2) through the dropping funnel, and continue as directed in the Limit Test Method I *Thorium Nitrate Colorimetric Method* beginning with "Distil until the temperature reaches 135^{o}." (See Volume 4)

METHOD OF ASSAY

Weigh accurately about 1 g of the sample, dissolve in 50 ml of water, add 50 ml of 0.05 M disodium EDTA and 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), and boil gently for 5 min. Cool, and add 50 ml of ethanol and 2 ml of dithizone TS. Titrate with 0.05 M zinc sulfate to a bright rose-pink colour, and perform a blank determination. Each ml of 0.05 M disodium EDTA is equivalent to 22.67 mg of $AlNH_4(SO_4)_2 \cdot 12H_2O$.

ALUMINIUM SILICATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group PTWI of 1 mg/kg bw for aluminium and its salts was established at the 67th JECFA (2006).

SYNONYMS Kaolin, light or heavy, INS No. 559

DEFINITION A native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. The article of commerce may be further specified as to chloride, foreign substances, particle size, loss on drying, loss on ignition and pH value.

DESCRIPTION A soft, whitish powder free from gritty particles; odourless

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, ethanol and mineral acids

Plasticity To 8 g of the sample add 5 ml of water and mix well. The mixture is plastic

Test for silicate Mix about 500 mg of the sample with about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 ml of water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it to a beaker with the aid of about 50 ml of water. Add gradually hydrochloric acid until no effervescence is observed, then add 10 ml more of the acid, and evaporate the mixture on a steam bath to dryness. Cool, add 20 ml of water, boil and filter the mixture through an ash-free filter paper. An insoluble residue of silica remains. (Note. Retain the filtrate for the test for aluminium). Transfer the gelatinous residue into a platinum dish, and cautiously add 5 ml of hydrofluoric acid (warning: toxic, corrosive, must not contact skin; work under fume hood). The precipitate dissolves. (If it does not dissolve, repeat the evaporation with hydrofluoric acid.) Heat and hold in the vapours a glass stirring rod with a drop of water on the tip. The drop becomes turbid.

Test for aluminium (Vol. 4) Add ammonia TS to the filtrate obtained in the test for silicate. A white gelatinous precipitate is formed which is insoluble in excess ammonia but dissolves in sodium hydroxide TS.

PURITY

Water soluble substances	Not more than 0.3% See description under TESTS
Acid soluble substances	Not more than 2% See description under TESTS
Asbestos	Absent Electron microscope method (tentative): Prepare a sample to be as homogeneous as possible. Examination of a specimen of the sample from a minimum of 100 fields of view using a transmission electron microscope fails to reveal any fibrous material.
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

Water soluble substances	Weigh 5 g of the sample to the nearest mg, and boil with 50 ml of water for 30 min., adding water from time to time to maintain approximately the original volume. Filter, evaporate the filtrate to dryness, dry at 105^{o} for 1 h, and weigh. % Water soluble substances = m/[10 x W] where m = the weight (mg) of the residue W = the weight (g) of the sample
Acid soluble substances	Weigh 2 g of the sample to the nearest mg, and boil with 100 ml of dilute hydrochloric acid TS under a reflux condenser for 15 min., cool, and filter. Evaporate 50 ml of the filtrate to dryness, then ignite gently to constant weight. % Acid soluble substances = m/[5 x W] where m = the weight (mg) of the residue W = the weight (g) of the sample

AMMONIUM ALGINATE

Prepared at the 49th JECFA (1997) , published in FNP 52 Add 5 superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). Metals and arsenic specifications revised at the 57th JECFA (2001) An ADI 'not specified' was established at the 39th JECFA (1992)

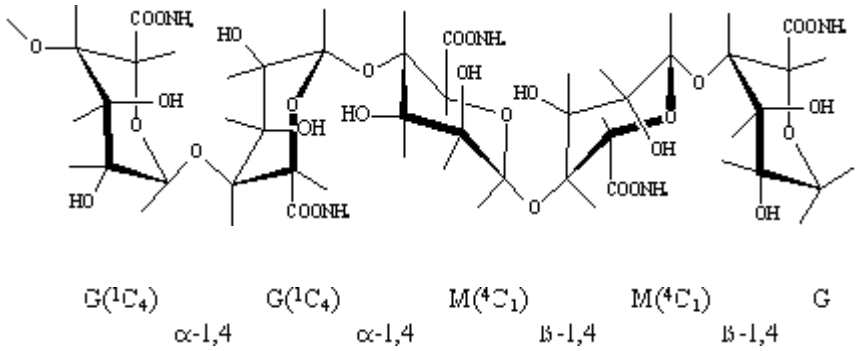
SYNONYMS INS No. 403

DEFINITION Ammonium salt of alginic acid.

C.A.S. number 9005-34-9

Chemical formula $(C_6 H_{11} NO_6)_n$

Structural formula Structural formula from Phillips, Wedlock and Williams: Gums and Stabilizers for the Food Industry 5 (1990) by permission of Oxford University Press.



The number and sequence of the Mannuronate and Glucuronate residues shown above vary in the naturally occurring alginate. The associated water molecules are not shown.

Formula weight Structural unit : 193.16 (theoretical), 217 (actual average)
Macromolecule : 10,000 - 600,000 (typical average)

Assay Yields, on the dried basis, not less than 18.0% and not more than 21.0% of carbon dioxide (CO_2), equivalent to not less than 88.7% and not more than 103.6% of ammonium alginate $C_6H_{11} NO_6)_n$.

DESCRIPTION White to yellowish brown filamentous, grainy, granular or powdered forms

FUNCTIONAL USES Stabilizer, thickener, gelling agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility Dissolves slowly in water forming a viscous solution; insoluble in ethanol, and ether

Precipitate formation with calcium chloride	To a 0.5% solution of the sample in sodium hydroxide TS add one-fifth of its volume of a 2.5% solution of calcium chloride. A voluminous, gelatinous precipitate is formed. This test distinguishes ammonium alginate from gum arabic, sodium carboxymethyl cellulose, carrageenan, gelatin, gum ghatti, karaya gum, carob bean gum, methyl cellulose and tragacanth gum.
Precipitate formation with ammonium sulfate	To a 0.5% solution of the sample in sodium hydroxide TS add one-half of its volume of a saturated solution of ammonium sulfate. No precipitate is formed. This test distinguishes ammonium alginate from agar, sodium carboxymethyl cellulose, carrageenan, de-esterified pectin, gelatin, carob bean gum, methyl cellulose and starch.
Test for alginate	Passes test Dissolve as completely as possible 0.1 g of sample by shaking with 0.15 ml of 0.1 N sodium hydroxide and add 1 ml of acid ferric sulfate TS. Within 5 min, a cherry-red colour develops that finally becomes deep purple.
Ammonium (Vol. 4)	Passes test
PURITY	
Loss on drying (Vol. 4)	Not more than 15% (105^{o}, 4 h)
Water-insoluble matter	Not more than 2% on the dried basis Disperse 2 g of the sample, weighed to the nearest 0.1 mg, in 800 ml of water in a 2,000-ml flask. Neutralize to pH 7 with sodium hydroxide TS and then add 3 ml in excess. Add 40 ml of hydrogen peroxide solution containing 30% by weight H_2O_2, cover the flask and boil for 1 h with frequent stirring. Filter while hot through a tared Gooch crucible provided with a glass fibre filter (2.4 cm, No. 934 AH, Reeve Angel & Co., Clifton, N.Y., or equivalent filter). If slow filtration is caused by high viscosity of the sample solution, boil until the viscosity is reduced enough to permit filtration. Wash the crucible thoroughly with hot water, dry the crucible and its contents at 105^{o} for 1 h, cool and weigh. Calculate as percentage of the dry weight.
Sulfated ash (Vol. 4)	Not more than 7% on the dried basis
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Microbiological criteria (Vol. 4)	Total plate count: Not more than 5,000 colonies per gram. Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield’s phosphate buffered dilution water and homogenizing in a high speed blender. Yeasts and moulds: Not more than 500 colonies per gram Coliforms: Negative by test. Salmonella: Negative by test

AMMONIUM CARBONATE

Prepared at the 26th JECFA (1982), published in FNP 25 (1982) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002)
An ADI 'not specified' was established at the 26th JECFA (1982)

SYNONYMS INS No. 503(i)

DEFINITION Consists of ammonium carbamate, ammonium carbonate and ammonium hydrogen carbonate in varying proportions

C.A.S. number 10361-29-2

Chemical formula $CH_6N_2O_2$,
$CH_8N_2O_3$
CH_5NO_3

Structural formula NH_2COONH_4
$(NH_4)_2HCO_3$
NH_4HCO_3

Formula weight Ammonium carbamate 78.06
Ammonium carbonate 98.73
Ammonium hydrogen carbonate 79.06

Assay Not less than 30.0% and not more than 34.0% of NH_3

DESCRIPTION White powder or hard, white or translucent masses of crystals with an odour of ammonia. On exposure to air it becomes opaque and is finally converted into white porous lumps or powder (of ammonium bicarbonate) due to loss of ammonia and carbon dioxide.

FUNCTIONAL USES Acidity regulator, raising agent

CHARACTERISTICS

IDENTIFICATION

Solubility Soluble in water

pH (Vol.4) About 8.6 (1 in 20 solution)

Test for carbonate (Vol. 4) Passes test

Test for ammonia (Vol. 4) Passes test

Heat test When heated, it volatilizes without charring and the vapour is alkaline to moist litmus

PURITY

Non-volatile residue (Vol. 4)

Not more than 500 mg/kg
Test 4 g of the sample in 10 ml of water

Chlorides

Not more than 30 mg/kg
Dissolve 500 mg of the sample in 10 ml of hot water, add about 5 mg of sodium carbonate, and evaporate to dryness on a steam bath. Test the residue as directed under the Limit Test. Any turbidity produced does not exceed that shown in a control containing 15 µg of chloride ion (Cl^-).

Sulfates

Not more than 50 mg/kg
Dissolve 4 g of the sample in 40 ml of water, add about 10mg of sodium carbonate and 1 ml of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Treat the residue as directed under the Limit Test. Any turbidity produced does not exceed that shown in a control containing 200 µg of sulfate ion (SO_4^{2-}).

Lead

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Place about 10 ml of water in a weighing bottle, tare the bottle and its contents, add about 2 g of the sample and weigh accurately. Transfer the contents of the bottle to a 250-ml flask and slowly add, with mixing, 50 ml of 1 N sulfuric acid. When solution has been effected, wash down the sides of the flask, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide. Each ml of 1 N sulfuric acid is equivalent to 17.03 mg of NH_3.

AMMONIUM CHLORIDE

Prepared at the 23rd JECFA (1979), published in FNP 12 (1979) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not limited' was established at the 23rd JECFA (1979)

SYNONYMS Sal ammoniac, ammonium muriate, INS No. 510

DEFINITION

Chemical names	Ammonium chloride
C.A.S. number	12125-02-9
Chemical formula	NH_4Cl
Formula weight	53.50
Assay	Not less than 99.0% on the dried basis

DESCRIPTION Colourless crystals, or a white, fine or coarse, crystalline powder; somewhat hygroscopic

FUNCTIONAL USES Dough conditioner, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility	Freely soluble in water, sparingly soluble in ethanol
pH (Vol. 4)	4.5 - 6.0 (1 in 20 soln)
Test for ammonium (Vol. 4)	Passes test
Test for chloride (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 2.0% (over silica gel, 4 h)
Sulfated ash (Vol. 4)	Not more than 0.5% Test 2 g of the sample (Method I)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Dry about 0.2 g of the sample over silica gel for 4 h, weigh accurately, and dissolve it in about 40 ml of water in a glass-stoppered flask. Add, while agitating, 3 ml of nitric acid, 5 ml of nitrobenzene, 50.0 ml of 0.1 N silver

nitrate, shake vigorously, then add 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate. Each ml of 0.1 N silver nitrate is equivalent to 5.349 mg of NH_4Cl.

AMMONIUM HYDROGEN CARBONATE

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not specified' was established at the 26th JECFA (1982)

SYNONYMS Ammonium bicarbonate, INS No. 503(ii)

DEFINITION

Chemical names	Ammonium hydrogen carbonate
C.A.S. number	1066-33-7
Chemical formula	CH_5NO_3
Structural formula	NH_4HCO_3
Formula weight	79.06
Assay	Not less than 99.0%

DESCRIPTION White crystals or a crystalline powder with a slight odour of ammonia.

FUNCTIONAL USES Raising agent

CHARACTERISTICS

IDENTIFICATION

Solubility	Freely soluble in water, insoluble in ethanol
pH (Vol. 4)	About 8 (1 in 20 soln)
Test for carbonate (Vol. 4)	Passes test
Test for ammonium (Vol. 4)	Passes test
Heat test	When heated, it volatilizes without charring and the vapour is alkaline to moist litmus

PURITY

Non-volatile residue (Vol. 4)	Not more than 500 mg/kg Test 4 g of the sample in 10 ml of water
Chlorides (Vol. 4)	Not more than 30 mg/kg Dissolve 500 mg of the sample in 10 ml of hot water, add about 5 mg of sodium carbonate, and evaporate to dryness on a steam bath. Test the residue as directed under the Limit Test. Any turbidity produced does not exceed that shown in a control containing 15 µg of chloride ion (Cl^-).

Sulfates (Vol. 4)

Not more than 70 mg/kg
Dissolve 4 g of the sample in 40 ml of water, add about 10mg of sodium carbonate and 1 ml of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Treat the residue as directed under the Limit Test. Any turbidity produced does not exceed that shown in a control containing 280 µg of sulfate ion (SO_4^{2-}).

Lead

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Place about 10 ml of water in a weighing bottle, tare the bottle and its contents, add about 2 g of the sample and weigh accurately. Transfer the contents of the bottle to a 250-ml flask and slowly add, with mixing, 50 ml of 1 N sulfuric acid. When solution has been effected, wash down the sides of the flask, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide. Each ml of 1 N sulfuric acid is equivalent to 79.06 mg of NH_4HCO_3.

AMMONIA SOLUTION

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 19 th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992) under the name Ammonium Hydroxide. Metals and arsenic specifications revised at the 59th JECFA (2002)
An ADI 'not limited' was established at the 9th JECFA (1965)

SYNONYMS Ammonium hydroxide, strong ammonia solution, aqueous ammonia, INS No. 527

DEFINITION

Chemical names	Ammonia solution
C.A.S. number	7664-41-7 (ammonia) 1336-21-6 (aqueous ammonia)
Chemical formula	NH_3 (aqueous)
Formula weight	17.03
Assay	Not less than 27% and not more than 30%

DESCRIPTION Clear, colourless liquid having an exceedingly pungent, characteristic odour. Upon exposure to air it loses ammonia rapidly

FUNCTIONAL USES Acidity regulator

CHARACTERISTICS

IDENTIFICATION

Test for ammonia	Hold a glass rod, wet with hydrochloric acid, near the sample. Dense white fumes are produced.
Specific gravity (Vol. 4)	d (25,25): about 0.90

PURITY

Non-volatile residue	Not more than 0.02% by the following procedure: Evaporate 11 ml (10 g) of the sample in a tared platinum or porcelain dish to dryness, dry at 105^{o} for 1 h, cool and weigh
Readily oxidizable substances	Dilute 4 ml of the sample with 6 ml of water, and add a slight excess of dilute sulfuric acid TS and 0.1 ml of 0.1N potassium permanganate. The pink colour does not completely disappear within 10 min.
Lead	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental

Methods."

METHOD OF ASSAY Tare accurately a 125-ml glass-stoppered conical flask containing 35.0 ml of 1N sulfuric acid. Cool the sample in the original bottle to 10^{o} or lower. Partially fill a 10-ml graduated pipet from near the bottom (do not use vacuum for drawing up the sample). Wipe off any liquid adhering to the outside of the pipet and discard the first ml. Hold the pipet just above the surface of the acid and transfer 2 ml into the flask, leaving at least 1 ml in the pipet. Stopper the flask, mix and weigh again to obtain the weight of the sample. Add methyl red TS and titrate the excess acid with 1N sodium hydroxide. Subtract the excess sulfuric acid from the total sulfuric acid (35.0 ml) to find the ml used to neutralize the sample. Each ml of 1N sulfuric acid used to neutralize the ammonia is equivalent to 17.03 mg of NH_3.

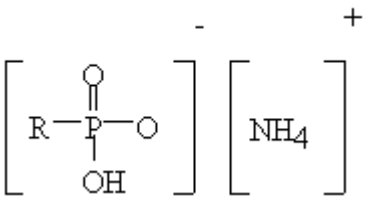
AMMONIUM SALTS of PHOSPHATIDIC ACID

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 17th JECFA (1973) and published in FNP 4 (1978) and in FNP 52 (1992). An ADI of 0-30 mg/kg bw was established at the 18th JECFA (1974).

SYNONYMS Ammonium phosphatides, Emulsifier YN, Mixed ammonium salts of phosphorylated glycerides; INS No. 442

DEFINITION The product consists essentially of a mixture of the ammonium compounds of phosphatidic acids derived from the edible fat (usually partially hardened rapeseed oil). A mono- or diglyceride moiety may be attached to phosphorus. Moreover, two phosphorus esters may be linked together as phosphatidyl phosphatides. The product is prepared by glycerolysis of the fat, followed by phosphorylation with phosphorus pentoxide, and neutralization with ammonia.
The article of commerce may be further specified as to water content, hexane-insoluble matter, inorganic hexane-insoluble matter, pH value and triglyceride content.

Structural formula (approximate composition)



where R may be a mono- or di-glyceride moiety

Assay The phosphorus content is not less than 3.0% and not more than 3.4% by weight; the ammonium N content is not less than 1.2% and not more than 1.5%.

DESCRIPTION Unctuous semisolid

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, partially soluble in ethanol and in acetone, soluble in fats

Test for phosphate Passes tests
See description under TESTS

Test for fatty acid Passes tests
See description under TESTS

Test for glycerol (Vol. 4) Passes test

PURITY

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

IDENTIFICATION TESTS

Test for phosphate — Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained.

Test for fatty acid — Reflux 1 g of the product for 1 h with 25 ml of 0.5 N ethanolic potassium fatty acid hydroxide. Ammonia is evolved from the end of the reflux condenser, recognizable by its odour and by its reaction on moist, red litmus paper. On cooling the residue to 0^{o}, a precipitate of potassium soap is obtained.

METHOD OF ASSAY

Determination of phosphorus — Absorption spectrophotometry

Reagents and working solutions
Sulfuric acid: sp gr 1.84
Nitric acid: sp gr 1.42
Perchloric acid: 60%, sp gr 1.54
Vanadate-molybdate solution: Separately dissolve in water 20 g of ammonium molybdate and 1 g of ammonium vanadate. Mix the two solutions, add 140 ml of concentrated nitric acid and dilute to 1000 ml with water. Mix well.
Standard phosphate solution: Stock solution: Dissolve 3.8346 g of potassium dihydrogen phosphate, previously dried at 110º, in water and dilute to 1000 ml; 1 ml of this solution = 2.0 mg P_2O_5.
Standard phosphate working solution: Transfer 50.0 ml of the standard phosphate solution (1ml = 2.0 mg P_2O_5) to a 500 ml volumetric flask and dilute to volume with water and mix well. 1 ml of standard phosphate working solution = 0.2 mg equivalents of P_2O_5

Procedure
Weigh accurately 1.5 to 1.6 g of a representative sample into a small glass capsule and transfer to a 300-ml Kjeldahl flask containing 5 ml of sulfuric acid and 10 ml of nitric acid. Heat the flask, gently at first, with continual swirling, and later more strongly over a bare flame. Add further measured amounts of nitric acid from time to time, cooling the flask prior to addition, and continue the heating until the stage where the digest is clear and assumes a golden colour. Cool, add 5 ml of 60% perchloric acid and continue the oxidation until white acid fumes form in the flask. Cool again and add 5 ml of water and continue heating until white fumes are again driven off. Cool, dilute carefully with water, cool again and transfer quantitatively to a 500-ml volumetric flask. Dilute to volume with water and mix well (Test solution).

Carry out a blank digestion exactly as above but omit the sample and use the same volume of acid as required to wet oxidize the sample (Blank digest solution).

Into separate 100-ml volumetric flasks, add by burette:
(a) 25.0 ml of Standard phosphate working solution (= 5.0 mg P_2O_5),
(b) 30.0 ml of Standard phosphate working solution (= 6.0 mg P_2O_5),
(c) a 25 ml aliquot of the test solution which will contain the equivalent of between 5 and 6 mg P_2O_5

Into each of the flasks containing the phosphorus standards, i.e. (a) and (b), transfer an aliquot of the blank digest solution equal in volume to (c), in order to compensate for possible traces of phosphorus derived from the acid digest reagents and which may be present in the Test Solution.

To each add 25 ml of the vanadate-molybdate reagent, mix, dilute to nearly 100 ml with water, mix well, adjust the temperature of the solution to 20º, dilute to the mark with water and re-mix.

After 10 min measure the absorbance of both the 6 mg P_2O_5 solution and the test solution against the 5 mg standard contained in the blank cell. Use optically matched 1 cm cells and measure at a wavelength of 420 nm, or with an Ilford 604 filter if using a photo-electric colorimeter.

Calculation

$$\% \text{ Phosphorus} = \left[5 + \frac{\text{A test}}{\text{A 6 mg}} \right] \times \frac{0.873}{\text{W}}$$

where
A test = absorbance difference between the 5 mg standard and the test solution
A 6 mg = absorbance difference between the 6 mg and 5 mg standards
W = weight of sample taken (g)

Determination of ammonium nitrogen

Apparatus for steam distillation

The apparatus consists of a 2-L flask fitted with a rubber bung through which pass an approximately 3" length of glass tubing, arranged so that the lower end is near the bottom of the flask, and a shorter L-shaped piece of tubing arranged such that the tube projects about 1/4" below the lower surface of the bung, to act as a steam outlet tube. The flask should be approximately 2/3 filled with distilled water made slightly acid with dilute sulfuric acid TS and contain a few pieces of sintered glass to prevent bumping when the contents of the flask are vigorously boiled to act as a steam generator. A tap funnel may be fitted to the flask if desired to facilitate replenishing the water in the flask between determinations.

The steam outlet tube is connected via a condensation trap to the inlet of a steam distillation head, fitted to a short necked 1-L round bottomed B34 necked flask. The distillation head should be such that the steam inlet tube reaches almost to the bottom of the 1-L flask and the outlet should be fitted with two splash traps, one near the top of the 1-L flask and the other near the top of B19 jointed verticle, single-surface condenser to which the distillation head connects. The vertical condenser should be fitted with an extended outlet tube, able to reach to the bottom of a 500-ml conical flask.

Reagents
Boric acid solution (2% w/v in water)
Sodium hydroxide solution (40 w/v in water)
0.02N Hydrochloric acid
Mixed indicator: Mix 5.0 of 0.1% w/v alcoholic solution of bromocresol green and 2.0 of a 0.1% w/v alcoholic solution of methyl red and dilute the mixture to 30 ml with 95% alcohol.
Silicone fluid 200/50 MS

Procedure
Assemble and thoroughly steam out the apparatus. Accurately weigh about 0.2 g of a representative sample of neutral Ammonium salts of phosphatidic acids into a small glass phial (approx. 3/4" diameter, 1/2" deep). Transfer the phial and weighed contents to the distillation flask and add approximately 250 ml distilled water. Connect the distillation head and splash traps to the distillation flask and vertical condenser, and arrange the condenser such that the outlet dips below the surface of 10 ml of 2% boric acid and 1 ml mixed indicator contained in a 500-ml conical flask.

Add to the distillation flask, via a funnel attached by means of a short piece of rubber tubing to the steam inlet tube, 75 ml 40% aqueous sodium hydroxide, and wash in with distilled water. Detach the funnel and connect the steam inlet to the steam supply. (Alternatively, the sodium hydroxide may be added to the flask through a tap funnel, fitted to the distillation flask if preferred and washed in with distilled water. If so a liquid seal should be maintained in the funnel during the addition and distillation). Vigorously steam distil the contents of the distillation flask and collect 200 ml distillate in the boric acid. During the distillation gently agitate the distillation flask if necessary, to avoid the sample being deposited around the upper surfaces of the flask. When the required amount of distillate has been collected, lower the receiving flask, stop the steam supply, and wash down the inside of the condenser, and the outside of the lower end, with a small quantity of distilled water, collecting the washings in the receiving flask.

Titrate the contents of the receiving flask with 0.02N hydrochloric acid. Carry out at least one blank determination in exactly the same way but omitting the sample.

During the distillation difficulty may be experienced with frothing of the contents of the distillation flask. If so, 2 drops of silicone fluid should be added to the distillation flask at the time of adding the sample; and a similar amount included in the blank determination.

Calculation
1 ml of 0.02 N HCl = 0.2802 mg of nitrogen.

$$\%\,\text{Nitrogen} = \frac{(\text{sample titre} - \text{blank titr e}) \times 28.02}{(\text{sample wt. in mg})}$$

α-AMYLASE from *ASPERGILLUS ORYZAE*, var.

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 31st JECFA (1987) and published in FNP 38 (1988) and in FNP 52 (1992). An ADI "Acceptable" was established at the 31st JECFA (1987).

SYNONYMS INS No. 1100

SOURCES Produced by the controlled fermentation of non-toxicogenic and non-pathogenic strains of *Aspergillus oryzae* and isolated from the growth medium

Active principles: alpha-Amylase (synonyms: diastase, ptyalin, glycogenase)

Systematic names and numbers: 1,4-alpha-D-Glucan glucanohydrolase (EC 3.2.1.1)

Reactions catalyzed: The enzyme preparations hydrolyze 1,4-alpha-glucosidic linkages in polysaccharides, yielding dextrins and oligo- and monosaccharides.

Secondary enzyme activities:
Amyloglucosidase
Proteases
Xylanase

DESCRIPTION Typically tan amorphous powders or tan to dark-brown liquids that may be dispersed in food-grade diluents and may contain stabilizers and preservatives; soluble in water and practically insoluble in ethanol and ether.

FUNCTIONAL USES
Enzyme preparation
Used in starch hydrolysis, syrup production, baking and brewing

GENERAL SPECIFICATIONS Must conform to the General Specifications for Enzyme Preparations Used in Food Processing (See Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

alpha-Amylase activity (Vol. 4): The sample shows fungal alpha-amylase activity

α-AMYLASE (thermostable) from *BACILLUS LICHENIFORMIS* containing a MODIFIED α-AMYLASE GENE from *B. LICHENIFORMIS*

New specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add. 11 (2003). An ADI "not specified" was established.

SOURCES

Produced by submerged fed-batch pure culture fermentation of a genetically modified nonpathogenic and nontoxigenic strain of *Bacillus licheniformis*. The sequence of the alpha-amylase gene, originally derived from *B. licheniformis,* has been modified to confer thermostability on the enzyme. The *B. licheniformis* production strain was developed using a host strain derived from a nonpathogenic and nontoxigenic *B. licheniformis* ancestral strain.

Active principles

Alpha-amylase (synonym: glycogenase)

Systematic names and numbers

1,4-alpha-D-glucan glucanohydrolase; E.C. 3.2.1.1; CAS No. 9000-90-2

Reactions catalysed

Endohydrolysis of 1,4-alpha-glucosidic linkages in amylose and amylopectin forming dextrins and oligosaccharides

DESCRIPTION

Brown liquid. Contains stabilizing and antimicrobial compounds.

FUNCTIONAL USES

Enzyme preparation.
Starch hydrolysis in the production of, e.g., sweeteners, ethanol, and beer.

GENERAL SPECIFICATIONS

Must conform to the General Specifications for Enzyme Preparations used in Food Processing (see Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Alpha-amylase

The sample shows alpha-amylase activity
See description under TESTS

TESTS

Alpha-amylase activity

Principle

Alpha-amylase catalyzes the hydrolysis of ethylidene-G_7PNP (4,6-ethylidene(G_7)-*p*-nitrophenyl(G_1)-α,D-maltoheptaoside) to, for example, G_2-PNP and G_3-PNP, where G = glucose and PNP = *p*-nitrophenol. G_2-PNP and G_3-PNP are then hydrolyzed to glucose and *p*-nitrophenol by added alpha-glucosidase. Para-nitrophenol is measured spectrophotometrically at 405 nm. Alpha-amylase activity is determined relative to an alpha-amylase standard with known activity and is expressed in Kilo Novo alpha-amylase Units (Termamyl) (KNU(T)). One KNU(T) is the amount of alpha-amylase which, under standard conditions (pH 7.1; 37^o), dextrinizes 5.26 g starch dry substance per hour. One KNU(T) corresponds to the amount of alpha-amylase that hydrolyses 672 micromoles of ethylidene-G_7PNP per minute under standard conditions (pH 7.1; 37^o).

The quantification limit of the method is approximately 0.3 KNU(T)/g.
(Note: The method can be adapted for manual execution. Any suitable spectrophotometer may be used in place of a centrifugal analyzer.)

Apparatus
Centrifugal analyzer (Cobas Fara, Roche, or equivalent)
Diluter (Hamilton Microlab or equivalent)

Reagents
(Note: Use only deionised water)
Alpha-amylase standard (available from Novozymes A/S)
Starch dry substance
Alpha-glucosidase reagent and the substrate, *4,6-ethylidene(G_7)-p-nitrophenyl(G_1)-α,D-maltoheptaoside* may be obtained as a kit for the centrifugal analyzer.
BRIJ 35 solution, 15%: Add 1000 ml BRIJ 35 solution (polyoxyethylene 23 lauryl ether) to a 2000 ml volumetric flask. Add water to volume.
Stabilizing stock solution: Place 882 g $CaCl_2 \cdot 2H_2O$ in a 2000ml volumetric flask. Add 33 ml of 15% BRIJ 35 solution and water to volume.
Stabilizing solution (1%): Pipette 20 ml of the stabilizing stock solution into a 2000 ml volumetric flask. Add water to volume.

Standard and sample solutions
Standard alpha-amylase solutions: Dilute the alpha-amylase standard to 0.450 KNU(T)/ml as follows:
Accurately weigh the calculated quantity of the standard. Place the standard in a 200 ml volumetric flask and add water to approximately two thirds of the volume. Add 2 ml of the stabilizing stock solution. Add water to volume. This is a standard stock solution. Prepare the standard working solutions by diluting the standard stock solution with the stabilizing solution (1%) as shown in the table below. Use the diluter and vials compatible with the centrifugal analyzer.

Sample No.	Enzyme Stock Solution (µl)	Stabilizing solution (1%) (µl)	KNU(T)/ml
1	20	580	0.0150
2	30	570	0.0225
3	40	560	0.0300
4	50	550	0.0375
5	60	540	0.0450

The standard solutions can be stored in a refrigerator for one day.
"Void" standard: In a vial, mix 85.5 microliters of the alpha-amylase standard stock solution with 514 microliters of the stabilizing solution (1%). The solution can be stored for one day in a refrigerator.

Alpha-amylase control sample: Accurately weigh an alpha-amylase preparation of known activity, add to a 250 ml volumetric flask and make up to volume with the stabilizing solution (1%). If necessary, dilute the sample with the stabilizing solution (1%) again to obtain the final alpha-amylase activity within the range of the standard curve. Place the solution in a vial. The solution can be stored in a refrigerator for one day.

Alpha-amylase test samples: Accurately weigh out each sample into individual 250 ml volumetric flasks and add the stabilizing solution (1%) to volume. If necessary, dilute the sample with the stabilizing solution (1%) again to obtain the final activity of approximately 0.03 KNU(T)/ml.

Procedure

1. Pour the substrate solution into a 4 ml reagent container placed in the reagent rack of the centrifugal analyzer.
2. Pour alpha-glucosidase solution into a 15 ml reagent container placed in the reagent rack.
3. Place vials containing standard solutions and the control sample in the calibration rack.
4. Place the vial containing the void standard in the first position in the calibration rack. (Note: The void standard is used to condition the pipette before use. It is not used in the determination of alpha-amylase activity).
5. Place vials containing the test samples in the sample rack.
6. Set up the analysis program and start the analysis

Analysis

The analysis is performed automatically by the centrifugal analyzer. The empty rotor of the analyzer rotates until the temperature reaches 37^{o}. Twenty microliters of the test sample or control sample, 10 microliters of water, and 250 microliters of the alpha-glucosidase reagent are pipetted into cavities in the rotor. The rotor rotates for 10 seconds. During that time, the reagents are centrifuged into horizontally-oriented cuvettes. Then, 25 microliters of the substrate and 20 microliters of water are added to each cuvette. The rotor rotates again and the substrate is mixed with the content of each cuvette. The absorption is measured for the first time after 120 seconds and then every five seconds. A total of 37 measurements are made for each cuvette.

Calculations

The analyzer reads the alpha-amylase activity of the test samples from the standard curve and calculates the results in KNU(T)/ml. Calculate the alpha-amylase activity of each test sample in KNU(T)/g using the following formula:

$$Activity(KNU(T))/g = \frac{S \times V \times F}{W}$$

Where

S = analysis result in KNU(T)/ml
V = volume of the volumetric flask used to dilute the test sample (ml)
F = dilution factor used in the second dilution of the test sample (ml/ml)
W = weight of the test sample (g)

α-AMYLASE from *BACILLUS MEGATERIUM* expressed in *BACILLUS SUBTILIS*

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). An ADI 'not specified' was established at the 37th JECFA (1990)

SYNONYMS Glycogenase

SOURCES Produced by the controlled fermentation of *Bacillus subtilis* containing the gene for alpha-amylase from *Bacillus megaterium*. The strain of *Bacillus subtilis* is non-pathogenic and non-toxicogenic (for example ATCC 39,701). When fermentation is complete, the broth is clarified by centrifugation or filtration. The clarified broth containing the soluble enzyme is ultrafiltered to produce the desired activity.

Active principles: alpha-Amylase

Systematic names and numbers: 1,4-alpha-D-Glucan glucanohydrolase (EC 3.2.1.1)

Reactions catalysed: Endohydrolysis of 1,4-alpha-D-glucosidic linkages in polysaccharides, containing three or more 1,4-alpha-linked D-glucose units

Secondary enzyme activities: Glycosyl transferase, protease

DESCRIPTION Typically tan to dark brown liquid containing the active enzyme

FUNCTIONAL USES Enzyme preparation
Used in starch hydrolysis

GENERAL SPECIFICATIONS Must conform to the General Specifications for Enzyme Preparations Used in Food Processing (See Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

alpha-Amylase activity (Vol. 4): The sample shows bacterial alpha-amylase activity

α-AMYLASE from *BACILLUS STEAROTHERMOPHILUS*

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). An ADI 'not specified' was established at the 37th JECFA (1990)

SYNONYMS Glycogenase; INS No. 1100

SOURCES Produced extracellularly by the controlled fermentation of *Bacillus stearothermophilus*

Active principles: alpha-Amylase

Systematic names and numbers: 1,4-alpha-D-Glucan glucanohydrolase (EC 3.2.1.1)

Reactions catalyzed: Endohydrolysis of 1,4-alpha-D-glucosidic linkages in polysaccharides, containing three or more 1,4-alpha-linked D-glucose units

DESCRIPTION Typically tan to dark brown liquids

FUNCTIONAL USES Enzyme preparation
Used in starch hydrolysis

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (See Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

alpha-Amylase activity: The sample shows bacterial alpha-amylase activity

α-AMYLASE from *BACILLUS STEAROTHERMOPHILUS* expressed in *BACILLUS SUBTILIS*

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). An ADI 'not specified' was established at the 37th JECFA (1990).

SYNONYMS Glycogenase

SOURCES Produced by the controlled fermentation of *Bacillus subtilis* containing the gene for alpha-amylase from *Bacillus stearothermophilus*. The strain of *Bacillus subtilis* is non-pathogenic and non-toxicogenic (for example ATCC 39,709). When fermentation is complete, the broth is clarified with calcium chloride. The broth is separated from the cells by filtration with diatomaceous earth, and the filtered, clarified broth containing the soluble enzyme is then ultrafiltered to concentrate the product to the desired activity.

Active principles: alpha-Amylase

Systematic names and numbers: 1,4-alpha-D-Glucan glucanohydrolase (EC 3.2.1.1)

Reactions catalysed: Endohydrolysis of 1,4-alpha-D-glucosidic linkages in polysaccharides, containing three or more 1,4-alpha-linked D-glucose units

DESCRIPTION Typically tan to dark brown liquid containing the active enzyme

FUNCTIONAL USES Enzyme preparation
Used in starch hydrolysis

GENERAL SPECIFICATIONS Must conform to the General Specifications for Enzyme Preparations Used in Food Processing (See Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

alpha-Amylase activity (Vol. 4): The sample shows bacterial alpha-amylase activity

α-AMYLASE from *BACILLUS SUBTILIS*

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). An ADI 'not specified' was established at the 37th JECFA (1990)

SYNONYMS Glycogenase; INS No. 1100

SOURCES Produced extracellularly by the controlled fermentation of *Bacillus subtilis*

Active principles: alpha-Amylase

Systematic names and numbers: 1,4-alpha-D-Glucan glucanohydrolase - EC 3.2.1.1

Reactions catalyzed: Endohydrolysis of 1,4-alpha-D-glucosidic linkages in polysaccharides containing three or more 1,4-alpha-linked D-glucose units

DESCRIPTION Typically brown liquids, granules or powders

FUNCTIONAL USES Enzyme preparation
Used in starch hydrolysis

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (See Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

alpha-Amylase activity (Vol. 4): The sample shows bacterial alpha-amylase activity

ANNATTO EXTRACTS (AQUEOUS-PROCESSED BIXIN)

Prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI for bixin of 0 – 12 mg/kg bw and a group ADI for norbixin and its disodium and dipotassium salts of 0 – 0.6 mg/kg bw expressed as norbixin were established at the 67th JECFA (2006). The colouring matters bixin and norbixin derived from annatto extracts (solvent-extracted bixin; solvent-extracted norbixin; aqueous-processed bixin; alkali-processed norbixin, acid-precipitated; and alkali-processed norbixin, not acid-precipitated) are included in the ADIs for bixin and norbixin. All previous ADIs for annatto extracts were withdrawn.

SYNONYMS Annatto E, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b(i)

DEFINITION Aqueous-processed bixin is prepared by removal of the outer coating of the seeds of the annatto tree (*Bixa orellana* L) by abrading the seeds in the presence of cold, mildly-alkaline water. The resultant preparation is acidified to precipitate bixin which is then filtered, dried and milled.

Aqueous-processed bixin contains several coloured components; the major colouring principle is *cis*-bixin, a minor colouring principle is *trans*-bixin; thermal degradation products of bixin may also be present as a result of processing.

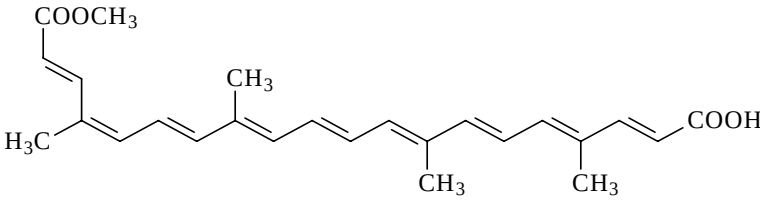
Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name *cis*-Bixin: Methyl (9-*cis*)-hydrogen-6,6'-diapo-Ψ,Ψ-carotenedioate

C.A.S. number *cis*-Bixin: 6983-79-5

Chemical formula $C_{25}H_{30}O_4$

Structural formula



cis-Bixin

Formula weight 394.5

Assay Not less than 25% colouring matter (expressed as bixin)

DESCRIPTION Dark red-brown to red-purple powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, slightly soluble in ethanol
UV/VIS absorption (Vol. 4)	The sample in acetone shows absorbance maxima at about 425, 457 and 487 nm
Thin Layer Chromatography	Activate a TLC plate (e.g. LK6D SILICA GEL 60 A (layer thickness: 250 μm, size: 5 x 20 cm)) for 1 h at 110°. Prepare a 5% solution of the sample in 95% ethanol and apply 10 µl to the plate. Allow to dry and develop using a mixture of n-butanol, methyl ethyl ketone and 10% aqueous ammonia (3:2:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry. Bixin and norbixin appear as yellow spots with R_f values of about 0.50 to 0.45, respectively. Spray with 5% sodium nitrite solution and then with 0.5 mol/l sulfuric acid and the spots immediately decolourise.

PURITY

Norbixin (Vol. 4)	Not more than 7 % of total colouring matters
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine using an ICP-AES/AAS-Hydride technique. Alternatively, determine arsenic using Method II of the Arsenic Limit Test. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS ICP-AES technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in Volume 4.
Mercury (Vol. 4)	Not more than 1 mg/kg Determine using cold vapour atomic absorption technique. Select sample size appropriate to the specified level.

METHOD OF ASSAY Proceed as directed in Food Colours, Colouring Matters Content by Spectrophotometry (Vol. 4), procedure 2, using 10 ml tetrahydrofuran to dissolve the sample and acetone in place of cyclohexane. Measure the absorbance at the A_{max} of about 487 nm. The specific absorbance ($A^{1\%}_{1\,cm}$) is 3090.

ASCORBIC ACID

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). A group ADI 'not specified' was established for ascorbic acid and its Ca, K and Na salts at the 25th JECFA (1981).

SYNONYMS Vitamin C; INS No. 300

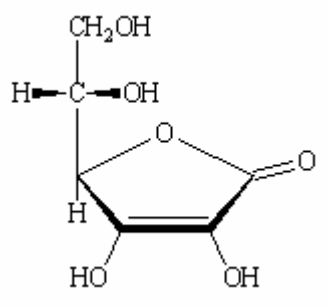
DEFINITION

Chemical names L-Ascorbic acid, ascorbic acid, 2,3-didehydro-L-threo-hexono-1,4-lactone, 3-keto-L-gulofuranolactone

C.A.S. number 50-81-7

Chemical formula $C_6H_8O_6$

Structural formula



Formula weight 176.13

Assay Not less than 99% on the dried basis

DESCRIPTION White to slightly yellow, odourless crystalline powder; melting point about 190° with decomposition

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water; sparingly soluble in ethanol; insoluble in ether

Colour reaction To 2 ml of a 2.0% solution in water, add 2 ml of water, 0.1 g of sodium hydrogen carbonate and about 0.02 g of ferrous sulfate. Shake and allow to stand. A deep violet colour is produced which disappears on addition of 5 ml of dilute sulfuric acid TS.

Reducing reaction A solution of the sample in water immediately reduces potassium permanganate TS without heating, producing a brown precipitate

A solution of the sample in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol TS.

PURITY

Loss on drying (Vol. 4)	Not more than 0.4% (over sulfuric acid in a vacuum, 24 h)
Specific rotation (Vol. 4)	[alpha] 25, D: Between +20.5 and +21.5^{o}
pH (Vol. 4)	2.4 - 2.8 (1 in 50 soln)
Sulfated ash (Vol. 4)	Not more than 0.1%
Lead (Vol. 4)	Not more than 2mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 0.400 g of the sample, previously dried in a vacuum desiccator over sulfuric acid for 24 hours, in a mixture of 100 ml of carbon dioxide-free water and 25 ml of dilute sulfuric acid TS. Titrate the solution at once with 0.1 N iodine, adding a few drops of starch TS as indicator as the end point is approached. Each ml of 0.1 N iodine is equivalent to 0.008806 g of $C_6H_8O_6$

ASCORBYL PALMITATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003)
An ADI of 0-1.25 mg/kg bw was established at the 17th JECFA (1973)

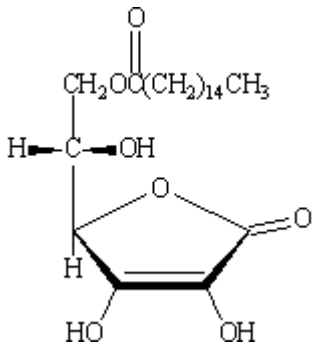
SYNONYMS Vitamin C palmitate; INS No. 304

DEFINITION

Chemical names Ascorbyl palmitate; L-ascorbyl palmitate; 2,3-didehydro-L-threo-hexono-1,4-lactone-6-palmitate; 6-palmitoyl-3-keto-L-gulofuranolactone

Chemical formula $C_{22}H_{38}O_7$

Structural formula



Formula weight 414.55

Assay Not less than 95% on the dried basis

DESCRIPTION White or yellowish-white solid, with a citrus-like odour

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very slightly soluble in water; freely soluble in ethanol

Melting range (Vol. 4) 107 ^{o} - 117^{o}

Reducing reaction A solution of the sample in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol TS (see Volume 4)

PURITY

Loss on drying (Vol.4) Not more than 2% (vacuum oven, 56 - 60^{o}, 1 h)

Specific rotation (Vol. 4)	[alpha] 25, D: Between +21° and +24° (10% (w/v) soln)
Sulfated ash (Vol. 4)	Not more than 0.1%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Add 0.800 g of the sample to a mixture of 50 ml of carbon dioxide-free water, 50 ml of chloroform and 25 ml of dilute sulfuric acid TS. Titrate the mixture at once with 0.1 N iodine making sure that the mixture is well shaken. Add a few drops of starch TS as indictor as the end point is approached. Each ml 0.1 N iodine is equivalent to 20.73 mg of $C_{22}H_{38}O_7$.

ASCORBYL STEARATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003)
An ADI of 0-1.25 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Vitamin C stearate; INS No. 305

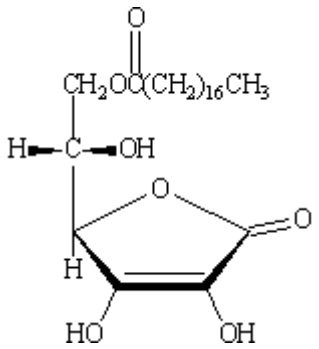
DEFINITION

Chemical names: Ascorbyl stearate, L-ascorbyl stearate, 2,3-didehydro-L-threo-hexono-1,4-lactone-6-stearate; 6-stearoyl-3-keto-L-gulofuranolactone

C.A.S. number: 25395-66-8

Chemical formula: $C_{24}H_{42}O_7$

Structural formula:



Formula weight: 442.6

Assay: Not less than 95%

DESCRIPTION White or yellowish-white solid, with a citrus-like odour

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water; soluble in ethanol

Melting point (Vol. 4): About 116°

Reducing reaction: A solution of the sample in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol TS (see Volume 4)

PURITY

Loss on drying (Vol. 4): Not more than 2% (vacuum oven, 56 - 60°, 1 h)

Sulfated ash (Vol. 4)	Not more than 0.1%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Add 0.800 g of the sample to a mixture of 50 ml of carbon dioxide-free water, 50 ml of chloroform and 25 ml of dilute sulfuric acid TS. Titrate the mixture at once with 0.1 N iodine, making sure that the mixture is well shaken. Add a few drops of starch TS as indicator as the end point is approached. Each ml of 0.1 N iodine is equivalent to 22.13 mg of $C_{24}H_{42}O_7$

ASPARTAME

Prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001)
An ADI of 0-40 mg/kg bw was established at the 25th JECFA (1981)

SYNONYMS Aspartyl phenylalanine methyl ester: APM; INS No. 951

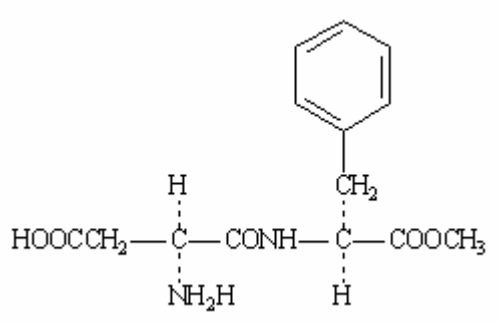
DEFINITION

Chemical names 3-Amino-N-(alpha-carbomethoxy-phenethyl)-succinamic acid, N-L-alpha-aspartyl-L-phenylalanine-1-methyl ester

C.A.S. number 22839-47-0

Chemical formula $C_{14}H_{18}N_2O_5$

Structural formula



Formula weight 294.31

Assay Not less than 98% and not more than 102% on the dried basis

DESCRIPTION White, odourless, crystalline powder, having a strong sweet taste

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Slightly soluble in water and in ethanol

Test for amine group Dissolve 2 g of ninhydrin in 75 ml of dimethylsulfoxide, add 62 mg of hydrindantin, dilute to 100 ml with 4 M lithium acetate buffer solution (pH 9), and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple colour is formed.

Test for ester Dissolve about 20 mg in 1 ml of methanol, add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, and then add 0.3 ml of 5 N potassium

hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the pH to between 1 and 1.5 with hydrochloric acid TS, and add 0.1 ml of ferric chloride TS. A burgundy colour is produced.

PURITY

Loss on drying (Vol. 4)	Not more than 4.5% (105°, 4 h)
pH (Vol. 4)	4.5 - 6.0 (1 in 125 soln)
Specific rotation (Vol. 4)	[alpha] 20, D: Between + 14.5 and + 16.5° (4% solution in 15 N formic acid; determine within 30 min after preparation of the sample solution)
Spectrophotometry (Vol. 4)	The transmittance of a 1 in 100, 2 N hydrochloric acid solution, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 N hydrochloric acid as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.
Sulfated ash (Vol. 4)	Not more than 0.2% Test 1 g of the sample (Method I)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
5-Benzyl-3,6-dioxo-2-piperazine acetic acid	Not more than 1.5% See description under TESTS
Other optical isomers	Passes test See description under TESTS

TESTS

5-Benzyl-3,6-dioxo- 2-piperazine acetic acid

Apparatus

Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector and designed for handling glass columns with on-column injection (Micro-Tex 220 or equivalent), containing a 1.83-meter (6 feet) x 4-mm (i.d.) glass column packed with 3% OV-1 on 80/100-mesh Supelcoport (Supelco, Inc. or equivalent). Condition the column overnight at 250° before readjustment and equilibration to the operating conditions. To preclude build-up of silicon oxide, clean the detector with acetone frequently.

Operating conditions

The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: Column temperature, 200°; Inlet temperature, 200°; Detector temperature, 275°; Carrier gas, helium, flowing at a rate of 75 ml per min; Hydrogen and air flow to burner, optimized to give maximum sensitivity;

Recorder, 1 mV full scale.
Note: For the Micro-Tex, the attenuation is 16 x 10.

Silation reagent
Just before use, dilute 3 parts, by volume, of N,O-bis-(trimethylsilyl) acetamide with 2 parts of dimethylformamide.

Standard preparation
Transfer about 25 mg of 5-Benzyl-3, 6-dioxo-2-piperazineacetic acid Reference Standard (available from Food Chemicals Codex, NAS/NRC, 2101 Constitution Avenue, N.W., Washington, D.C. 20418, USA), accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Pipet 10 ml of this solution into a second 100-ml volumetric flask, dilute to volume with methanol, and mix. Pipet 3 ml of the second solution into a 2-dram vial, with Teflon-lined cap, and evaporate to dryness on a steam bath. Add 1 ml of the Silylation reagent to the residue, cap the vial tightly, shake and heat in an oven at 80^{o} for 30 min. Remove the vial from the oven, shake for 15 sec, and cool to room temperature.

Sample preparation
Transfer about 10 mg of the aspartame sample, accurately weighed, into a 2-dram vial, with Teflon-lined cap, add 1 ml of the Silylation reagent, cap tightly, shake, and heat in an oven at 80^{o} for 30 min. Remove the vial from the oven, shake for 15 sec, and cool to room temperature.

Procedure
Inject a 3-µl portion of the Standard preparation into the gas chromatograph, obtain the chromatogram, measure the height of the peak produced by the 5-benzyl-3,6-dioxo-2-piperazineacetic acid, and record it as P. Under the stated conditions, the elution time is about 7-9 min. Similarly, inject a 3-µl portion of the Sample preparation, obtain the chromatogram, measure the height of the peak produced by any 5-benzyl-3,6-dioxo-2-piperazineacetic acid contained in the sample, and record it as p.

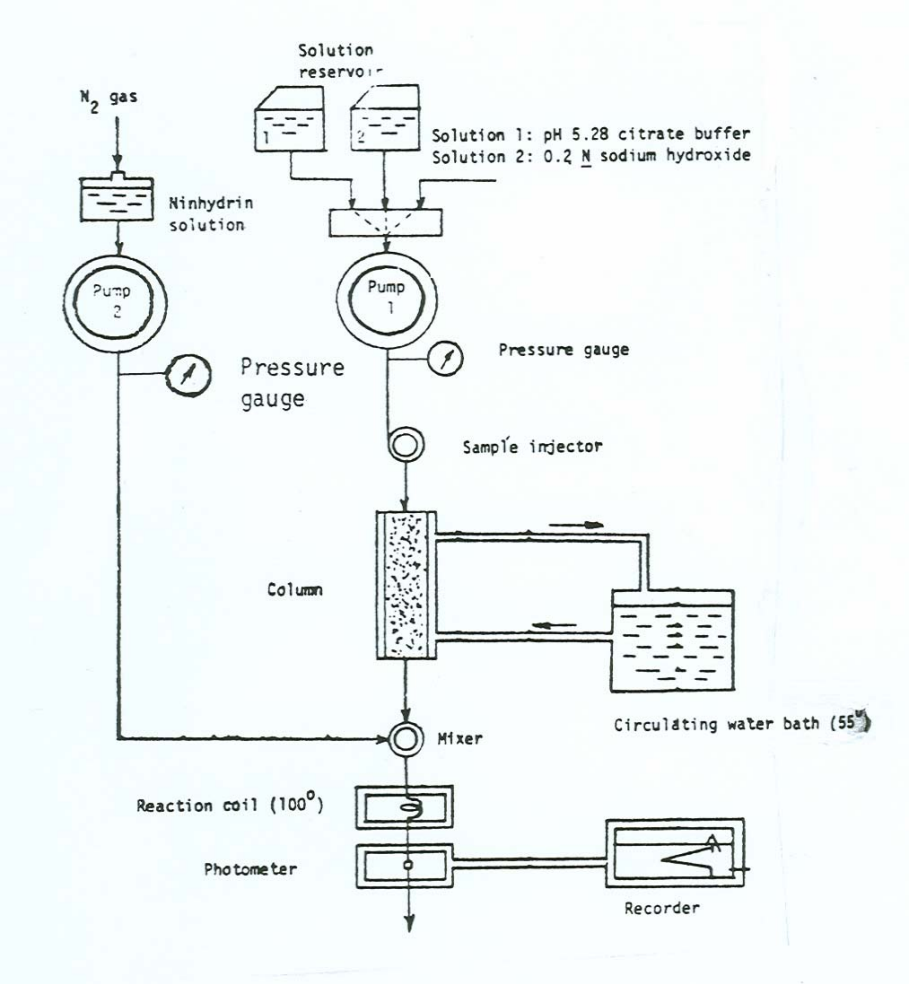
Calculation
Calculate the percent of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the sample by the formula:

$$\frac{3 \times W \times p}{500 \times w \times p} \times 100$$

where
W = the exact weight in mg of the reference standard taken
w = the exact weight in mg of the aspartame taken

Other optical isomers

Apparatus
Use a suitable amino acid analyzer (such as Hitachi KLA-5, or equivalent) which is equipped with a 550-mm x 9-mm (i.d.) column packed with approximately 50 g of strong cation exchange resin (Hitachi Custom Ion-Exchange Resin No. 2613, or equivalent), 29-m x 0.5 mm (i.d.) reaction coil, a ninhydrin supply, and a photometer with an interference filter for 570 nm and selenium photocell detector (see Figure).



Operating conditions

The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

- Column temperature: 55°
- Reaction coil temperature: 100°
- Eluant: pH 5.28 citrate buffer solution
- Pressure of eluant: 8-10 kg/cm^2
- Flow rate of eluant: 60 ml/h
- Pressure of ninhydrin solution: 2-5 kg/cm^2
- Flow rate of ninhydrin solution: 30 ml/h
- Photometric detector: measuring wavelength: 570 nm
- Recorder full scale: absorbance: 0-0.1

Reagents and solutions

- pH 5.28 citrate buffer solution: Dissolve 34.3 g of sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in about 400 ml of water, add 7.5 ml of hydrochloric acid TS (35%) and 5 ml of benzyl alcohol, and add sufficient water to make 1,000 ml.
- pH 2.2 citrate buffer solution: Dissolve 1.4 g of sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), 13.0 g of citric acid ($C_6H_8O_7H_2O$) and 10.9 g of sodium chloride in about 400 ml of water and add sufficient water to make 1,000 ml.

- Ninhydrin solution: Pour 140 ml of water into a 500-ml beaker, add 82.0 g of sodium acetate ($C_2H_3NaO_2$), stir to dissolve completely, add 25 ml of glacial acetic acid TS and dilute to 250 ml with water. Adjust the pH of the solution to 5.51 ± 0.03 with glacial acetic acid TS or sodium acetate TS (Acetate Buffer Solution). Pour 750 ml of methyl-cellosolve into a 1,000-ml brown glass bottle and add 250 ml of Acetate Buffer Solution. Supply nitrogen gas through the solution, while mixing, dissolving 20 g of ninhydrin and then 0.38 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in the solution. Allow the solution to stand for at least 24 h before use.

Preparation

- Standard Preparation: Transfer 2.50 mg of L-alpha-aspartyl-D-phenylalanine methyl ester Reference Standard (Available from Ajinomoto Co. Inc., 1-5-8 Kyobashi, Chuo-ku, Tokyo 104, Japan.) into a 100-ml volumetric flask, dissolve and dilute to volume with water (Solution A).Transfer 250 mg of L-alpha-aspartyl-L-phenylalanine methyl ester Reference Standard into another 100-ml volumetric flask, dissolve in pH 2.2 citrate buffer solution, add 10.0 ml of Solution A and dilute to volume with pH 2.2 citrate buffer solution. Store this preparation below 5^o.
- Sample Preparation: Transfer 250 mg of the sample into a 100-ml volumetric flask, dissolve and dilute to volume with pH 2.2 citrate buffer solution.

Procedure

Regenerate the column with 0.2 N sodium hydroxide TS and then buffer with pH 5.28 citrate buffer solution. After conducting ninhydrin solution to the system, inject a 500-µl portion of the Standard Preparation into the amino acid analyzer and obtain the chromatogram. Under the stated conditions, the retention time is about 100 min for L-alpha-aspartyl-D-phenylalanine methyl ester and about 115 min for L-alpha-aspartyl-L-phenylalanine methyl ester, respectively.

Similarly, inject a 500-µl portion of the Sample Preparation and obtain the chromatogram. Compare this chromatogram with that of the Standard Preparation and identify the component by comparing the retention time. No peak corresponding to L-alpha-aspartyl-D-phenylalanine methyl ester is observed. (The same method is applicable to D-alpha-aspartyl-L-phenylalanine methyl ester.

The detection limit for the sum of L-alpha-aspartyl-D-phenylalanine methyl ester and D-alpha-aspartyl-L-phenylalanine methyl ester in this method is about 1 µg/ml.)

METHOD OF ASSAY

Weigh accurately about 150 mg of the sample, previously dried at 105^o for 4 h dissolve in 35 ml of dimethylformamide, add 5 drops of thymol blue TS, and titrate with a microburette to a dark blue end-point with 0.1 N lithium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1 N lithium methoxide is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

Caution: Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminium foil while dissolving the sample and during the titration.

ASPARTAME-ACESULFAME SALT

Revised specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005), superseding specifications prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000). The ADIs for aspartame (0-40 mg/kg bw) established at the 25th JECFA (1981) and for acesulfame K (0-15 mg/kg bw) established at the 37th JECFA (1990) cover the aspartame and acesulfame moieties of the salt.

SYNONYMS Aspartame-acesulfame, INS No. 962

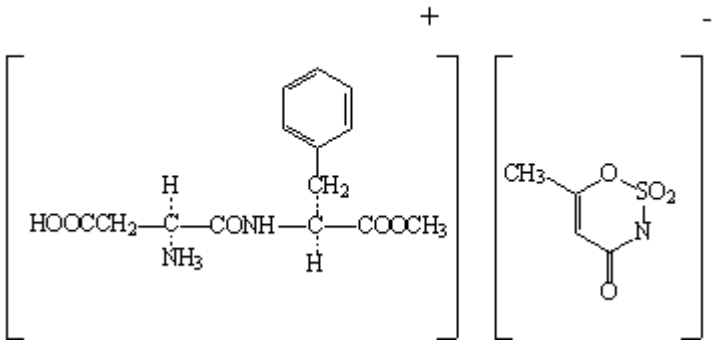
DEFINITION The salt is prepared by heating an approximately 2:1 ratio (w:w) of aspartame and acesulfame K in solution at acidic pH and allowing crystallization to occur. The potassium and moisture are eliminated. The product is more stable than aspartame alone.

Chemical names 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L-α-aspartic acid.
[2-carboxy-β-(*N*-(*b*-methoxycarbonyl-2-phenyl)ethylcarbamoyl)]ethanaminium-6-methyl-4-oxo-1,2,3-oxathiazin-3-ide-2,2-dioxide.

C.A.S. number 106372-55-8

Chemical formula $C_{18}H_{23}O_9N_3S$

Structural formula



Formula weight 457.46

Assay 63.0% to 66.0% aspartame (dried basis) and 34.0% to 37.0% acesulfame (acid form on a dried basis).

DESCRIPTION A white, odourless, crystalline powder

FUNCTIONAL USES Sweetening agent, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)) Sparingly soluble in water, and slightly soluble in ethanol.

PURITY

Loss on drying (Vol. 4) — No more than 0.5% (105°, 4 h)

Transmittance (Vol. 4) — The transmittance of a 1% solution in water determined in a 1 cm cell at 430 nm with a suitable spectrophotometer using water as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.

Specific Rotation (Vol. 4) — $[\alpha]_D^{20}$ +14.5 to +16.5.
After preparing a solution of 6.2 g of sample in 100 ml formic acid (15N), make the measurement within 30 min of preparation of the solution. Divide the calculated specific rotation by 0.646 to correct for the aspartame content of the aspartame-acesulfame salt.

5-Benzyl-3,6-dioxo-2-piperazineacetic acid — Not more than 0.5%
See description under TESTS

Lead (Vol. 4) — Not more than 1 mg/kg.
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

5-Benzyl-3,6-dioxo- 2-piperazine acetic acid

Principle
5-benzyl-3,6-dioxo- 2-piperazine acetic acid is determined in aspartame-acesulfame salt dissolved in methanol-water by comparison to an authentic standard after separation by HPLC.

Apparatus
Use a suitable high-pressure liquid chromatograph equipped with UV detector for measuring absorbance at 210 nm and a 250 x 4.6 mm column packed with octyldecyl silanized silica (10-μm Partisil ODS-3 or equivalent) and operated under isocratic conditions at 40°.

Mobile phase
Dissolve 5.6 g of potassium phosphate monobasic into 820 ml of water in a 1-l flask and adjust the pH to 4.3 with phosphoric acid. Add 180 ml of methanol and mix. Filter through a 0.45 μm filter and de-gas.

Standard
Accurately weigh approximately 25 mg of authentic 5-benzyl-3,6-dioxo-2-piperazine acetic acid into a 100-ml volumetric flask; add 10 ml of methanol to dissolve the material and dilute to volume with water and mix. Accurately transfer 15 ml of this solution to a 50-ml flask and dilute to volume with a 1:9 (v:v) mixture of methanol:water prepared on the day of use.

Sample
Accurately weigh approximately 50 mg of sample into a 10 ml volumetric flask and dilute to volume with a 1:9 (v:v) mixture of methanol:water prepared on the day of use.

Procedure

Separately inject 20 μl portions of the standard and the sample into the chromatograph (The flow rate of the mobile phase is about 2 ml/min.) and record the peak areas in standard and sample chromatograms (under the conditions described, the retention time of 5-benzyl-3,6-dioxo-2-piperazine acetic acid and aspartame are approximately 4 and 11 min, respectively). Measure the peak area response of 5-benzyl-3,6-dioxo- 2-piperazine acetic acid in each chromatogram and calculate the percentage of 5-benzyl-3,6-dioxo-2-piperazine acetic acid as follows:

$$\% = 1000(A_U C_S)/(A_S W_u)$$

Where A_U and A_S are the peak areas of 5-benzyl-3,6-dioxo-2-piperazine acetic acid in the sample and standard, respectively, C_S is the concentration of 5-benzyl-3,6-dioxo-2-piperazine acetic acid in the standard in mg/ml and W_u is the weight, in mg, of aspartame-acesulfame salt taken in the sample preparation.

METHOD OF ASSAY

Principle

Aspartame-acesulfame salt is dissolved in methanol and potentiometrically titrated with tetrabutylammonium hydroxide.

Apparatus

Use a suitable autotitrator (e.g., Metrohm 670, or equivalent) equipped with a glass pH electrode and a silver-silver chloride double liquid junction reference electrode (e.g., Yokogawa pH electrode SM 21-AL4 or equivalent and reference electrode SR 20-AS52 or equivalent).

Standard tetrabutylammonium hydroxide solution

Prepare a 0.1 M solution in a 1:1 (v:v) mixture of 2-propanol:methanol. Weigh 24 and 98 mg benzoic acid with 0.01 mg accuracy and dissolve each into two 50-ml volumetric flasks and dilute to volume with 2-propanol. Titrate both solutions with the 0.1 M tetrabutylammonium hydroxide and record the volume required to reach the equivalence point with 0.001 ml accuracy. Perform a blank titration on 50 ml of 2-propanol. Determine the standard factor (F) for each titration. and average the two factors as follows:

$$F = [(W_S \times 1000)/(122 \times (V_S - V_O))]$$

Where: W_S = weight of primary benzoic acid (g)
V_S = volume of equivalence point (ml)
V_o = volume of equivalence for the blank (ml)
122 = molecular weight of benzoic acid

Procedure

Weigh accurately 100 to 150 mg of sample and dissolve it in 50-ml methanol. Titrate with the standardized 0.1 M tetrabutylammonium hydroxide. Determine the volume (ml) of the standard solution needed to reach the first (V1) and second (V2) equivalency points. Perform a blank titration on the methanol. Calculate the acesulfame and aspartame content as follows:

$$\text{Acesulfame content (\% m/m)} = [(V_1 - V_B) \times N \times 163/(10 \times W)]$$

$$\text{Aspartame content (\% m/m)} = [(V_2 - V_1) \times N \times 294/(10 \times W)]$$

Where: W = Weight of sample (g)

V_1 = volume of first equivalence point (ml)
V_2 = volume of second equivalence point (ml)
V_B = volume of equivalence point of blank (m)l
N = normality of the standard 0.1 M tetrabutylammonium hydroxide
163 and 294 = formula weights of acesulfame and aspartame moieties, respectively
10 = conversion of g to % (m/m)

AZODICARBONAMIDE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004)
An acceptable level of treatment of 0-45 mg/kg was established at the 9th JECFA (1965)

SYNONYMS Azobisformamide; INS No. 927a

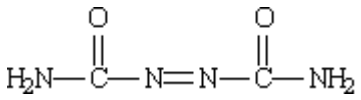
DEFINITION

Chemical names: Azodicarbonamide, azodicarboxylic acid diamide

C.A.S. number: 123-77-3

Chemical formula: $C_2H_4N_4O_2$

Structural formula:



Formula weight: 116.08

Assay: Not less than 98.6%; not less than 47.2% and not more than 48.7% of N on the dried basis

DESCRIPTION Yellow to orange-red, odourless, crystalline powder

FUNCTIONAL USES Maturing agent for flour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Practically insoluble in water and in ethanol; slightly soluble in dimethyl sulfoxide

Melting range (Vol. 4): Above 180° with decomposition

Test for oxidation: Liberates iodine from potassium iodide TS solution in the presence of 10% sulfuric acid

Test for carbon dioxide: Heat about 10 mg of the sample in a crucible. A drop of barium hydroxide solution held above the sample by means of a glass rod becomes turbid

PURITY

Loss on drying (Vol. 4): Not more than 0.5% (50°, 2h in vacuo)

pH (Vol. 4)

Not less than 5.0 (1 in 50 suspension made by adding 2 g of sample to 100 ml of water and agitating the mixture with a power stirrer for 5 min).

Sulfated ash (Vol. 4)

Not more than 0.15%
Test 1.5 g of the sample (Method I)

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Titrimetric method/azodicarbonamide:
Transfer about 225 mg of the accurately weighed sample, previously dried in a vacuum oven at 50° for 2 h, into a 250-ml glass-stoppered iodine flask. Add about 23 ml of dimethyl sulfoxide to the flask, washing any adhered sample down with the solvent, stopper the flask, and place about 2 ml of the solvent in the cup or lip of the flask. Swirl occasionally until complete solution of the sample is effected, and then loosen the stopper to drain the remainder of solvent into the flask and to rinse down any dissolved sample into the solution. Add 5 g of potassium iodide followed by 15 ml of water, immediately pipet 10 ml of 0.5 N hydrochloric acid into the flask, and stopper quickly. Swirl until the potassium iodide dissolves, and allow to stand for 20-25 min protected from light.

Titrate the liberated iodine with 0.1 N sodium thiosulfate to the disappearance of the yellow colour. Titrate with additional thiosulfate if any yellow colour appears within 15 min. Perform a blank determination on a solution consisting of 25 ml of dimethyl sulfoxide, 5 g of potassium iodide, 15 ml of water, and 5 ml of 0.5 N hydrochloric acid, and make any necessary correction. Each ml of 0.1 N sodium thiosulfate is equivalent to 5.804 mg of $C_2H_4N_4O_2$.

Kjeldahl method (semimicro)/nitrogen:
Transfer about 50 mg of the accurately weighed sample, previously dried in a vacuum oven at 50° for 2 h, into a 100-ml Kjeldahl flask, add 3 ml of hydriodic acid (min. 57%) and digest the mixture for 75 min adding sufficient water, when necessary, to maintain the original volume. Increase the heat at the end of the digestion period and continue heating until the volume is reduced by about one-half. Cool to room temperature, add 1.5 g of potassium sulfate and 3 ml of water. Carefully add 4.5 ml of concentrated sulfuric acid and heat until iodine fumes are no longer evolved. Allow the mixture to cool, wash down the sides of the flask with water, heat until charring occurs, and again cool to room temperature. To the charred material add 40 mg of mercuric oxide, heat until the colour of the solution is pale yellow, then cool, wash down the sides of the flask with a few ml of water and digest the mixture for 3 h. Cool the digest, add 20 ml ammonia-free water, 16 ml of a 50% sodium hydroxide solution and 5 ml of a 44% sodium thiosulfate solution. Connect the flask to a distillation apparatus and distil, collecting the distillate in 10 ml of a 4% boric acid solution. Add a few drops of methyl red-methylene blue TS to the distillate and titrate with 0.05 N sulfuric acid. Perform a blank determination. Each ml of 0.05 N sulfuric acid is equivalent to 0.7004 mg of N.

BEESWAX

Revised specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005), superseding specifications prepared at the 39th JECFA (1992) and published in FNP 52 Add 1 (1992), and incorporating the decisions on the metals and arsenic specifications agreed at the 63rd JECFA (2004) and published in FNP 52 Add 12 (2004). The 65th JECFA (2005) considered the additive to be of no toxicological concern for the functional uses listed.

SYNONYMS INS No. 901

DEFINITION Beeswax is obtained from the honeycombs of bees (Fam. *Apidae*, e.g. *Apis mellifera L*) after the honey has been removed by draining or centrifuging. The combs are melted with hot water, steam or solar heat; the melted product is filtered and cast into cakes of yellow beeswax. White beeswax is obtained by bleaching the yellow beeswax with oxidizing agents, e.g. hydrogen peroxide, sulfuric acid, or sunlight.
Beeswax consists of a mixture of esters of fatty acids and fatty alcohols, hydrocarbons and free fatty acids; minor amounts of free fatty alcohols are also present.

C.A.S. number 8006-40-4 (yellow beeswax)
8012-89-3 (white beeswax)

DESCRIPTION Yellow beeswax: yellow or light-brown solid that is somewhat brittle when cold and presents a dull, granular, non-crystalline fracture when broken; it becomes pliable at about 35°. It has a characteristic odour of honey.

White beeswax: white or yellowish white solid (thin layers are translucent) having a faint and characteristic odour of honey

FUNCTIONAL USES Glazing agent; release agent; stabilizer; texturizer for chewing gum base; carrier for food additives (including flavours and colours); clouding agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; sparingly soluble in alcohol; very soluble in ether

PURITY

Melting range (Vol. 4) 62 - 65°

Acid value (Vol. 4) 17 - 24

Peroxide value Not more than 5
See description under TESTS

Saponification value (Vol. 4) 87 -104

Carnauba wax Passes test
See description under TESTS

Ceresin, paraffins, and Passes test

certain other waxes	See description under TESTS
Fats, Japan wax, rosin and soap	Passes test See description under TESTS
Glycerol and other polyols	Not more than 0.5 % (calculated as glycerol) See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, "Instrumental Methods".

TESTS

PURITY TESTS

Peroxide value

Weigh accurately 5 g of the sample into a 200-ml conical flask. Add 30 ml of a 2:3 solution of chloroform and acetic acid TS and close the flask with a stopper. Heat with warm water and swirl to dissolve the sample. Cool to room temperature and add 0.5 ml of saturated potassium iodide solution. Close the flask with the stopper and shake vigorously for 60±5 sec. Add 30 ml of water and titrate immediately with 0.01 N sodium thiosulfate using starch TS as indicator. Carry out a blank determination.

Peroxide value = $(a-b) \times N \times 1000/W$
where
a = volume (ml) of sodium thiosulfate used for the sample
b = volume (ml) of sodium thiosulfate used for the blank
N = normality of the sodium thiosulfate
W = weight of sample (g)

Carnauba wax

Transfer 100 mg of the sample into a test tube, and add 20 ml of *n*-butanol. Immerse the test tube in boiling water, and shake the mixture gently until the sample dissolves completely. Transfer the test tube to a beaker of water at 60 °, and allow the water to cool to room temperature. A loose mass of fine, needle-like crystals separates from a clear mother liquor. Under the microscope, the crystals appear as loose needles or stellate clusters, and no amorphous masses are observed, indicating the absence of carnaúba wax.

Ceresins, paraffins and certain other waxes

Transfer 3.0 g of the sample to a 100 ml round-bottomed flask, add 30 ml of a 4% w/v solution of potassium hydroxide in aldehyde-free ethanol and boil gently under a reflux condenser for 2 h. Remove the condenser and immediately insert a thermometer. Place the flask in water at 80° and allow to cool, swirling the solution continuously. No precipitate is formed before the temperature reaches 65°, although the solution may be opalescent.

Fats, Japan wax, rosin and soap

Boil 1 g of the sample for 30 min with 35 ml of a 1 in 7 solution of sodium hydroxide, maintaining the volume by the occasional addition of water, and cool the mixture. The wax separates and the liquid remains clear. Filter the cold mixture and acidify the filtrate with hydrochloric acid. No precipitate is formed.

Glycerol and other polyols

To 0.20 g of the sample in a round-bottom flask, add 10 ml of ethanolic potassium hydroxide TS, attach a reflux condenser to the flask and heat in

a water bath for 30 min. Add 50 ml of dilute sulfuric acid TS, cool and filter. Rinse the flask and filter with dilute sulfuric acid TS. Combine the filtrate and washings and dilute to 100.0 ml with dilute sulfuric acid TS. Place 1.0 ml of the solution in a tube, add 0.5 ml of a 1.07 % (w/v) solution of sodium periodate, mix and allow to stand for 5 min. Add 1.0 ml of decolourized fuchsin solution (see below) and mix. Any precipitate disappears. Place the tube in a beaker containing water at 40^{o}. Allow to cool while observing for 10 to 15 min. Any bluish-violet colour in the solution is not more intense than a standard prepared at the same time in the same manner using 1.0 ml of a 0.001 % (w/v) solution of glycerol in dilute sulfuric acid TS.

Decolourized Fuchsin Solution

Dissolve 0.1 g of basic fuchsin in 60 ml of water. Add a solution of 1 g of anhydrous sodium sulfite (Reagent grade) in 10 ml of water. Slowly and with continuous shaking of the solution add 2 ml of hydrochloric acid. Dilute to 100 ml with water. Allow to stand protected from light for at least 12 h, decolourize with activated charcoal and filter. If the solution becomes cloudy, filter before use. If on standing the solution becomes violet, decolourize again by adding activated charcoal. Store protected from light.

BEET RED

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not specified' was established at the 31st JECFA (1987)

SYNONYMS Beetroot Red; INS No. 162

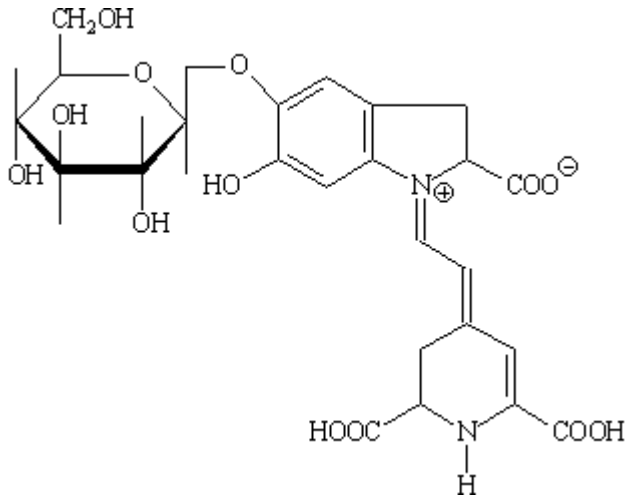
DEFINITION Obtained from the roots of red beets (*Beta vulgaris* L var rubra) as press juice or by aqueous extraction of shredded beet roots; composed of different pigments all belonging to the class betalaine; main colouring principle consists of betacyanins (red) of which betanine accounts for 75-95%; minor amounts of betaxanthine (yellow) and degradation products of betalaines (light brown) may be present; the betanine content in extracts of beetroot will suffer a progressive degradation which is accelerated by raising the pH, temperature and water activity; it is therefore expected that all commercial products will slowly lose their colour and alter their shade according to the conditions of storage.
Besides the colour pigments the juice or extract consists of sugars, salts and/or proteins naturally occurring in red beets. The solution may be concentrated and some products may be refined in order to remove most of the sugars, salts and proteins. Food grade acids (e.g., citric, lactic, L-ascorbic) may be added as pH controlling agents and stabilizers and carriers (e.g., maltodextrin) may be added as aids for manufacturing dry powders.

Chemical names [S-(R^*,R^*)-4-[2-[2-Carboxy-5-(ß-D-glucopyranosyloxy)-2,3-dihydro-6-hydroxy-1H-indol-1-yl)ethenyl]-2,3-dihydro-2,6-pyridine-dicarboxylic acid; 1-[2-(2,6-dicarboxy-1,2,3,4-tetrahydro-4-pyridylidene) ethylidene]-5- ß-D-glucopyrano-syloxy)-6-hydroxyindolium-2- carboxylate

C.A.S. number 7659-95-2 (betanine)

Chemical formula Betanine: $C_{24}H_{26}N_2O_{13}$

Structural formula



Formula weight Betanine: 550.48

Assay Content of red colour (expressed as betanine) is not less than 0.4%.

DESCRIPTION

Red or dark red liquid, paste, powder or solid.

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in or miscible with water; insoluble in or immiscible with ethanol
Colour reaction	Addition of an aqueous 10% w/v sodium hydroxide solution to an aqueous solution of the sample successively changes the colour from red to reddish violet to yellow.
Spectrophotometry (Vol. 4)	Betanine in water at pH 5.4 has an absorbance maximum at about 530 nm and at pH 8.9 exhibits a broadened maximum at about 545 nm.
Thin layer chromatography	Passes test See description under TESTS

PURITY

Nitrate	Not more than 2 g nitrate anion/g of red colour (as calculated from assay) See description under TESTS
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Basic colouring	To 1 g of the sample add 100 ml of 1% sodium hydroxide solution, and mix well. Extract 30 ml of this solution with 15 ml of diethyl ether. When extracted wash the ether layer twice with 5 ml of dilute acetic acid TS; the dilute acetic acid layer does not produce a colour.
Other acidic colouring matters	To 1 g of the sample add 1 ml of ammonia TS and 8 ml of water, and shake well. Discard an oily layer when separated. Proceed as directed under *Determination by Paper Chromatography (Ascending chromatography)*, using 2 µl of the solution as the sample solution, and a mixture of pyridine and ammonia TS (2:1 by volume) as the developing solvent. Stop the development when the solvent front has advanced about 15 cm from the point of application. No spot is observed at the solvent front after drying under daylight, or, if any spot is observed, it shall be decolourized when sprayed with a solution of stannous chloride (2 parts of stannous chloride by weight in 5 parts of water).

TESTS

IDENTIFICATION TESTS

Thin layer chromatography

(a) On cellulose plates (0.25 mm) with Sørensen's phosphate buffer (pH 5.6) as solvent, Beet Red colour gives a number of spots in various colours (yellow, orange, red, purple, violet). Betanine appears as a purple spot with an R_f value of about 0.7.
Sørensen's phosphate buffer (pH 5.6):
- Solution A: 1/15 M potassium dihydrogen phosphate: Dissolve 9.08 g of KH_2PO_4 in water and dilute to 1000 ml.
- Solution B: 1/15 M disodium hydrogen phosphate: Dissolve 11.88 g of $Na_2HPO_4 \cdot 2H_20$ in water and dilute to 1000 ml.
Sørensen's phosphate buffer is composed of a mixture of solutions A and B in the following proportions: 94.8 parts of solution A + 5.2 parts of solution B.
(b) On cellulose plates (0.10 mm) in the solvent (2 g sodium citrate + 78.5 ml water + 21.5 ml ammonia TS), betanine follows the front of the solvent as distinct from acidic water-soluble synthetic dyes. In this solvent betanine is yellow.

PURITY TESTS

Nitrate

Apparatus
A suitably sensitive potentiometric instrument, such as a pH/mV meter, with nitrate - selective electrode and reference electrode as prescribed by the manufacturer.

Solutions
- Standard nitrate solution (10,000 mg/l): Dissolve 16.31 g of potassium nitrate (KNO_3), previously dried at 105º, 24 h in 1000 ml of water
- Buffer solution: Dissolve 6.66 g of aluminium sulfate octahydrate, $Al_2(SO_4)_3 \cdot 8H_2O$, 3.12 g of silver sulfate (Ag_2SO_4), 1.24 g of boric acid (H_3BO_3) and 1.94 g of sulfamic acid (NH_2HSO_3) in 900 ml water, adjust to pH 3.0 with 1 M sulfuric acid and dilute with water to 1000 ml
- Diluted buffer solution: Dilute the Buffer solution with an equal amount of water
- Calibration solutions: Dilute the standard solution with the Diluted buffer solution in order to prepare the following solutions: 0, 100, 200, 300, 400 and 500 mg nitrate/l.

Procedure
Accurately weigh about 0.5 g of the sample in a conical flask, add 50 ml of Diluted buffer solution and dissolve by swirling.
Measure the potential of the calibration solutions and also of the sample solution. Plot the calibration curve from the potential figures against the corresponding nitrate concentrations using antilog paper with the nitrate concentrations along the linear axis. From the calibration curve read the nitrate concentration of the sample.

Calculation

$$Nitrate\ content = \frac{a}{200 \times w \times A}\ g / g\ colouring\ matters$$

where
a = nitrate concentration of sample, mg/l
w = weight of sample
A = % red colour as calculated from assay

METHOD OF ASSAY

Dissolve a quantity of Beet Red accurately weighed in buffer TS (pH 5) and dilute to a suitable volume with the buffer solution (V ml in total); the maximum absorption shall be within the range of 0.2 to 0.8. Centrifuge the solution if necessary, and measure the absorption, correcting for a blank composed of Buffer TS (pH 5). The colour content is calculated on the basis of the maximum absorption A (at about 530 nm), using the specific absorbance for betanine, A (1%, 1 cm) = 1120.

$$\%\ Red\ colour = \frac{A \times V}{1120 \times L \times W}$$

where
A = maximum absorption
V = volume of test solution measured in ml
L = length of cell measured in cm
W = weight of sample in g.

BENZOIC ACID

Prepared at the 49th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI of 0-5 mg/kg bw for benzoic acid and its salts was established at the 27th JECFA (1983) was maintained at the 46th JECFA (1996)

SYNONYMS INS No. 210

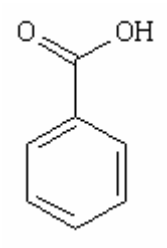
DEFINITION

Chemical names: Benzoic acid, benzenecarboxylic acid, phenylcarboxylic acid

C.A.S. number: 65-85-0

Chemical formula: $C_7H_6O_2$

Structural formula:



Formula weight: 122.12

Assay: Not less than 99.5% on the dried basis

DESCRIPTION White crystalline solid, usually in the form of scales or needles, having not more than a faint characteristic odour

FUNCTIONAL USES Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water, freely soluble in ethanol

Melting range (Vol. 4): 121 - 123º

Test for benzoate (Vol. 4): Passes test
Use 0.1 g of the sample with 0.1 g of calcium carbonate and 5 ml of water

pH (Vol. 4): About 4.0 (solution in water)

PURITY

Loss on drying (Vol. 4)	Not more than 0.5% (over sulfuric acid, 3 h)
Sublimation test	Place a small amount of the sample in a dry test tube. Wrap the test tube about 4 cm from the bottom with moistened filter paper. Heat the test tube over a low flame. Benzoic acid sublimes and crystals deposit in the colder part of the test tube leaving no residue at the bottom.
Sulfated ash (Vol. 4)	Not more than 0.05%
Lead	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."
Readily carbonizable substances	Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 ml of sulfuric acid TS. The colour produced should not be darker than a light pink (Matching Fluid Q).
Readily oxidizable substances	Add 1.5 ml of sulfuric acid to 100 ml of water, heat to boiling and add 0.1N potassium permanganate in drops, until the pink colour persists for 30 sec. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1N potassium permanganate to a pink colour that persists for 15 sec. Not more than 0.5 ml should be required.
Chlorinated organic compounds	Not more than 0.07% (as Cl_2) Test 0.25 g of the sample dissolved in 10 ml of 0.1 N sodium hydroxide, using 0.5 ml of 0.01N hydrochloric acid in the control.

METHOD OF ASSAY Weigh, to the nearest mg, 2.5 g of the dried sample. Dissolve in 15 ml of warm ethanol previously neutralized using phenol red TS as indicator. Add 20 ml of water and titrate with 0.5N sodium hydroxide, using phenolphthalein TS as indicator. Each ml 0.5N sodium hydroxide is equivalent to 61.06 mg of $C_7H_6O_2$.

SODIUM BENZOATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 17th JECFA (1974), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI of 0-5 mg/kg bw for benzoic acid and salts was established at the 27th JECFA (1983), and maintained at the 57th JECFA (2001)

SYNONYMS INS No. 211

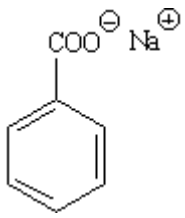
DEFINITION

Chemical names: Sodium benzoate, sodium salt of benzenecarboxylic acid, sodium salt of phenylcarboxylic acid

C.A.S. number: 532-32-1

Chemical formula: $C_7H_5NaO_2$

Structural formula:



Formula weight: 144.11

Assay: Not less than 99.0% on the dried basis

DESCRIPTION White, almost odourless, crystalline powder, flakes or granules

FUNCTIONAL USES Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, sparingly soluble in ethanol

Test for benzoate (Vol. 4): Passes test
Use a 10% solution of the sample

Test for sodium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 1.5% (105°, 4 h)

Acidity or alkalinity: Dissolve 2 g of the sample, weighed to the nearest mg, in 20 ml of freshly

boiled water. Not more than 0.5 ml of either 0.1N sodium hydroxide or 0.1N hydrochloric acid should be required for neutralization, using phenolphthalein TS as indicator.

Readily carbonizable substances

Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 ml of sulfuric acid TS. The colour produced should not be darker than a light pink ("*Matching Fluid Q"*)

Chlorinated organic compounds (Vol. 4)

Not more than 0.07% (as chlorine)
Test 0.25 g of the sample using 0.5 ml of 0.01N hydrochloric acid in the control

Readily oxidizable substances

Add 1.5 ml of sulfuric acid to 100 ml of water, heat to boiling and add 0.1N potassium permanganate, dropwise, until the pink colour persists for 30 sec. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1N potassium permanganate to a pink colour that persists for 15 sec. Not more than 0.5 ml should be required.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh, to the nearest mg, 3 g of the sample previously dried for 4 h at 105° and transfer to a 250-ml Erlenmeyer flask. Add 50 ml of water to dissolve the sample. Neutralize the solution, if necessary, with 0.1N hydrochloric acid, using phenolphthalein TS as indicator. Add 50 ml of ether and a few drops of bromophenol blue TS, and titrate with 0.5N hydrochloric acid, shaking the flask constantly, until the colour of the indicator begins to change. Transfer the lower aqueous layer to another flask. Wash the ethereal layer with 10 ml of water, and add the washing and an additional 20 ml of ether to the separated aqueous layer. Complete the titration with the 0.5N hydrochloric acid, shaking constantly the flask.

Each ml of 0.5N hydrochloric acid is equivalent to 72.05 mg of $C_7H_5NaO_2$.

POTASSIUM BENZOATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 17th JECFA (1974), published in FNP 4 (1978). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI of 0-5 mg/kg bw for benzoic acid and salts was established at the 27th JECFA (1983) and maintained at the 46th JECFA (1996).

SYNONYMS INS No. 212

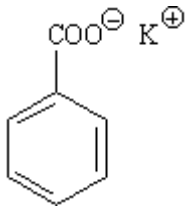
DEFINITION

Chemical names: Potassium benzoate, potassium salt of benzenecarboxylic acid, potassium salt of phenylcarboxylic acid

C.A.S. number: 582-25-2 (anhydrous)

Chemical formula: $C_7H_5KO_2 \cdot 3H_2O$

Structural formula:



Formula weight: 160.22 (anhydrous)
214.27 (trihydrate)

Assay: Not less than 99.0% on the dried basis

DESCRIPTION White crystalline powder

FUNCTIONAL USES Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, soluble in ethanol

Test for benzoate (Vol. 4): Passes test
Use a 10% solution of the sample

Test for potassium (Vol. 4): Passes test
Use a 10% solution of the sample

PURITY

Loss on drying (Vol. 4): Not more than 26.5% (105°, 4 h)

Acidity or alkalinity	Dissolve 2 g of the sample, weighed to the nearest mg, in 20 ml of freshly boiled water. Not more than 0.5 ml of either 0.1N sodium hydroxide or 0.1N hydrochloric acid should be required for neutralization, using phenolphthalein TS as indicator.
Readily carbonizable substances	Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 ml of sulfuric acid TS. The colour produced should not be darker than a light pink ("Matching Fluid Q")
Chlorinated organic compounds (Vol. 4)	Not more than 0.07% (as chlorine) Test 0.25 g of the sample using 0.5 ml of 0.01N hydrochloric acid in the control
Readily oxidizable substances	Add 1.5 ml of sulfuric acid to 100 ml of water, heat to boiling and add 0.1N potassium permanganate, dropwise, until the pink colour persists for 30 sec. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1N potassium permanganate to a pink colour that persists for 15 sec. Not more than 0.5 ml should be required.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh to the nearest 0.1 mg, 2.5 to 3 g of the dried sample, and transfer to a 250-ml Erlenmeyer flask. Add 50 ml of water to dissolve the sample. Neutralize the solution, if necessary, with 0.1N hydrochloric acid, using phenolphthalein TS as indicator. Add 50 ml of ether and a few drops of bromophenol blue TS and titrate with 0.5N hydrochloric acid, shaking constantly the flask, until the colour of the indicator begins to change. Transfer the lower aqueous layer to another flask. Wash the ethereal layer with 10 ml of water, and add the washing and an additional 20 ml of ether to the separated aqueous layer. Complete the titration with the 0.5N hydrochloric acid, shaking constantly the flask. Each ml of 0.5N hydrochloric acid is equivalent to 80.11 mg of $C_7H_5KO_2$.

CALCIUM BENZOATE

Prepared at the 49th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 27th JECFA (1983), published in FNP 28 (1983). Metals and arsenic specifications revised at the 63rd JECFA (2004). *A group ADI of 0-5 mg/kg bw for benzoic acid and its salts was established at the 27th JECFA (1983) and was maintained at the 46th JECFA (1996)*

SYNONYMS Monocalcium benzoate, INS No. 213

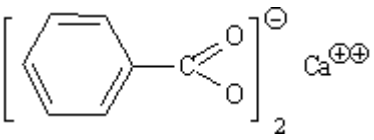
DEFINITION

Chemical names: Calcium benzoate

C.A.S. number: 2090-05-3

Chemical formula: Anhydrous: $C_{14}H_{10}CaO_4$
Monohydrate: $C_{14}H_{10}CaO_4 \cdot H_2O$
Trihydrate: $C_{14}H_{10}CaO_4 \cdot 3H_2O$

Structural formula:



(anhydrous)

Formula weight: Anhydrous: 282.31
Monohydrate: 300.32
Trihydrate: 336.36

Assay: Not less than 99.0% on the dried basis

DESCRIPTION White or colourless crystals, or white powder

FUNCTIONAL USES Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Sparingly soluble in water

Test for benzoate (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 17.5% (105º, 4 h)

Water insoluble matter

Not more than 0.3%
Dissolve 10 g of the sample, weighed to the nearest mg, in 100 ml of hot water. Filter through a Gooch crucible, tared to an accuracy of ±0.2 mg, and wash any residue with hot water. Dry the crucible for 2 h at 105º. Cool, weigh and calculate as percentage.

Acidity or alkalinity

Passes test
Dissolve 2 g of the sample, weighed to the nearest mg, in 20 ml of freshly boiled water. Not more than 0.5 ml of either 0.1N sodium hydroxide or 0.1N hydrochloric acid should be required for neutralization, using phenolphthalein TS as indicator.

Fluoride (Vol. 4)

Not more than 10 mg/kg
Weigh 5 g of the sample to the nearest mg and proceed as directed in the Limit Test (Method I or III)

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

Chlorinated organic compounds (Vol. 4)

Not more than 0.07% (as Cl_2)
Test 0.25 g of the sample, using 0.5 ml of 0.01N hydrochloric acid in the control.

Readily oxidizable substances

Passes test
Add 1.5 ml of sulfuric acid to 100 ml of water, heat to boiling and add 0.1N potassium permanganate in drops, until the pink colour persists for 30 sec. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1N potassium permanganate to a pink colour that persists for 15 sec. Not more than 0.5 ml should be required.

METHOD OF ASSAY

Weigh accurately 0.6 g of the dried sample, dissolve in a mixture of 20 ml of water and 2 ml of dilute hydrochloric acid TS, and dilute to 100 ml with water. While stirring (preferably with a magnetic stirrer) add about 30 ml of 0.05M disodium ethylenediaminetetraacetate from a 50-ml buret, then add 15 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation (an alternative indicator is hydroxynaphthol blue, of which 0.25 g is used - in this case the naphthol green TS is omitted) and 3 ml of naphthol green TS, and continue the titration until the solution is deep blue in colour. Each ml of 0.05M disodium ethylenediamine tetraacetate is equivalent to 14.116 mg of $C_{14}H_{10}CaO_4$.

BENZOYL PEROXIDE

Prepared at the 63rd JECFA (2004), published in FNP 52 Add 12 (2004) superseding specifications prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000). Treatment of whey with benzoyl peroxide at a maximum concentration of 100 mg/kg does not pose a safety concern (63rd JECFA, 2004).

SYNONYMS Benzoyl superoxide, INS No. 928

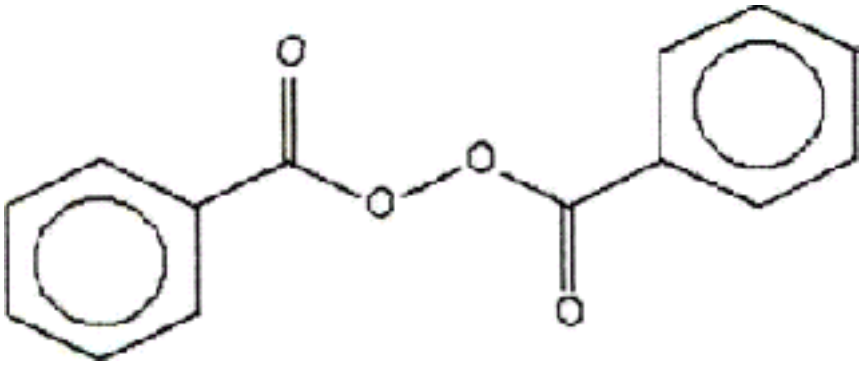
DEFINITION Benzoyl peroxide is manufactured by the reaction of benzoyl chloride, sodium hydroxide and hydrogen peroxide.

Chemical name: Dibenzoyl peroxide

C.A.S. number: 94-36-0

Chemical formula: $C_{14}H_{10}O_4$

Structural formula



Formula weight: 242.23

Assay: Not less than 96.0%

DESCRIPTION Colourless, crystalline solid having a faint odour of benzaldehyde.
Caution: Benzoyl peroxide, especially in the dry form, is a dangerous, highly reactive, oxidizing material and has been known to explode spontaneously

FUNCTIONAL USES Bleaching agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water, slightly soluble in ethanol and soluble in ether.

Melting range (Vol. 4): 103 - 106° with decomposition

Decomposition to benzoic acid: To 0.5 g of the sample add 50 ml of 0.5 N ethanolic potassium hydroxide, heat gradually to boiling and continue boiling for 15 min. Cool and dilute with 200 ml of water. Add sufficient 0.5 N hydrochloric acid to make strongly acidic and extract with ether. Dry the ether solution over anhydrous sodium sulfate, and then evaporate to dryness on a steam bath. The benzoic acid so obtained melts

between 121° and 123°.

PURITY

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods".

METHOD OF ASSAY Dissolve about 250 mg of the sample, accurately weighed, in 15 ml of acetone in a 100-ml glass-stoppered bottle. Add 3 ml of 50% (w/v) potassium iodide solution and swirl for 1 min. Titrate immediately with 0.1 N sodium thiosulfate (without addition of starch as an indicator). Each ml of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of $C_{14}H_{10}O_4$.

BRILLIANT BLUE FCF

Prepared at the 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-12.5 mg/kg bw was established at the 13th JECFA (1969).

SYNONYMS CI Food Blue 2, FD&C Blue No.1, CI (1975) No. 42900, INS No. 133

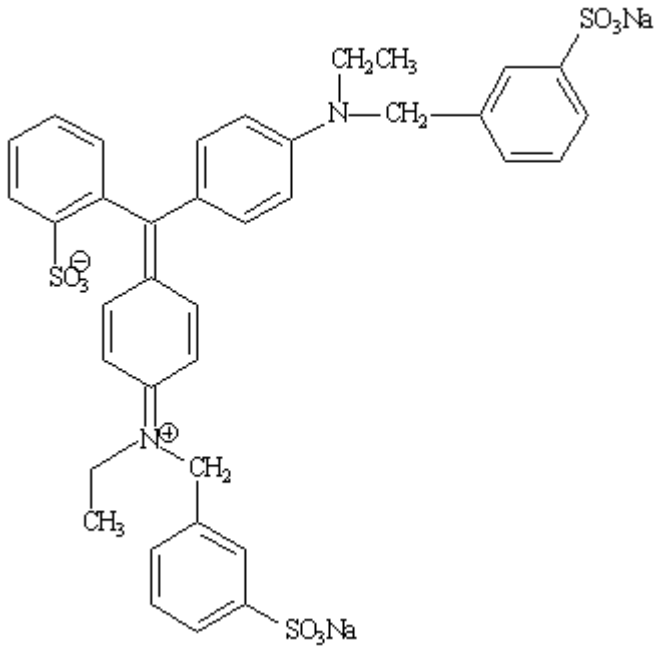
DEFINITION Consists essentially of Disodium 3-[N-ethyl-N-[4-[[4-[N-ethyl-N-(3-sulfonatobenzyl)-amino] phenyl] (2-sulfonatophenyl)methylene]-2,5-cyclohexadiene-1-ylidene] ammoniomethyl] benzenesulfonate and its isomers and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply

Chemical names Disodium 3-[N-ethyl-N-[4-[[4-[N-ethyl-N-(3-sulfonatobenzyl)-amino]phenyl](2-sulfonatophenyl)methylene]-2,5-cyclohexa-diene-1-ylidene]ammoniomethyl]-benzenesulfonate;
Disodium I-[4-(N-ethyl-3-sulfonatobenzylamino)phenyl]-I- [4-(N-ethyl-3-sulfonatobenzyliminio)cyclohexa-2,5-dienyli-dene]toluene-2-sulfonate (an alternative chemical name)

C.A.S. number 3844-45-9

Chemical formula $C_{37}H_{34}N_2Na_2O_9S_3$

Structural formula



Formula weight 792.86

Assay	Not less than 85% total colouring matter

DESCRIPTION Blue powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; slightly soluble in ethanol
Identification of colouring matters (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 15% at 135° together with chloride and sulfate calculated as sodium salts
Water insoluble matter (Vol. 4)	Not more than 0.2%
Lead (Vol. 4)	Not more than 2mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Chromium (Vol. 4)	Not more than 50 mg/kg
Subsidiary colouring matters (Vol. 4)	Not more than 6% Use the following conditions: Developing solvent: No. 4 Develop chromatogram for approximately 20 hours
Organic compounds other than colouring matters (Vol. 4)	Not more than 1.5%, sum of 2-, 3- and 4-formylbenzenesulfonic acids Not more tan 0.3% 3-[[N-ethyl-N-(4-sulfophenyl) amino] methyl] benzenesulfonic acid Proceed as directed under *Column Chromatography* The following absorptivities may be used: 3-formylbenzenesulfonic acid: 0.0495 mg/L/cm at 246 nm in dilute HCl 3-[[N-ethyl-N-(4-sulfophenyl)amino] methyl] benzenesulfonic acid: 0.078 mg/L/cm at 277 nm in dilute ammonia.
Leuco base (Vol. 4)	Not more than 5% Weigh accurately 120±5 mg of sample and proceed as directed under *Leuco Base in Sulfonated Triarylmethane Colours* Absorptivity (a) = 0.164 mg/L/cm at approximately 630 nm Ratio = 0.9706

Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether extractable matter (Vol. 4)	Not more than 0.2%
METHOD OF ASSAY	Proceed as directed under *Total Content by Titration with Titanous Chloride,* Volume 4, using the following: Weight of sample: 1.8 - 1.9 g Buffer: 15 g sodium hydrogen tartrate Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N $TiCl_3$: 39.65 mg

BROMELAIN

Prepared at the 15th JECFA (1971), published in NMRS 50B (1972) and in FNP 52 (1992). An ADI 'not limited' was established at the 15th JECFA (1971)

SYNONYMS INS No. 1101(iii)

SOURCES Purified proteolytic substances derived from *Ananas comosus* and *Ananas bracteatus* (L).

Active principles: Bromelain (cystein proteinase)

Systematic names and numbers: None (EC 3.4.22.4)

Reactions catalyzed: The enzyme hydrolyzes polypeptides, amides and esters, especially at linkages involving basic amino acids, or leucine or glycine, yielding peptides of lower molecular weight.

DESCRIPTION White to light tan amorphous powder; soluble in water, the solutions being colourless to light yellow and somewhat opalescent; practically insoluble in alcohol, chloroform and ether

FUNCTIONAL USES Enzyme preparation
Used in the chillproofing of beer, tenderizing of meat, preparation of precooked cereals, and production of protein hydrolysates.

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Bromelain activity (Vol. 4): The sample shows plant proteolytic activity (see *Proteolytic Activity, Plant)*

BUTYLATED HYDROXYANISOLE

Prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003) An ADI of 0-0.5 mg/kg bw was established at the 33rd JECFA (1988)

SYNONYMS BHA; INS No. 320

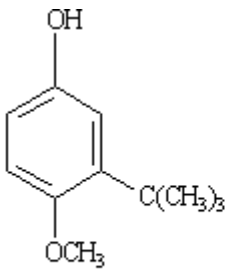
DEFINITION

Chemical names 3-Tertiary-butyl-4-hydroxyanisole, a mixture of 3- and 2-tertiary-butyl-4-hydroxyanisole

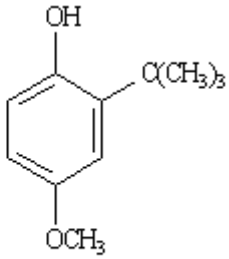
C.A.S. number 25013-16-5

Chemical formula $C_{11}H_{16}0_2$

Structural formula



3-isomer



2-isomer

Formula weight 180.25

Assay Not less than 98.5% of $C_{11}H_{16}0_2$ and not less than 85% of 3-tertiary-butyl-4-hydroxyanisole

DESCRIPTION White or slightly yellow crystals or waxy solid, with a faint characteristic odour

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; freely soluble in ethanol and propane-1,2-diol

Colour reaction To 5 ml of a 1 in 10,000 solution of the sample in 72% ethanol, add 2 ml of sodium borate TS and 1 ml of a 1 in 10,000 solution of 2,6-dichloroquinonechlorimide in absolute ethanol, and mix. A blue colour appears.

PURITY

Sulfated ash (Vol. 4) Not more than 0.05%
Test 5 g of the sample (Method I)

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

Phenolic impurities

Not more than 0.5%
See description under TESTS

TESTS

PURITY TESTS

Phenolic impurities

Determine by *Thin-Layer Chromatography*, (see Volume 4) using silica gel G plates.

Solution 1: Dissolve 0.25 g of the sample in 10 ml of ether.
Solution 2: Dilute 1 ml of Solution 1 to 10 ml with ether, and then dilute 1 ml of the resulting solution to 20 ml with ether. Use the final dilution as solution 2.

Procedure
Spot 2 µl each of Solution 1 and of Solution 2 on separate TLC plates. Place each plate in a developing chamber containing chloroform as solvent, and allow the solvent front to ascend to a point 15 cm above the sample spots. Develop the chromatograms by spraying with an aqueous mixture of equal volumes of 2% ferric chloride solution and 1% potassium ferricyanide solution mixed prior to use. The blue colours produced may be intensified by spraying with 2N hydrochloric acid. Any blue spots appearing (other than the major spot and the spot at R_f 0.35) are not more intense than the major spot appearing on Chromatogram 2.

METHOD OF ASSAY

Gas Chromatographic Method

Internal standard solution (either diphenylamine or 4-tertiary-butylphenol):
Accurately weigh 500 mg, dissolve in acetone and make up to 250 ml with acetone.

Standard solution:
Accurately weigh 90 mg of 3-butylated hydroxyanisole and 10 mg of 2-butylated hydroxyanisole and dissolve in Internal standard solution to make 100 ml.

Procedure:
Dissolve 10 mg of the sample, accurately weighed, in the internal standard solution to make 50 ml. Inject aliquots of the solution into a gas chromatograph equipped with a hydrogen flame ionization detector. Either of the following GC conditions or equivalent may be used:

A: The internal standard elutes after 3-tert-butyl-4-hydroxyanisole:
Column
- length: 1.5 m
- inner diameter: 2 mm

- material: glass
- packing: 10% XE-60 on 100-200 mesh
Temperatures
- injector: 250°
- column: 155°
- detector: 250°
Carrier gas: nitrogen
Flow rate: 30 ml/min

B: The internal standard elutes before 3-ter-butyl-4-hydroxyanisole:
Column
- length: 2 m
- inner diameter: 3 mm
- material: glass
- packing: 5% Versamide-900 on 80/100 mesh Chromosorb W-AW-DMCS
Temperatures
- injector: 225°
- column: 170°
- detector: 250°
Carrier Gas: nitrogen
Flow rate: 30 ml/min

Prepare a standard curve of 2- and 3-butylated hydroxyanisole peak height/internal standard peak height versus concentration, using internal standard solutions having various concentrations of butylated hydroxyanisole. Determine the concentrations of 2- and 3-butylated hydroxyanisole by reference to a standard curve. The sum of per cent 2-isomer and per cent 3-isomer gives per cent of total in the sample.

Alternative gas chromatographic method

Assay preparation:
Dissolve about 100 mg of the sample, accurately weighed, in Internal standard solution, and dilute with Internal standard solution to 10 ml.

Chromatographic system:
The gas chromatograph is equipped with a flame-ionization detector, and contains a 1.8-m x 2-mm stainless steel column packed with 10 percent liquid phase on the support, the column is maintained isothermally at a temperature between 175° and 185°, and helium is used as the carrier gas. Chromatograph a sufficient number of injections of the Standard preparation, and record the areas as directed under Procedure, to ensure that the relative standard deviation does not exceed 2.0% for the 3-tert-butyl-4- hydroxyanisole isomer and 6.0% for the 2-tert-butyl-4- hydroxyanisole isomer. The resolution between the isomers is not less than 1.3 and the tailing factor does not exceed 2.0.
Liquid phase: 25% 2-cyanoethyl : 75% methyl-polysiloxane.
Support:
Siliceous earth for chromatography has been fluxcalcined by mixing diatomite with Na_2CO_3 flux and calcining above 900°. The siliceous earth is acid-washed, then water-washed until neutral, but not base-washed. The siliceous earth is silanized by treating with an agent such as dimethyldichlorosilane to mask the surface silanol group.

Procedure:
Separately inject suitable portions (about 5 µl) of the Standard preparation and the Assay preparation into the gas chromatograph, and record the chromatograms. Measure the areas under the peaks for each isomer and the internal standard in each chromatogram, and calculate the quantity, in mg, of each isomer in the sample taken by the formula $10C_S(R_U/R_S)$, in which C_S is the concentration, in mg, of the isomer in the Standard solution, R_S is the ratio of the area of each isomer standard to that of the Internal standard in the chromatogram from the Standard preparation, and R_U is the ratio of the area of each isomer to that of the internal standard in the chromatogram from the Assay preparation.

Calculate the weight, in mg, of $C_{11}H_{16}0_2$ in the sample taken by adding the quantities of the two isomers.

BUTYLATED HYDROXYTOLUENE

Prepared at the 37th JECFA (1990), published in FNP 52 (1992) superseding specifications prepared at the 30th JECFA (1986), published in FNP 37 (1986). Metals and arsenic specifications revised at the 61st JECFA (2003) An ADI of 0-0.3 mg/kg bw was established at the 44th JECFA (1995)

SYNONYMS BHT; INS No. 321

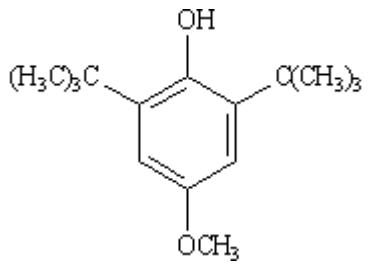
DEFINITION

Chemical names 2,6-Ditertiary-butyl-p-cresol, 4-methyl-2,6-ditertiary-butyl-phenol

C.A.S. number 128-37-0

Chemical formula $C_{15}H_{24}O$

Structural formula



Formula weight 220.36

Assay Not less than 99.0%

DESCRIPTION White, crystalline or flaked solid, odourless or having a characteristic faint aromatic odour

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and propane-1,2-diol; freely soluble in ethanol

Melting range (Vol. 4) 69^{o} - 72^{o}

Spectrophotometry (Vol. 4) The absorption in the range 230 to 320 nm of a 2 cm layer of a 1 in 100,000 solution in dehydrated ethanol exhibits a maximum only at 278 nm

Colour reaction To 10 ml of a 1 in 100,000 solution of the sample in methanol add 10 ml of water, 2 ml of sodium nitrite solution (3 in 1000) and 5 ml of dianisidine dihydrochloride solution (200 mg of 3,3-dimethoxy-benzidine dihydrochloride dissolved in a mixture of 40 ml of methanol and 60 ml of 1 N hydrochloric acid). An orange red colour develops within 3 min. Add 5 ml of chloroform, and shake. The chloroform layer exhibits a purple or magenta colour that fades

when exposed to light.

PURITY

Solidification (Vol. 4) — Not lower than 69.2°

Sulfated ash (Vol. 4) — Not more than 0.005%
Test 20 g of the sample (Method I)

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

Phenolic impurities — Not more than 0.5%
See description under TESTS

TESTS

PURITY TESTS

Phenolic impurities — Determine by *Thin-Layer Chromatography*, (see Volume 4) using silica gel G plates.

Solution 1: Dissolve 0.25 g of the sample in 10 ml of ether.
Solution 2: Dilute 1 ml of Solution 1 to 10 ml with ether, and then dilute 1 ml of the resulting solution to 20 ml with ether. Use the final dilution as solution 2.

Procedure
Spot 2 µl each of Solution 1 and of Solution 2 on separate TLC plates. Place each plate in a developing chamber containing chloroform as solvent, and allow the solvent front to ascend to a point 15 cm above the sample spots. Develop the chromatograms by spraying with an aqueous mixture of equal volumes of 2% ferric chloride solution and 1% potassium ferricyanide solution mixed prior to use. The blue colours produced may be intensified by spraying with 2N hydrochloric acid. Any blue spots appearing (other than the major spot and the spot) are not more intense than the major spot appearing on Chromatogram 2.

METHOD OF ASSAY

Gas Chromatography Method (see Volume 4)

Internal standard solution (diphenylamine or 4-tertiary butylphenol): Accurately weigh 500 mg, dissolve in acetone and make up to 250 ml with acetone.
Standard solution: Accurately weigh 100 mg of butylated hydroxytoluene and dissolve in acetone to make 50 ml.

Procedure:
Dissolve 10 mg of the sample, accurately weighed, in the internal standard solution to make 50 ml. Inject aliquots of the solution into a gas chromatograph, using the following conditions:
Column
- length: 1.5 m

- inner diameter: 3 mm
- material: glass
- packing: 10% XE-60 on 100-200 mesh

Temperatures
- injector: 225°
- column: 155°
- detector: 250°

Carrier gas: nitrogen
Flow rate: 30 ml/min
Detector type: FID

Prepare a standard curve of butylated hydroxytoluene peak height/internal standard peak height versus concentration, using internal standard solutions having various concentrations of butylated hydroxytoluene. Determine the concentration of butylated hydroxytoluene in the sample from the standard curve.

CALCIUM 5'-GUANYLATE

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for 5'guanylic acid and its Ca & Na salts was established at the 18th JECFA (1974)

SYNONYMS Calcium guanylate, INS No. 629

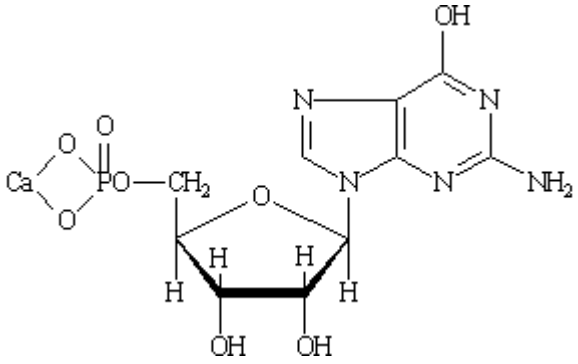
DEFINITION

Chemical names Calcium guaosine-5'-monophosphate

C.A.S. number 38966-30-2

Chemical formula $C_{10}H_{12}CaN_5O_8P \cdot xH_2O$

Structural formula



Formula weight 401.20 (anhydrous)

Assay Not less than 97.0% and not more than 102.0% of after drying

DESCRIPTION Odourless, white or off-white crystals, or powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Sparingly soluble in water

Spectrophotometry (Vol. 4) A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 256±2nm. The ratio A250/A260 is between 0.95 and 1.03, and the ratio A280/260 is between 0.63 and 0.71.

Test for calcium (Vol. 4) Passes test

Test for ribose (Vol. 4) Passes test

Test for organic Passes test

phosphate (Vol. 4) — Test 5 ml of a 1 in 2000 solution

PURITY

Loss on drying (Vol. 4) — Not more than 23% (120^{o}, 4 h)

pH (Vol. 4) — 7.0 - 8.0 (1 in 2,000 soln)

Water soluble matter — To 1 g of the sample, add 50 ml of water, allow to stand for 10 min with occasional shaking, filter through analytical grade filter paper (Whatman No. 42 or equivalent). Evaporate a 25 ml portion of the solution to dryness on a water bath and dry the residue at 105º for 1 h. Residue weighs less than 80 mg.

Amino acids — Not detectable by the following test: To 5 ml of a 1 in 2,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.

Related foreign substances — Chromatographically not detectable
Test 10 µl of a 1 in 2,000 solution of the sample.

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Weigh accurately about 500 mg of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10.0 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 260 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of $C_{10}H_{12}CaN_5O_8P$, in % in the sample by the formula:

$$\frac{A}{294.1} \times \frac{250{,}000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{loss on drying (\%)}} \times 100$$

CALCIUM 5'-INOSINATE

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for inosinic acid and its Ca, K & Na salts, was established at the 29th JECFA (1985)

SYNONYMS Calcium inosinate, INS No. 633

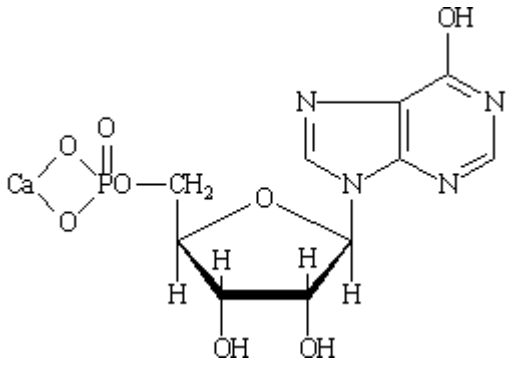
DEFINITION

Chemical names: Calcium inosine-5'-monophosphate

C.A.S. number: Ca salt: 38966-29-9
Ca (1:1) salt: 3387-37-9
Ca (1:1) salt hydrate: 76079-57-7

Chemical formula: $C_{10}H_{11}CaN_4O_8P \cdot xH_2O$

Structural formula



Formula weight: 386.19 (anhydrous)

Assay: Not less than 97.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION Odourless, white or off-white crystals, or powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Sparingly soluble in water

Spectrophotometry (Vol. 4): A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 250±2 nm. The ratio A250/A260 is between 1.55 and 1.65, and the ratio A280/260 is between 0.20 and 0.30.

Test for calcium (Vol. 4): Passes test

Test for ribose (Vol. 4): Passes test

Test for organic phosphate (Vol. 4)	Passes test Test 5 ml of a 1 in 2,000 solution
PURITY	
Water (Vol. 4)	Not more than 23% (Karl Fischer Method)
pH (Vol. 4)	7.0 - 8.0 (1 in 2,000 soln)
Water soluble matter	To 1 g of the sample, add 50 ml of water, allow to stand for 10 min with occasional shaking, filter through analytical grade filter paper (Whatman No. 42 or equivalent). Evaporate a 25 ml portion of the solution to dryness on a water bath and dry the residue at 105° for 1 h. Residue weighs less than 450 mg.
Amino acids	Not detectable by the following test: To 5 ml of a 1 in 1,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.
Related foreign substances	Chromatographically not detectable Test 5 µl of a 1 in 1,000 solution.
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Weigh accurately about 500 mg of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10.0 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 260 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of $C_{10}H_{12}CaN_5O_8P$, in % in the sample by the formula:

$$\frac{A}{314.9} \times \frac{250{,}000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{water (\%)}} \times 100$$

CALCIUM 5'-RIBONUCLEOTIDES

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 18th JECFA (1974).

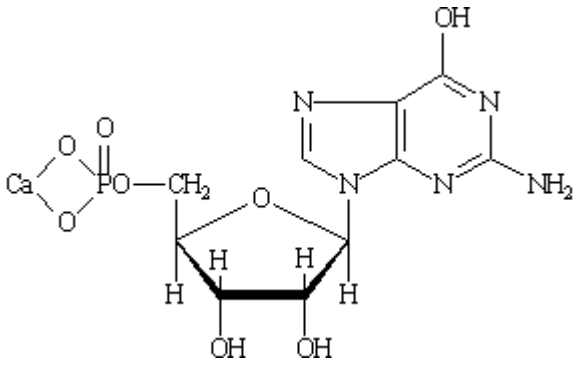
SYNONYMS Calcium ribonucleotides, INS No. 634

DEFINITION

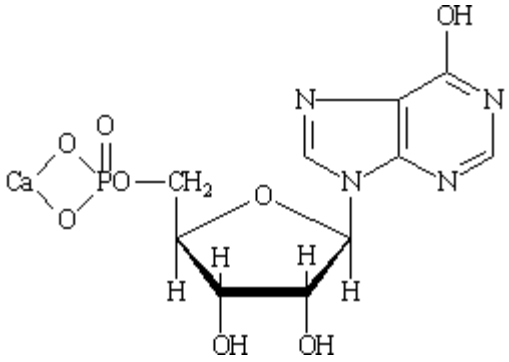
Chemical names (Mixture of) calcium inosine-5'-monophosphate and calcium guanosine-5'-monophosphate

Chemical formula $C_{10}H_{11}CaN_4O_8P \cdot x\ H_2O$ and
$C_{10}H_{12}CaN_5O_8P \cdot x\ H_2O$

Structural formula



Calcium 5'-guanylate



Calcium 5'-inosinate

Assay Not less than 97% and not more than the equivalent of 102% of $C_{10}H_{11}CaN_4O_8P$ and $C_{10}H_{12}CaN_5O_8P$, calculated on the anhydrous basis. The proportion of $C_{10}H_{11}CaN_4O_8P$ or $C_{10}H_{12}CaN_5O_8P$ to the sum of them is between 47% and 53%.

DESCRIPTION Odourless, white or off-white crystals or powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Sparingly soluble in water
Test for ribose (Vol. 4)	Passes test
Test for organic phosphate (Vol. 4)	Passes test Test 5 ml of a 1 in 2,000 solution
Test for inosinic acid	To 2 ml of a 1 in 2,000 solution add 2 ml of 10% hydrochloric acid and 0.1 g of zinc powder, heat in a water bath for 10 min, and filter. Cool the filtrate in ice water, add 1 ml of a 3 in 1,000 sodium nitrite solution, shake well, and allow to stand for 10 min. Add 1 ml of a 1 in 200 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution. A violet red colour is produced.
Test for guanylic acid	To 1 ml of a 1 in 5,000 solution add 1 ml of 10% hydrochloric acid, heat in a water bath for 10 min, cool, and add 0.5 ml of Folin-Ciocalteu TS and 2 ml of saturated sodium carbonate solution. A blue colour is produced.
Test for calcium (Vol. 4)	Passes test

PURITY

Water (Vol. 4)	Not more than 23% (Karl Fischer Method)
pH (Vol. 4)	7.0 - 8.0 (1 in 2,000 soln)
Water soluble matter	To 1 g of the sample, add 50 ml of water, allow to stand for 10 min with occasional shaking, filter through analytical grade filter paper (Whatman No. 42 or equivalent). Evaporate a 25 ml portion of the solution to dryness on a water bath and dry the residue at 1057 for 1 h. Residue weighs less than 80 mg.
Amino acids	Not detectable by the following test: To 5 ml of a 1 in 2,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.
Related foreign substances	Chromatographically not detectable Test 5 µl of a 1 in 1,000 soln. Only inosinic acid and 5'-guanylic acid are detected
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Calculate the contents of calcium inosine-5'-monophosphate (I) and calcium guanosine-5'-monophosphate (G) in the sample by the following equation, using values for I_{ca} and G_{ca} obtained as described below.

$$Content\ (\%) = \frac{I_{ca} + G_{ca}}{100 - water\ (\%)} \times 100$$

Weigh accurately about 650 mg of the sample, and dissolve in water to make 500 ml (Solution A).

To determine I_{ca} (calcium inosine-5'-monophosphate), take a 1-ml portion of Solution A, add 4 ml of 6 N hydrochloric acid and water to make to 10 ml. Heat in a water bath for 40 min, cool, add 0.4 g of zinc powder, allow to stand for 50 min, shaking occasionally and vigorously, and add water to make to 20 ml. Filter through filter paper. To a 10-ml portion of the filtrate add 1 ml of 6 N hydrochloric acid, and add 1 ml of a 1 in 1,000 sodium nitrite solution, cooling in an ice-water bath. Shake well, allow to stand for 10 min, add 1 ml of a 1 in 200 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution, shake well, allow to stand for 15 min at room temperature, and add water to make to 20 ml (Sample solution). For the control, prepare in the same manner as the sample, using 1 ml of water instead of Solution A. Determine the absorbance of the sample solution at 515 nm against the control solution.

To prepare calibration curves, weigh accurately about 3 mg each of disodium 5'-inosinate and disodium 5'-guanylate, and dissolve respectively in 100 ml of 0.01 N hydrochloric acid. Determine the absorbance at 250 nm on the solution of disodium 5'-inosinate and at 260 nm on the solution of disodium 5'-guanylate, using 0.01 N hydrochloric acid as the control. Determine the molecular extinction coefficients E_I and E_G, and calculate the contents of (I) disodium inosine-5'-monophosphate and (G) disodium guanosine-5'-monophosphate by the equations:

$$Content\ (\%)\ of\ I = \frac{E_I}{12,160} \times 100$$

$$Content\ (\%)\ of\ G = \frac{E_G}{11,800} \times 100$$

Weigh accurately a quantity of each which is equivalent to about 50 mg, combine and dissolve in water to make 200 ml (Solution B). To 1-ml, 2-ml and 3-ml portions of Solution B add 4 ml of 6 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed for preparing Sample Solution from Solution A. Determine the absorbance of each Standard Solution at 515 nm and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of I (disodium inosine-5'-monophosphate) from the calibration curve and the absorbance of Sample Solution.

From the content of I, calculate the content of I_{ca} (calcium inosine 5'-monophosphate) as 0.985 x I.

To determine G_{ca} (calcium guanosine-5'-monophosphate), take 1 ml of Solution A, add 4 ml of 2 N hydrochloric acid and water to make to 10 ml.

Heat in a water bath for 30 min, cool, add 2 ml of Folin-Ciocalteu TS and 5 ml of a 4 in 5 sodium carbonate solution. Allow to stand for 15 min, and add water to make to 50 ml. Centrifuge if necessary, and use the supernatant for the test (Sample Solution).

Prepare the control in the same manner as the Sample Solution, using 1 ml of water instead of Solution A. Determine the absorbance of the Sample Solution at 750 nm.

To 1-ml, 2-ml and 3-ml portions of Solution B, add 4 ml of 2 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed in preparing Sample Solution. Determine the absorbance of each Standard Solution at 750 nm, and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of G (disodium guanosine-5'-monophos-phate) from the calibration curve and the absorbance of Sample Solution.

From the content of G calculate the content of G_{ca} (calcium guanosine-5'-monophosphate) as 0.986 x G.

CALCIUM ACETATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS INS No. 263

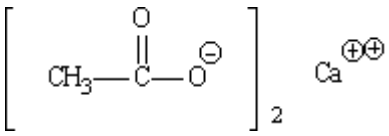
DEFINITION

Chemical names: Calcium acetate

C.A.S. number: 62-54-4

Chemical formula: Anhydrous: $C_4H_6CaO_4$
Hydrates: $C_4H_6CaO_4 \cdot H_2O$; $C_4H_6CaO_4 \cdot xH_2O$ ($x < 1$)

Structural formula:



Formula weight: Anhydrous: 158.17; Monohydrate: 176.18

Assay: Not less than 98% after drying

DESCRIPTION White, hygroscopic, bulky, crystalline solid; a slight odour of acetic acid may be present; the monohydrate may be needles, granules or powder.

FUNCTIONAL USES Antimold and antirope agent, stabilizer, buffer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, insoluble in ethanol

Test for acetate (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 11% (155° to constant weight; monohydrate)

pH (Vol. 4): 6 - 9 (1 in 10 soln)

Water insolubles: Not more than 0.3%
Dissolve 10 g of the sample, weighed to the nearest mg, in 100 ml of hot water. Filter through a Gooch crucible, tared to an accuracy of ±0.2 mg, and wash any residue with water. Dry the crucible for 2 h at 105°. Cool, weigh and calculate as percentage. (The weight of the dried residue should not exceed 30 mg).

Formic acid and oxidizable impurities

Not more than traces

Dissolve 1 g of the sample in 5 ml of water. Add 2.5 ml of 0.1 N potassium dichromate and 6 ml of sulfuric acid and allow to stand for 1 min. Add 20 ml of water, cool to 15° and add 1 ml of potassium iodide TS. A faint yellow or brown colour should be produced immediately.

Aldehydes

Not more than traces

Dissolve 2 g of the sample in 10 ml of water and distil. To the first 5 ml of the distillate, add 10 ml of mercuric chloride TS and make alkaline with N sodium hydroxide. Allow to stand for 5 min, and acidify with dilute sulfuric acid TS. The solution should show no more than a faint turbidity.

Lead (Vol. 4)

Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

1.Calcium content:

Dissolve in a beaker 2.5 g of the sample, weighed to the nearest mg, in 5 ml of hot dilute hydrochloric acid TS. Cool, transfer to a 250-ml volumetric flask, dilute to volume with water, and mix. Transfer 50 ml of the solution to a 400-ml beaker, add 100 ml of water, 25 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation (an alternative indicator is hydroxynaphtol blue, of which 0.25 g is used - in this case the naphthol green TS is omitted), and 3 ml of naphthol green TS. Titrate with 0.05 M disodium ethylenediamine-tetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 7.909 mg of $C_4H_6CaO_4$.

2. Acid content:

Half fill a chromatographic column (1.5 cm in diameter, 20 cm long) with a strong cation-exchange resin (Amberlite IR 120, Amberlite IR 100, Duolit C III, Dorvex 50, Lewatit KS, Ion Exchanger I Merck). Add 0.1 N hydrochloric acid through the top of the column, with the outflow orifice closed until the resin is completely covered and let stand 1-2 h. Drain the acid and rinse the column with water (about 1 liter) until 20 ml of eluate forms a red colour, when one drop each of 0.02 N sodium hydroxide and phenolphthalein TS is added. Weigh, to the nearest mg, 0.05 g of the sample, previously dried at 155° to constant weight, into a flask. Dissolve in 15 ml of water and pour slowly on to the column. Wash the flask and the column with about 200 ml of water and collect the total filtrate in a conical flask. Add two drops of phenolphthalein TS and titrate with 0.1 N sodium hydroxide using a microburette. Each ml of 0.1 N sodium hydroxide is equivalent to 7.909 mg of $C_4H_6CaO_4$.

CALCIUM ALGINATE

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP52, Add 3 (1995). An ADI 'not specified' was established at the 39th JECFA (1992)

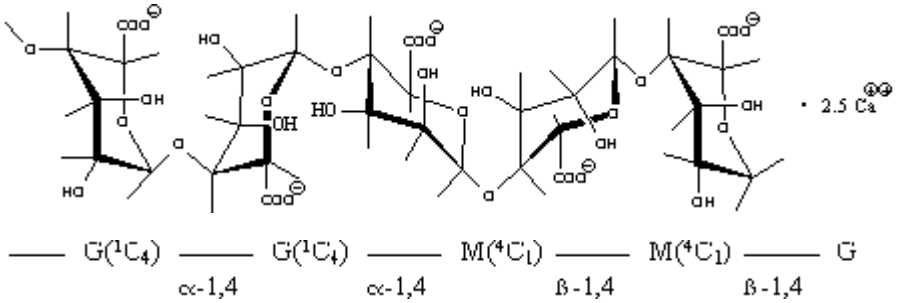
SYNONYMS INS No. 404

DEFINITION Calcium salt of alginic acid.

C.A.S. number 9005-35-0

Chemical formula $(C_6 H_7 Ca_{1/2} O_6)_n$

Structural formula Structural formula from Phillips, Wedlock and Williams: Gums and Stabilizers for the Food Industry 5 (1990) by permission of Oxford University Press.



The number and sequence of the Mannuronate and Glucuronate residues shown above vary in the naturally occurring alginate. The associated water molecules are not shown.

Formula weight Structural unit : 195.16 (theoretical), 219 (actual average)
Macromolecule: 10,000 - 600,000 (typical average)

Assay Not less than 18.0% and not more than 21.0% of carbon dioxide (CO_2), equivalent to not less than 89.6% and not more than 104.5% of calcium alginate $(C_6H_7Ca_{1/2}O_6)_n$ on the anhydrous basis.

DESCRIPTION White to yellowish brown filamentous, grainy, granular and powdered forms

FUNCTIONAL USES Stabilizer, thickener, gelling agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ether; slightly soluble in ethanol; slowly soluble in solutions of sodium polyphosphate, sodium carbonate, and substances that combine with calcium ions.

Precipitate formation with calcium chloride To a 0.5% solution of the sample in sodium hydroxide TS add one-fifth of its volume of a 2.5% solution of calcium chloride. A voluminous, gelatinous precipitate is formed. This test distinguishes calcium alginate from gum

arabic, sodium carboxymethyl cellulose, carrageenan, gelatin, gum ghatti, karaya gum, carob bean gum, methyl cellulose and tragacanth gum.

Precipitate formation with ammonium sulfate — To a 0.5% solution of the sample in sodium hydroxide TS add one-half of its volume of a saturated solution of ammonium sulfate. No precipitate is formed. This test distinguishes calcium alginate from agar, sodium carboxymethyl cellulose, carrageenan, de-esterified pectin, gelatin, carob bean gum, methyl cellulose and starch.

Test for alginate — Passes test
Dissolve as completely as possible 0.1 g of sample by shaking with 0.15 ml of 0.1 N sodium hydroxide and add 1 ml of acid ferric sulfate TS. Within 5 min, a cherry-red colour develops that finally becomes deep purple.

Calcium (Vol. 4) — Passes test

PURITY

Loss on drying (Vol. 4) — Not more than 15% (105^{o}, 4 h)

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

Microbiological criteria (Vol. 4) — Total plate count: Not more than 5,000 colonies per gram.
Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield’s phosphate buffered dilution water and homogenizing in a high speed blender.

Yeasts and moulds: Not more than 500 colonies per gram
Coliforms: Negative by test
Salmonella: Negative by test

METHOD OF ASSAY Proceed as directed under Carbon Dioxide Determination by Decarboxylation in the *General Methods* (See Volume 4). Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2) or 27.38 mg of calcium alginate (equivalent weight 219).

CALCIUM ALUMINIUM SILICATE

Prepared at the 28th JECFA (1984), published in FNP 31/2 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group PTWI of 1 mg/kg bw for aluminium and its salts was established at the 67th JECFA (2006).

SYNONYMS Aluminium calcium silicate, calcium aluminosilicate, calcium silicoaluminate, sodium calcium silicoaluminate; INS No. 556

DEFINITION

Chemical names: Calcium aluminosilicate

Assay:
Not less than 44% and not more than 50% of silicon dioxide (SiO_2)
Not less than 3% and not more than 5% of aluminium oxide (Al_2O_3)
Not less than 32% and not more than 38% of calcium oxide (CaO)
Not less than 0.5% and not more than 4% of sodium oxide (Na_2O)

DESCRIPTION Fine, white, free-flowing powder

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water and ethanol

PURITY

Loss on ignition (Vol. 4): Not less than 14% and not more than 18% (ignition at 1000° to constant weight)

Loss on drying (Vol. 4): Not more than 10% (105°, 2 h)

Fluoride (Vol. 4): Not more than 50 mg/kg
Weigh 1 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III).

Lead (Vol. 4): Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Silicon dioxide:

Transfer 500 mg of the sample, previously dried at 105° for 2 h and weighed accurately, into a 250 ml beaker. Wash the walls of beaker with a few ml of water, then add 30 ml of 72% perchloric acid and 15 ml of hydrochloric acid. Heat on a hot-plate until dense white fumes appear. Let cool. Add 15 ml of hydrochloric acid and reheat until dense fumes appear. Let cool, add 70 ml of water, and filter through Whatman No. 40 filter paper or equivalent.
Wash the paper and the precipitate with hot water to remove perchloric acid. Then transfer the paper and the precipitate to a tared platinum crucible, and ignite at 900° to constant weight. Moisten the residue with a few drops of water, then add 15 ml of hydrochloric acid and 8 drops of sulfuric acid, and heat on a hotplate until white fumes of sulfur trioxide appear. Let cool. Add 5 ml of water, 10 ml of hydrofluoric acid (warning: toxic, corrosive, must not contact skin; work under fumehood) and 3 drops of sulfuric acid, then evaporate to dryness on a hotplate. Carefully heat over a flame until fumes of sulfur trioxide no longer appear. Then ignite at 900° to constant weight. The weight loss after the addition of hydrofluoric acid represents the weight of SiO_2 in the sample taken.

Aluminium oxide:

Fuse the residue obtained in the silicon dioxide determination with 2 g of potassium pyrosulfate for 5 min. Cool, dissolve the fusion in water, and dilute to 250 ml in a volumetric flask. Transfer 100 ml of the solution into a 600 ml-beaker, add 100 ml of water and 5 drops of bromotymol blue TS, and heat to as low boil. Add ammonium hydroxide, dropwise, until a blue colour appear, then boil the solution for 5 min. to expel the excess ammonia. Filter through Whatman No. 41, or equivalent filter paper, and wash the precipitate with six portions of a 1-in-50 hot ammonium chloride solution. Transfer the filter and precipitate into a tared platinum crucible, char the paper, and ignite over a Meker burner to constant weight. The weight of the residue, corrected for the ash content of the filter paper and multiplied by 2.5, represents the weight of Al_2O_3 in the original sample.

Calcium oxide:

To the combined filtrate and washings retained in the silicon dioxide determination, add, while stirring, about 30 ml of 0.05 M disodium ethylendiaminetetraacetate from a 50-ml burette. Then add 15 ml of sodium hydroxide TS and 300 g of hydroxynaphthol blue indicator, and continue the titration to a blue end point. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 2.804 mg of CaO.

Sodium oxide:

Transfer about 500 mg of the sample, previously dried at 105° for 2 h, and accurately weighed, into a tared platinum dish, and moisten with 8 to 10 drops of water. Add 25 ml of 70% perchloric acid and 10 ml of hydrofluoric acid (warning: toxic, corrosive, must not contact skin; work under fumehood) and heat on a hot plate in a hood until dense white fumes of perchloric acid appear. Add 10 ml of hydrofluoric acid (warning: toxic, corrosive, must not contact skin; work under fumehood), heat again to dense white fumes, and dissolve the residue in sufficient water to make 250 ml. Set a suitable flame photometer to a wavelength of 589 nm. Adjust the instrument to zero transmittance against water, then adjust it to 100% transmittance with a standard solution containing 200 µg of sodium, in the form of the chloride, per

ml. Read the percent transmittance of three other standard solutions containing 50, 100 and 150 µg each of sodium per ml, and plot the standard curve as % transmittance vs. concentration of sodium. Place a portion of the sample solution in the photometer, read the percent transmittance in the same manner, and by reference to the standard curve determine the concentration (C) of sodium, in T per ml in the sample solution. Calculate the quantity, in mg, of Na_2O in the sample taken by the formula:

$$250 \times C \times \frac{1.348}{1000} - F$$

where F is the quantity of sodium oxide equivalent to any sodium sulfate present in the sample, is found as follows:

Correction for sodium sulfate content:

Weigh accurately 12.5 g of the sample, previously dried at 105° for 2 h and stir it with 240 ml of water for at least 5 min. with a high speed mixer. Transfer the mixture into a 250-ml graduated cylinder, and wash the mixer container with water, adding the washings to the cylinder to make 250 ml. Stopper the cylinder, invert it several times to mix the sample, and determine the conductivity of the slurry using a suitable conductance bridge assembly. By means of a standard curve, obtained from solutions containing 50, 100, 200 and 500 mg of sodium sulfate per 100 ml, determine the concentration (C), in mg per 100 ml, of sodium sulfate in the sample slurry, and calculate the correction factor (F) by the formula:

$$F = 0.437 \times 2.5 \times C \times \frac{w}{W}$$

where w is the weight of the sample taken for the sodium oxide determination, and W is the weight of the sample taken for the preparation of the slurry.

CALCIUM ASCORBATE

Prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). A group ADI 'not specified' was established for ascorbic acid and its Ca, K and Na salts at the 25th JECFA (1981).

SYNONYMS INS No. 302

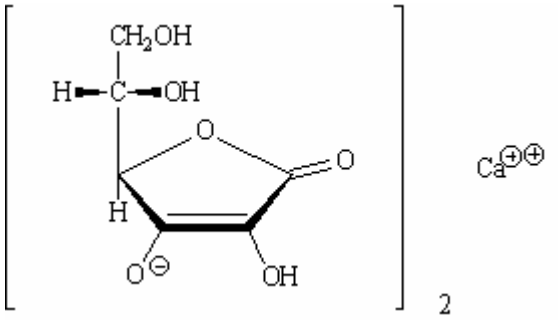
DEFINITION

Chemical names: Calcium ascorbate dihydrate, calcium salt of 2,3-didehydro-L-threo-hexono-1,4-lactone dehydrate

C.A.S. number: 5743-27-1

Chemical formula: $C_{12}H_{14}O_{12}Ca \cdot 2H_2O$

Structural formula:



Formula weight: 426.35

Assay: Not less than 98% of $C_{12}H_{14}O_{12}Ca \cdot 2H_2O$

DESCRIPTION White to slightly yellow odourless crystalline powder

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water; slightly soluble in ethanol and insoluble in ether

Test for calcium (Vol. 4): Passes test

Specific rotation (Vol. 4): [alpha] 25, D: Between +95 and +97° (5% (w/w) soln)

PURITY

pH (Vol. 4)	6.0 - 7.5 (1 in 10 soln)
Fluoride (Vol. 4)	Not more than 10 mg/kg (Method I)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh accurately about 0.4 g of the sample into a 250 ml flask and add 50 ml of carbone dioxide free water. Immediately titrate with 0.1 N iodine, adding a few drops of starch TS as indicator as the end point is approached. Each ml of 0.1 N iodine is equivalent to 10.66 mg of $C_{12}H_{14}O_{12}Ca \cdot 2H_2O$.

CALCIUM CARBONATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004) An ADI 'not limited' was established at the 9th JECFA (1965)

SYNONYMS Chalk; INS No. 170 (i)

DEFINITION

Chemical names	Calcium carbonate, carbonic acid calcium salt
C.A.S. number	471-34-1
Chemical formula	$CaCO_3$
Formula weight	100.09
Assay	Not less than 98.0% after drying

DESCRIPTION Odourless, white micro-crystalline powder

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water and ethanol
Test for carbonate (Vol. 4)	Passes test
Test for calcium (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 2% (200°, 4 h)
Acid insoluble substances	Not more than 0.2% Weigh 5 g of the sample to the nearest mg and suspend in 25 ml of water. Cautiously add with agitation 25 ml of a 1 in 2 solution of hydrochloric acid and add water to make a volume of about 200 ml. Heat the solution to boiling, cover, digest on a steam bath for 1 h, cool, and filter. Wash the precipitate with water until the last washing shows no chloride with silver nitrate TS, and then ignite it. Cool, weigh and calculate as percentage. (The weight of the residue should not exceed 10 mg).
Barium	Not more than 0.03% See description under TESTS
Fluoride (Vol. 4)	Not more than 50 mg/kg Weigh 1 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method III)

Free alkali

Not more than 0.05%
Add 3 g of the sample to 30 ml of freshly boiled and cooled water, stir for 3 min. and filter. To 20 ml of the filtrate add 2 drops of phenolphthalein TS. Though a red colour is produced, it should disappear when 0.2 ml of 0.1 N hydrochloric acid is added.

Magnesium and alkali salts

Not more than 1%
Mix 1 g of the sample with 40 ml of water, carefully add 5 ml of hydrochloric acid, mix and boiled for 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline and cool. Transfer the mixture to a 100-ml cylinder, dilute with water to 100 ml, let stand for 4 h or overnight and then decant the clear supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. Cool, weigh and calculate as percentage. (The weight of the residue should not exceed 5 mg).

Arsenic (Vol. 4)

Not more than 3mg/kg (Method II)

Lead (Vol. 4)

Not more than 3 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Barium

Weigh 1 g of the sample to the nearest mg and mix with 10 ml of water. Add 15 ml of dilute hydrochloric acid TS and dilute to 30 ml with water and filter. To 20 ml of the filtrate add 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allow to stand for 15 min. The solution should show no more turbidity than a comparison solution containing 1 mg barium/ml. To prepare the comparison solution, add 20 ml of water to 0.3 ml of barium standard solution (1.799 g barium chloride in 1 L), and then 2 g of sodium acetate TS, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS.

METHOD OF ASSAY

Weigh, to the nearest 0.1 mg, 200 mg of the dried sample. Transfer into a 400-ml beaker, add 10 ml of water and swirl to form a slurry. Cover the beaker with a watch glass and introduce 2 ml of dilute hydrochloric acid TS from a pipette inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the sample. Wash down the sides of the beaker, the outer surface of the pipette and the watch glass, and dilute to about 100 ml with water. While stirring, preferably with a magnetic

stirrer, add about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml burette, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxynaphthol blue indicator, and continue the titration to a blue end point. Each ml of 0.05 M disodium ethylenediamine- tetraacetate is equivalent to 5.004 mg of $CaCO_3$.

CALCIUM CHLORIDE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS

INS No. 509

DEFINITION

Chemical names: Calcium chloride

C.A.S. number: 10043-52-4

Chemical formula:
Anhydrous: $CaCl_2$
Dihydrate: $CaCl_2 \cdot 2H_2O$
Hexahydrate: $CaCl_2 \cdot 6H_2O$

Formula weight:
Anhydrous: 110.99
Dihydrate: 147.02
Hexahydrate: 219.08

Assay:
Anhydrous: Not less than 93%
Dihydrate: Not less than 99.0% and not more than the equivalent of 107.0% of $CaCl_2 \cdot 2H_2O$
Hexahydrate: Not less than 98.0% and not more than the equivalent of 110% of $CaCl_2 \cdot 6H_2O$

DESCRIPTION

Anhydrous: White, deliquescent lumps or porous masses
Dihydrate: White, hard, deliquescent fragments or granules
Hexahydrate: Colourless, very deliquescent crystals

FUNCTIONAL USES

Firming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4):
Anhydrous: Freely soluble in water and ethanol
Dihydrate: Freely soluble in water; soluble in ethanol
Hexahydrate: Very soluble in water and ethanol

Test for chloride (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test

PURITY

Free alkali:
Not more than 0.15% as $Ca(OH)_2$
Dissolve 1 g of the sample in 20 ml of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS. If the solution is pink, the pink colour is discharged by adding 2 ml of 0.02 N hydrochloric acid.

Magnesium and alkali salts

Not more than 5%

Dissolve 1 g of anhydrous calcium chloride, or the corresponding weight of a hydrate, in about 50 ml of water, add 500 mg of ammonium chloride, mix and boil for about 1 min. Quickly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS dropwise until the mixture is just alkaline, and cool. Transfer the mixture into a 100-ml cylinder, dilute with water to 100 ml, let stand for 4 h or overnight, and then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 25 mg.

Fluoride (Vol. 4)

Not more than 40 mg/kg (Method III)

Lead (Vol. 4)

Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 1 g of anhydrous calcium chloride, or the corresponding weight of a hydrate, transfer to a 250-ml beaker, and dissolve in a mixture of 100 ml of water and 5 ml of dilute hydrochloric acid TS. Transfer the solution to a 250-ml volumetric flask, dilute with water to volume and mix. Pipet 50 ml of the solution into a suitable container, add 100 ml of water, 15 ml of sodium hydroxide TS, 40 mg of murexide indicator (amm. purpurate) and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetra-acetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 5.55 mg of $CaCl_2$; 7.35 mg of $CaCl_2 \cdot 2H_2O$; or 10.95 mg of $CaCl_2 \cdot 6H_2O$.

CALCIUM DI-L-GLUTAMATE

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001)
A group ADI 'not specified' for glutamic acid and its Ammonium, Ca, K, Mg & Na salts, was established at the 31st JECFA (1987)

SYNONYMS Calcium glutamate, INS No. 623

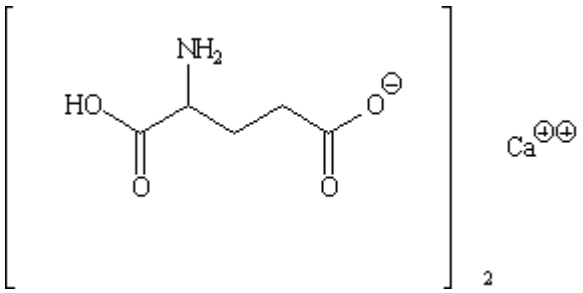
DEFINITION

Chemical names: Monocalcium di-L-glutamate

C.A.S. number: 19238-49-4

Chemical formula: $C_{10}H_{16}CaN_2O_8 \cdot xH_2O$ (x = 0, 1, 2 or 4)

Structural formula:



Formula weight: 332.32 (anhydrous)

Assay: Not less than 98.0% and not more than 102.0 % on the anhydrous basis

DESCRIPTION White, practically odourless crystals or crystalline powder

FUNCTIONAL USES Flavour enhancer, salt substitute

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water

Test for glutamate (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test

PURITY

Water (Vol. 4): Not more than 19% (Karl Fischer Method)

Specific rotation (Vol. 4): [alpha] 20, D: Between +27.4 and +29.2° (10% w/v solution in 2N hydrochloric acid)

Chlorides (Vol. 4) — Not more than 0.2%
Test 0.07 g of the sample as directed in the Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control

Pyrrolidone carboxylic acid (Vol. 4) — Passes test

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Dissolve about 250 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank. Each ml of 0.1 N perchloric acid is equivalent to 8.308 mg of $C_{10}H_{16}CaN_2O_8$. Calculate the content on the anhydrous basis.

CALCIUM GLUCONATE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and republished in FNP 52 (1992). Group ADI "not specified" for glucono-delta-lactone and gluconates, excluding ferrous gluconate, established at the 51st JECFA in 1998.

SYNONYMS INS No. 578

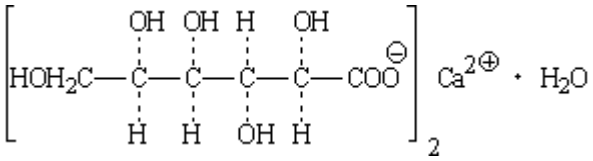
DEFINITION

Chemical names: Calcium di-D-gluconate monohydrate

C.A.S. number: 299-28-5

Chemical formula: $C_{12}H_{22}CaO_{14} \cdot H_2O$

Structural formula:



Formula weight: 448.39

Assay: Not less than 98% and not more than 102% on the dried basis

DESCRIPTION Odourless, white, crystalline granules or powder, stable in air

FUNCTIONAL USES Acidity regulator, firming agent, sequestrant, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water; insoluble in ethanol

Test for calcium (Vol. 4): Passes test

Test for gluconate (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 2.0% (105°, 16 h)

Reducing substances (Vol. 4): Not more than 1.0% calculated as D-glucose (Method I)

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified

level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.5 g of the dried sample and dissolve in 5 ml of dilute hydrochloric acid. Add 50 ml of water, 25 ml of sodium hydroxide TS and about 0.1 g of 2-hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid. Titrate with 0.05 M EDTA immediately. At the end-point, the red colour changes completely to blue. Each ml of 0.05 M EDTA is equivalent to 22.42 mg of $C_{12}H_{22}CaO_{14} \cdot H_2O$.

CALCIUM HYDROXIDE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). *An ADI 'not limited' was established at the 9th JECFA (1965)*

SYNONYMS Slaked lime; INS No. 526

DEFINITION

Chemical names	Calcium hydroxide
C.A.S. number	1305-62-0
Chemical formula	$Ca(OH)_2$
Formula weight	74.09
Assay	Not less than 92.0%

DESCRIPTION White powder

FUNCTIONAL USES Neutralizing agent, buffer, firming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Slightly soluble in water, insoluble in ethanol, soluble in glycerol.
Test for alkali	The sample is alkaline to moistened litmus paper
Test for calcium (Vol. 4)	Passes test

PURITY

Barium	Not more than 0.03% Mix 1.5 g of the sample with 10 ml of water, add 15 ml of dilute hydrochloric acid TS and dilute to 30 ml with water and filter. To 20 ml of the filtrate, add 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS, and allow to stand for 15 min. The turbidity of the solution is not greater than that of a control prepared by adding water to 0.3 ml of barium standard solution (1.779 g barium chloride in 1000 ml of water) to make to 20 ml, adding 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allowing to stand for 15 min.
Magnesium and alkali salts	Not more than 6% Dissolve 500 mg of the sample in a mixture of 30 ml of water and 10 ml of dilute hydrochloric acid TS and boil for 1 min. Quickly add 40 ml of oxalic acid TS and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS dropwise until the mixture is just alkaline and cool. Transfer the mixture to a 100-ml cylinder, dilute to volume with water, let stand for 4 h or overnight, then decant the clear,

supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a flame, and continue the heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 15 mg.

Acid insoluble ash

Not more than 1.0%
Dissolve 2 g of the sample in 30 ml of dilute hydrochloric acid (1 in 3) and heat to boiling. Filter the mixture, wash the residue with hot water and ignite. The weight of the residue does not exceed 20 mg.

Fluoride (Vol. 4)

Not more than 50 mg/kg (Method I or III)

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 1.5 g of the sample, transfer to a beaker, and gradually add 30 ml of dilute hydrochloric acid TS. When solution is complete, transfer to a 500-ml volumetric flask, rinse the beaker thoroughly, adding the rinsings to the flask, dilute to volume with water, and mix. Pipet 50 ml of the solution into a suitable container and add 50 ml of water and 15 ml of sodium hydroxide TS, 40 mg of murexide indicator (amm. purpurate) and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 3.705 mg of $Ca(OH)_2$.

CALCIUM LACTATE

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS INS No. 327

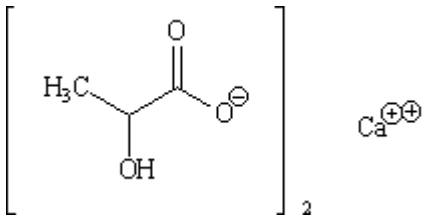
DEFINITION

Chemical names: Calcium dilactate, calcium dilactate hydrate, 2-Hydroxypropanoic acid calcium salt

C.A.S. number: 814-80-2

Chemical formula: $C_6H_{10}CaO_6 \cdot xH_2O$ (x = 0 - 5)

Structural formula:



Formula weight: 218.22 (anhydrous)

Assay: Not less than 98.0% of on the dried basis

DESCRIPTION White to cream coloured, almost odourless, crystalline powder or granules. The pentahydrate is somewhat efflorescent.

FUNCTIONAL USES Buffer, dough conditioner, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, practically insoluble in ethanol

Test for lactate (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 30% (120°, 4 h)

pH (Vol. 4): 6.0-8.0 (1 in 20 soln)

Acidity: Dissolve 1 g of the sample in 20 ml of water, add 3 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Not more than 0.5 ml should be required.

Magnesium and alkali salts	Not more than 1% mg/kg Dissolve 1 g of the sample in 40 ml of water, add 0.5 g of ammonium chloride, boil, and add about 20 ml of ammonium oxalate TS. Heat the solution on a water bath for 1 h, cool, add water to 100 ml, and filter. To 50 ml of the filtrate, add 0.5 ml of sulfuric acid, evaporate to dryness, and ignite to constant weight. The residue should not exceed 5 mg.
Fluoride (Vol. 4)	Not more than 30 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 350 mg of previously dried sample, accurately weighed, in 150 ml of water containing 2 ml of dilute hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml buret. Then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy-naphthol blue indicator, and continue the titration to a blue end-point. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 10.91 mg of $C_6H_{10}CaO_6$.

CALCIUM DL-MALATE

Prepared at the 67th JECFA (2006), published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 27th JECFA (1983) and published in FNP 52 (1992) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). Metals and arsenic specifications were revised at the 59th JECFA (2002). A group ADI 'not specified' for malic acid and its Ca, K and Na salts was established at the 23rd JECFA (1979).

SYNONYMS DL-Monocalcium malate; INS No. 352(ii)

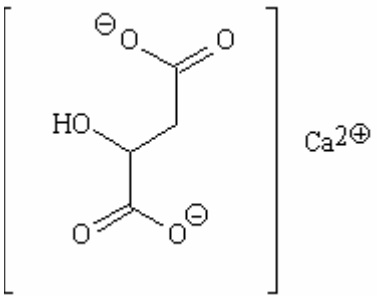
DEFINITION

Chemical names: Monocalcium DL-malate, 2-hydroxybutanedioic acid monocalcium salt

C.A.S. number: 17482-42-7

Chemical formula: $C_4H_4CaO_5$

Structural formula:



Formula weight: 172.1

Assay: Not less than 97.5% after drying

DESCRIPTION White, colourless powder

FUNCTIONAL USES Buffering agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water, insoluble in ethanol

Test for malate (Vol. 4): Passes test
Test 100 ml of a saturated solution of the sample

Test for calcium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 2% (110^{o}, 3 h)
Fluoride (Vol. 4)	Not more than 30 mg/kg (Method III)
Fumaric acid and maleic acid (Vol. 4)	Not more than 1.0% of fumaric acid and not more than 0.05% of maleic acid
Lead (Vol.4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4.

METHOD OF ASSAY Weigh accurately about 0.4 g of the sample, previously dried at 110^{o} for 3 h, dissolve in a mixture of 10 ml of water and 2 ml of dilute hydrochloric acid TS, and dilute to about 100 ml with water. While stirring (preferably with a magnetic stirrer) add about 30 ml of 0.05 M disodium ethylenediaminetetra-acetate from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxynaphtol blue indicator, and continue the titration to a blue end-point. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 8.607 mg of $C_4H_4CaO_5$.

CALCIUM OXIDE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'Not limited' was established at the 9th JECFA (1965).

SYNONYMS Lime; INS No. 529

DEFINITION

Chemical names	Calcium oxide
C.A.S. number	1305-78-8
Chemical formula	CaO
Formula weight	56.08
Assay	Not less than 95.0% after ignition

DESCRIPTION Odourless, hard, white or greyish white masses or granules, or white to greyish white powder

FUNCTIONAL USES Alkali, dough conditioner, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Slightly soluble in water, insoluble in ethanol, soluble in glycerol (Caution: Protect eyes when adding water)
Reaction with water	Moisten the sample with water; heat is generated (Caution: Protect eyes when adding water).
Test for alkali	The sample is alkaline to moistened litmus paper
Test for calcium (Vol. 4)	Passes test

PURITY

Loss on ignition (Vol. 4)	Not more than 10% (1 g, about 800^{o} to constant weight)
Barium	Not more than 0.03% Cautiously mix 1.5 g of the sample with 10 ml water, add 15 ml of dilute hydrochloric acid TS, dilute to 30 ml with water and filter. To 20 ml of the filtrate add 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allow to stand for 15 min. The turbidity of the

solution is not greater than that of a control prepared by adding water to 0.3 ml of barium standard solution (1.779 g barium chloride in 1000 ml of water) to make to 20 ml, adding 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allowing to stand for 15 min.

Magnesium and alkali salts

Not more than 3.6%
Dissolve 500 mg of the sample in 30 ml of water and 15 ml of dilute hydrochloric acid TS. Heat the solution and boil for 1 min. Add rapidly 40 ml of oxalic acid TS and stir vigorously. Add 2 drops of methyl red TS and neutralize the solution with ammonia TS to precipitate the calcium completely. Heat the mixture on a steam bath for 1 h, cool, dilute to 100 ml with water, mix well and filter. To 50 ml of the filtrate carefully add 0.5 ml of concentrated sulfuric acid, evaporate to dryness and ignite to constant weight in a tared platinum crucible.

Acid insoluble matter

Not more than 1%
Slake 5 g of the sample, mix with 100 ml of water and sufficient hydrochloric acid, added dropwise, to effect solution. Boil the solution, cool, add hydrochloric acid, if necessary, to make the solution distinctly acid, and filter through a tared crucible. Wash the residue with water until free of chlorides, dry at 105^{o} for 1 h, cool, and weigh.

Fluoride (Vol. 4)

Not more than 50 mg/kg (Method I or III)

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Ignite at approximately 800^{o} about 1 g of the sample to constant weight, accurately weigh the residue and dissolve it in 20 ml of dilute hydrochloric acid TS. Cool the solution, dilute with water to 500 ml and mix. Pipet 50 ml of this solution into a suitable container and add 50 ml of water, then add 15 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediamine tetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediamine tetraacetate is equivalent to 2.804 mg of CaO.

CALCIUM PROPIONATE

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Addendum 3 (1995). These specifications were reviewed and maintained at the 51st JECFA (1998) and republished in FNP 52 Add 6 (1998). ADI "not limited"established at the 17th JECFA in 1973.

SYNONYMS Calcium propanoate, INS No. 282

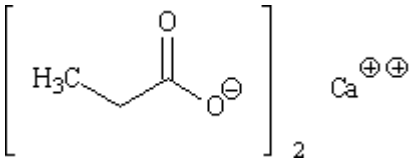
DEFINITION

Chemical names: Calcium propionate

C.A.S. number: 4075-81-4

Chemical formula: $C_6H_{10}CaO_4$

Structural formula:



Formula weight: 186.22

Assay: Not less than 98.0% on the dried basis

DESCRIPTION White crystals, powder or granules with not more than a faint odour of propionic acid

FUNCTIONAL USES Preservative, antimould and antirope agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, soluble in ethanol

Test for calcium (Vol. 4): Passes test

Test for propionate: Warm the sample with sulfuric acid. The propionic acid evolved may be recognized by its odour.

Test for alkali salt of organic acid: Ignite the sample at a relatively low temperature. The alkaline organic acid residue effervesces with acid.

PURITY

Loss on drying (Vol. 4): Not more than 4% (105^{o}, 2 h)

pH (Vol. 4): 7.5 - 10.5 (1 in 10 soln)

Water insoluble matter — Not more than 0.3%
Weigh 5 g of the sample to the nearest mg, transfer into a 100-ml beaker and add 50 ml of water. Stir until all the sample appears to be completely dissolved. Filter through a Gooch crucible, tared to an accuracy of ±0.2 mg. Rinse the beaker with 20 ml of water. Dry the crucible with its contents in a 60^{o}-oven to constant weight. Cool in a desiccator, weigh, and calculate as percentage.

Fluoride (Vol. 4) — Not more than 30 mg/kg
Weigh 5 g of the sample to the nearest mg and proceed as directed in the Limit Test (Method I or III)

Iron (Vol. 4) — Not more than 50 mg/kg
Test 0.5 g of the sample as described in the Limit Test using 2.5 ml of Iron Standard Solution (25 µg) in the control

Lead (Vol. 4) — Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve in a beaker 2.5 g of the sample, weighed to the nearest mg, in 5 ml of hot dilute hydrochloric acid TS. Cool, transfer to a 250-ml volumetric flask, dilute to volume with water, and mix. Transfer 50 ml of the solution to a 400-ml beaker, add 100 ml of water, 25 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation and 3 ml of naphthol green TS. An alternative indicator is hydroxynaphthol blue, of which 0.25 g is used. In this case the naphthol green TS is omitted. Titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 9.311 mg of $C_6H_{10}CaO_4$.

CALCIUM SILICATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' for silicon dioxide and certain silicates was established at the 29th JECFA (1985).

SYNONYMS INS No. 552

DEFINITION A synthetic hydrous calcium silicate or polysilicate prepared by a various reactions between siliceous material (e.g. diatomaceous earth) and natural calcium compounds (e.g. lime with varying proportions of other elements, such as magnesium, etc). The article of commerce may be further specified as to calcium and silicon dioxide contents, loss on drying, loss on ignition, pH of a 10% water slurry, bulk density, moisture, sulfate and chloride.

Chemical names Calcium silicate

C.A.S. number 1344-95-2

DESCRIPTION A very fine, white or off-white powder with low bulk density and high physical water absorption

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol

Test for silicate Mix about 500 mg of the sample with about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 ml of water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it to a beaker with the aid of about 50 ml of water. Add gradually hydrochloric acid until no effervescence is observed, then add 10 ml more of the acid, and evaporate the mixture on a steam bath to dryness. Cool, add 20 ml of water, boil and filter the mixture through an ash-free filter paper. An insoluble residue of silica remains. (Note. Retain the filtrate for the test for calcium). Transfer the gelatinous residue into a platinum dish, and cautiously add 5 ml of hydrofluoric acid (Warning: toxic, corrosive, must not contact skin; work under fume hood). The precipitate dissolves. (If it does not dissolve, repeat the evaporation with hydrofluoric acid.) Heat and hold in the vapours a glass stirring rod with a drop of water on the tip. The drop becomes turbid.

Test for calcium Neutralize the filtrate obtained in the Test for silicate with ammonia TS using 2 drops methyl red TS as indicator. Then add dilute hydrochloric acid TS dropwise until the solution is acid. Upon the addition of ammonium oxalate TS a white granular precipitate of calcium oxalate forms. This precipitate is

insoluble in acetic acid but dissolves in hydrochloric acid.

PURITY

Fluoride (Vol. 4)

Not more than 50 mg/kg
Weigh 1 g of the sample to the nearest mg, and proceed as directed in the Limit Test (Method I or II).

Asbestos

Absent
Electron microscope method (Tentative): Prepare a sample to be as homogeneous as possible. Examination of a specimen of the sample from a minimum of 100 fields of view using a transmission electron microscope fails to reveal any fibrous material. (Information needed on detection limit; information also requested on a simpler, more practical method).

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

CALCIUM SULFATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). ADI 'not limited' was established at the 17th JECFA (1973).

SYNONYMS INS No. 516

DEFINITION

Chemical names	Calcium sulfate
C.A.S. number	7778-18-9
Chemical formula	Anhydrous: $CaSO_4$ Dihydrate: $CaSO_4 \cdot 2H_2O$
Formula weight	Anhydrous: 136.14 Dihydrate: 172.18
Assay	Not less than 99.0% after drying

DESCRIPTION Fine, white to slightly yellow-white, odourless powder

FUNCTIONAL USES Yeast food, dough conditioner, firming agent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Slightly soluble in water; insoluble in ethanol
Test for calcium (Vol. 4)	Passes test
Test for sulfate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 1.5% (250° to constant weight) Dihydrate: Between 19 and 23% (250° to constant weight)
Fluoride (Vol. 4)	Not more than 30 mg/kg (Method I or III)
Selenium (Vol. 4)	Not more than 30 mg/kg Test 0.2 g of the sample as directed in the Limit Test Method II)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF Dissolve about 250 mg of the sample, accurately weighed, in 100 ml of water

ASSAY

and 4 ml of dilute hydrochloric acid TS, boil if necessary to effect solution, and cool. Add 15 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetra-acetate is equivalent to 6.807 mg of $CaSO_4$.

CANDELILLA WAX

Revised specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005), superseding specifications prepared at the 39th JECFA (1992) and published in FNP 52 Add 1 (1992), and incorporating the decisions on the metals and arsenic specifications agreed at the 63rd JECFA (2004) and published in FNP 52 Add 12 (2004). The 65th JECFA (2005) considered the additive to be of no toxicological concern for the functional uses listed.

SYNONYMS INS no. 902

DEFINITION Crude candelilla wax is obtained by first boiling the dried stalks of the candelilla plant (*Euphorbia antisyphilitica*) in water acidified with sulfuric acid to release the wax. The molten wax is then skimmed off and allowed to solidify and refined by further treatment with sulfuric acid and subsequent passage through filter-presses.
Candelilla wax consists primarily of odd-numbered n-alkanes (C_{29} to C_{33}), together with esters of acids and alcohols with even-numbered carbon chains (C_{28} to C_{34}). Free acids, free alcohols, sterols, neutral resins, and mineral matter are also present.

C.A.S. number 8006-44-8

DESCRIPTION Yellowish-brown hard, brittle, lustrous solid with an aromatic odour when heated

FUNCTIONAL USES Glazing agent, texturizer for chewing gum base, surface-finishing agent, carrier for food additives (including flavours and colours), clouding agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in toluene

Infrared absorption The infrared spectrum of the sample, melted and prepared for analysis on a potassium bromide plate, corresponds to that of a candelilla wax standard (see Appendix).

PURITY

Melting range (Vol. 4) 68.5^{o} - 72.5^{o}

Acid value (Vol. 4) Between 12 and 22

Saponification value (Vol. 4) Between 43 and 65

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, “Instrumental Methods”.

Infrared spectrum of Candelilla Wax

CANTHAXANTHIN

Prepared at the 51st JECFA (1998), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). Corrected at the 69th JECFA. An ADI of 0-0.03 mg/kg bw was established at the 44th JECFA (1995).

SYNONYMS CI Food Orange 8; INS No. 161 g; CI (1975) No 40850

DEFINITION Consists predominantly of trans-ß-carotene-4,4'-dione together with minor amounts of other isomers; diluted and stabilized forms are prepared from canthaxanthin meeting these specifications and include solutions or suspensions of canthaxanthin in edible fats or oils, emulsions and water dispersible powders; these preparations may have different cis/trans isomer ratios; the analytical methods described for the parent colour are not necessarily suitable for the assay of or determination of impurities in the stabilized forms (appropriate methods should be available from the manufacturer).

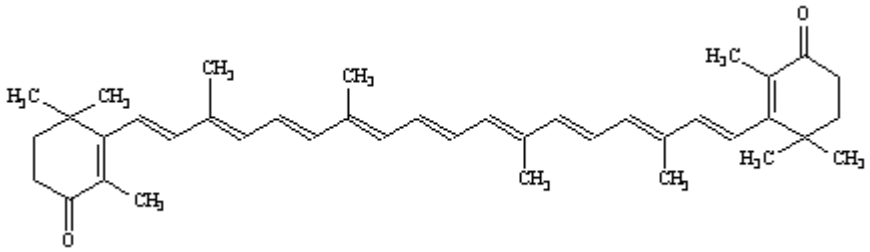
These specifications define only synthetic canthaxanthin and do not cover any commercially available food colour from natural sources.

Chemical names ß-Carotene-4,4'-dione; canthaxanthin; 4,4'-dioxo-ß-carotene

C.A.S. number 514-78-3

Chemical formula $C_{40}H_{52}O_2$

Structural formula



Formula weight 564.86

Assay Not less than 96% total colouring matters (expressed as canthaxanthin)

DESCRIPTION Deep violet crystals or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, insoluble in ethanol, practically insoluble in vegetable oils, very slightly soluble in acetone
Spectrophotometry (Vol. 4)	A solution in cyclohexane has an absorbance maximum between 468 and 472 nm
Positive test for carotenoids	The colour of a solution of canthaxanthin in acetone disappears after successive additions of a 5 % solution of sodium nitrite and 1 N sulfuric acid

PURITY

Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Subsidiary colouring matters	Carotenoids other than canthaxanthin: not more than 5% of total colouring matters See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, “Instrumental Methods”.

TESTS

PURITY TESTS

Subsidiary colouring matters

Dissolve about 80 mg of sample in 100 ml chloroform. Apply 400 µl of this solution as a streak 2 cm from the bottom of a TLC-plate (Silicagel 0.25 mm). Immediately develop the chromatogram with a solvent mixture of 95 parts dichloromethane and 5 parts diethyl ether in a saturated chamber, suitably protected from light, until the solvent front has moved 15 cm above the initial streak. Remove the plate, allow the main part of the solvent to evaporate at room temperature and mark the principal band as well as the bands corresponding to other carotenoids. Remove the silicagel adsorbent that contains the principal band, transfer it to a glass-stoppered 100 ml centrifuge tube and add 40.0 ml chloroform (solution 1).
Remove the silicagel adsorbent that contains the combined bands corresponding to the other carotenoids, transfer it to a glass-stoppered, 50 ml centrifuge tube and add 20.0 ml chloroform (solution 2).
Shake the centrifuge tubes by mechanical means for 10 min and centrifuge for 5 min. Dilute 10.0 ml of Solution 1 to 50.0 ml with chloroform (solution 3).
Determine, with a suitable spectrophotometer, the absorbances of Solutions 2 and 3 in 1-cm cells at the wavelength maximum about 485 nm, using chloroform as blank.
Calculation
Carotenoids other than canthaxantin (%) =

$$\frac{A_2 \times 100}{10\,A_1 + A_2}$$

where
A_2 = absorbance of Solution 2
A_3 = absorbance of Solution 3

METHOD OF ASSAY Proceed as directed under *Total Content by Spectrophotometry* (see Volume 4) using the following conditions:

W = 0.1 g
$V_1 = V_2 = V_3$ = 100 ml
$v_1 = v_2$ = 5 ml
$A^{1\%}_{1\ cm}$ = 2200
wavelength $_{max}$ = about 470 nm

CARAMEL COLOURS

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 55th JECFA (2000), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI for Class I 'Not specified' was established at the 29th JECFA (1985), for Class II of 0-160 mg/kg bw was established at the 55th JECFA (2000) and an ADI for Class III of 0-200 mg/kg bw (0-150 mg/kg bw on solids basis) was established at the 29th JECFA (1985) and an ADI for Class IV of 0-200 mg/kg bw (0-150 mg/kg bw on solids basis) was established at the 29th JECFA (1985).

SYNONYMS

Caramel colours are divided into four classes. The synonyms for each class are:

Class I: Plain caramel; INS No.150a
Class II: Sulfite caramel; INS No.150b
Class III: Ammonia caramel; INS No.150c
Class IV: Sulfite ammonia caramel; INS No.150d

DEFINITION

Complex mixtures of compounds, some of which are in the form of colloidal aggregates, manufactured by heating carbohydrates either alone or in the presence of food-grade acids, alkalis or salts; classified according to the reactants used in their manufacture as follows:

Class I: Prepared by heating carbohydrates with or without acids or alkalis; no ammonium or sulfite compounds are used.
Class II: Prepared by heating carbohydrates with or without acids or alkalis in the presence of sulfite compounds; no ammonium compounds are used.
Class III: Prepared by heating carbohydrates with or without acids or alkalis in the presence of ammonium compounds; no sulfite compounds are used.
Class IV: Prepared by heating carbohydrates with or without acids or alkalis in the presence of both sulfite and ammonium compounds.

In all cases the carbohydrate raw materials are commercially available food-grade nutritive sweeteners consisting of glucose, fructose and/or polymers thereof. The acids and alkalis are food-grade sulfuric or citric acids and sodium, potassium or calcium hydroxides or mixtures thereof.

Where ammonium compounds are used they are one or any of the following: ammonium hydroxide, ammonium carbonate and ammonium hydrogen carbonate, ammonium phosphate, ammonium sulfate, ammonium sulfite and ammonium hydrogen sulfite.

Where sulfite compounds are used they are one or any of the following: sulfurous acid, potassium, sodium and ammonium sulfites and hydrogen sulfites.

Food-grade anti-foaming agents may be used as processing aids during manufacture.

DESCRIPTION

Dark brown to black liquids or solids having an odour of burnt sugar

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Miscible with water

Identification of colouring matters (Vol. 4)

Passes test

Classification

Class I: Not more than 50% of the colour is bound by DEAE Cellulose and not more than 50% of the colour is bound by Phosphoryl Cellulose.
Class II: More than 50% of the colour is bound by DEAE Cellulose and it exhibits an Absorbance Ratio of more than 50.
Class III: Not more than 50% of the colour is bound by DEAE Cellulose and more than 50% of the colour is bound by Phosphoryl Cellulose.
Class IV: More than 50% of the colour is bound by DEAE Cellulose and it exhibits an Absorbance Ratio of not more than 50.
See description under TESTS

PURITY

Note: Arsenic and lead metals limits apply to all classes of caramel and are expressed on the basis of the product as is:
Other limits and ranges apply to the individual classes as indicated and, unless otherwise stated, are expressed on a solids basis.

Solid content

Class I: 62-77%
Class II: 65-72%
Class III: 53-83%
Class IV: 40-75%
See description under TESTS

Colour intensity

Class I: 0.01-0.12
Class II: 0.06-0.10
Class III: 0.08-0.36
Class IV: 0.10-0.60
See description under TESTS

Total nitrogen (Vol. 4)

Class I: max 0.1%
Class II: max 0.2%
Class III: 1.3 -6.8%
Class IV: 0.5-7.5%

Determine as directed under Nitrogen Determination (Kjeldahl Method) using Method II

Total sulfur	Class I: max 0.3% Class II: 1.3 -2.5% Class III: max 0.3% Class IV: 1.4-10.0% See description under TESTS
Sulfur dioxide	Class I: - Class II: max 0.2% Class III: - Class IV: max 0.5% See description under TESTS
Ammoniacal nitrogen	Class I: - Class II: - Class III: max 0.4% Class IV: max 2.8% See description under TESTS
4-Methylimidazole (MEI)	Class I: - Class II: - Class III: max 300 mg/kg & max 200 mg/kg on an equivalent colour basis Class IV: max 1000 mg/kg & max 250 mg/kg on an equivalent colour basis See description under TESTS
2-Acetyl-4-tetrahydroxy-butylimidazole (THI)	Class I: - Class II: - Class III: max 40 mg/kg & max 25 mg/kg on an equivalent colour basis. Class IV: - See description under TESTS
Arsenic (Vol.4)	Not more than 1 mg/kg (Method II)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, “Instrumental Methods”.

TESTS

IDENTIFICATION TESTS

Classification/Colour bound by DEAE Cellulose	For the purposes of this specification, colour bound by DEAE cellulose is defined as the percentage of decrease in absorbance of a caramel colour solution at 560 nm after treatment with DEAE Cellulose.

Special reagent
DEAE (diethylaminoethyl) Cellulose of 0.7 meq/gram capacity, e.g. Cellex D from Bio-Rad or equivalent DEAE Celluloses of higher or lower capacities in proportionately higher or lower quantities.

Procedure
Prepare a caramel colour solution of approximately 0.5 absorbance unit at 560 nm by transferring an appropriate amount of caramel colour into a 100-ml volumetric flask with the aid of 0.025 N hydrochloric acid. Dilute to volume with 0.025 N hydrochloric acid and centrifuge or filter, if solution is cloudy. Take a 20 ml aliquot of the caramel colour solution, add 200 mg of DEAE Cellulose, mix thoroughly for several min, centrifuge or filter, and collect the clear supernatant. Determine the absorbance of the caramel colour solution and the supernatant in a 1-cm cell at 560 nm, with a suitable spectrophotometer previously standardized using 0.025 N hydrochloric acid as reference. Calculate the percentage of Colour Bound by DEAE Cellulose by the formula:

$$\frac{(X_1 - X_2)}{X_1} \times 100$$

where
X_1 is the absorbance of the caramel colour solution at 560 nm; and
X_2 is the absorbance of the supernatant after DEAE Cellulose treatment at 560 nm.

Classification/Colour bound by phosphoryl cellulose

For the purposes of this specification colour bound phosphoryl cellulose is defined as the percentage of decrease in absorbance of a caramel colour solution at 560 nm after treatment with Phosphoryl Cellulose.

Special reagent
Phosphoryl Cellulose of 0.85 meq/gram capacity, e.g. Cellex P from Bio-Rad or equivalent Phosphoryl Celluloses of higher or lower capacities in proportionately higher or lower quantities.

Procedure
Transfer 200-300 mg of caramel colour into a 100-ml volumetric flask, dilute to volume with 0.025 N hydrochloric acid, and centrifuge or filter, if solution is cloudy. Take a 40 ml aliquot of the caramel colour solution, add 2.0 g Phosphoryl Cellulose and mix thoroughly for several min. Centrifuge or filter, and collect the clear supernatant. Determine the absorbance of the caramel colour solution and the supernatant in a 1-cm cell at 560 nm, with a suitable spectrophotometer previously standardized using 0.025 N hydrochloric acid as reference. Calculate the percentage of Colour Bound by Phosphoryl Cellulose by the formula:

$$\frac{(X_1 - X_2)}{X_1} \times 100$$

where
X_1 is the absorbance of the caramel colour solution at 560 nm;

and

X_2 is the absorbance of the supernatant after Phosphoryl Cellulose treatment at 560 nm.

Classification/ Absorbance ratio

For the purposes of this specification, Absorbance Ratio is defined as the absorbance of caramel colour at 280 nm divided by the absorbance of caramel colour at 560 nm.

Procedure

Transfer 100 mg of caramel colour into a 100-ml volumetric flask with the aid of water, dilute to volume, mix and centrifuge if solution is cloudy. Pipet a 5.0 ml portion of the clear solution into a 100-ml volumetric flask, dilute to volume with water, and mix. Determine the absorbance of the 0.1% solution in a 1-cm cell at 560 nm and that of the 1:20 diluted solution at 280 nm with a suitable spectrophotometer previously standardized using water as reference. (A suitable spectrophotometer is one equipped with a monochromator to provide a band width of 2 nm or less and of such quality that the stray-light characteristic is 0.5% or less.) Calculate the Absorbance Ratio of the caramel colour by dividing the absorbance units at 280 nm multiplied by 20 (dilution factor) by the absorbance units at 560 nm.

PURITY TESTS

Solids content

The solids content of Caramel Colour is determined by drying a sample upon a carrier composed of pure quartz sand that passes a No. 40 but not a No. 60 sieve and has been prepared by digestion with hydrochloric acid, washed acid-free, dried and ignited. Mix 30.0 g of prepared sand accurately weighed with 1.5-2.0 g Caramel Colour accurately weighed and dry to constant weight at 60^{o} under reduced pressure 50 mm/Hg (6.7 kPa). Record the final weight of the sand plus caramel. Calculate the % solids as follows:

$$\% \text{ solids} = \frac{(w_F - w_S)}{w_C} \times 100$$

where

w_F is the final weight of sand plus caramel;

w_S is the weight of sand; and

w_C is the weight of caramel initially added.

Calculation on a solids basis

The contents of Total Nitrogen, Total sulfur, Ammoniacal nitrogen, sulfur dioxide, 4-MEI and THI are expressed on a solids basis. The concentration (C_I) of each impurity is determined on an "as is" basis; the concentration (C_S) on a solid basis is then calculated using the formula:

$$C_S = \frac{C_i \times 100}{\% \text{ solids}}$$

Colour Intensity

For the purpose of this specification, Colour Intensity is defined as the absorbance of a 0.1% (w/v) solution of Caramel Colour solids in water in a 1 cm cell at 610 nm.

Procedure

Transfer 100 mg of Caramel Colour into a 100 ml volumetric flask, dilute to volume with water, mix and centrifuge if the solution is cloudy. Determine the absorbance (A_{610}) of the clear solution in a 1 cm cell at 610 nm with a suitable spectrophotometer previously standardized using water as a reference. Calculate the Colour Intensity of the Caramel Colour as follows:

$$\text{Colour intensity} = \frac{A_{610} \times 100}{\%\ \text{solids}}$$

Determine % solids as described under Solids content.
Calculation on an equivalent colour basis: Where additional limits for 4-MEI and THI are expressed on an equivalent colour basis the concentrations are first calculated on a solids basis as directed under "Calculations on a solids basis", and then expressed on an equivalent colour basis according to the formula:

$$\text{Equivalent colour basis} = \frac{C_S}{\text{Colour intensity}} \times 0.1$$

where

C_S is the concentration on a solids basis.

This gives content expressed in terms of a product having a Colour Intensity of 0.1 absorbance units.

Total sulfur

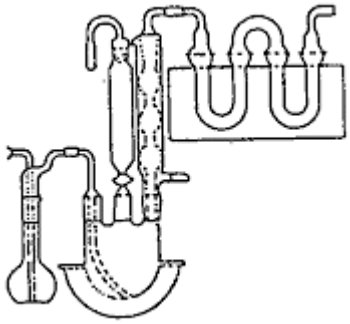
In the largest available casserole that fits in an electric muffle furnace, place 1-3 g MgO or equivalent quantity of $Mg(NO_3)_2 \cdot 6H_2O$ (6.4 - 19.2 g), 1 g powdered sucrose, and 50 ml HNO_3. Add 5-10 g caramel colour. Place same quantities of reagents in another casserole for blank. Evaporate on steam bath to paste. Place casserole in cold electric muffle (25º) and gradually heat until all NO_2 fumes are driven off. Cool, dissolve and neutralize with HCl (1+2.5), adding excess of 5 ml. Filter, heat to boiling, and add 5 ml 10% $BaCl_2 \cdot 2H_2O$ solution dropwise. Evaporate to 100 ml, let stand overnight, filter, wash, ignite, and weigh the $BaSO_4$. Correct result for $BaSO_4$ obtained in blank and report as mg S/100 g. Commercial instruments that analyse for total sulfur such as, the Leco-Combustion/Titration procedure can also be used and are recommended for sample amounts of about 200 mg.

Sulfur dioxide

Apparatus

Use a modified Monier-Williams apparatus (available from 5GA Scientific, Inc., Bloomfield, N.J., USA) for the determination of sulfurous acid, or construct the apparatus as shown in the figure. The assembly consists of a 1000-ml three-neck round-bottom distillation flask having 24/40 standard-taper ground-glass joints. A 30-cm Allihn condenser is attached in the reflux position to an outer neck of the flask, and the other end of the condenser is connected with 1/4-inch. Tygon or silicon tubing (preboiled with 1 in 20 hydrochloric acid

solution and rinsed with water) to the absorption tube assembly (having 35/20 ball joints or the equivalent). Connect the centre neck of the flask with a 125-ml cylindrical separator, and attach a piece of tubing to a short U-tube inserted through a rubber stopper in the neck of the separator. Attach a curved glass inlet tube, reaching nearly to the bottom of the flask, to the other outer neck of the flask, and connect the inlet tube to a 250-ml gas-washing bottle with a piece of the tubing. The gas-washing bottle, in turn, is connected by tubing to a nitrogen cylinder.



Grind 4.5 g of pyrogallol (pyrogallic acid) with 5 ml of water in a small mortar, and transfer the slurry to the gas-washing bottle. Grind the residue again, and transfer it quantitatively to the bottle with two 5-ml portions of water. Pass nitrogen from the cylinder to the bottle to flush out air, and then add to the bottle, through a long-stem funnel, a cooled solution of 65 g of potassium hydroxide in about 85 ml of water. Place the head of the bottle in position, and bubble nitrogen through it to remove air from the headspace. Clamp off the tubing on both sides of the bottle, and connect it to the glass inlet tube of the distillation flask. The gas-washing bottle must be prepared with fresh pyrogallol solution as described on the day of use.

To each U-tube of the absorption tube assembly add the following: two pieces of 8-mm glass rod about 25 mm in length, 10 ml of 3-mm glass beads at the exit side, 10.0 ml of 3% hydrogen peroxide solution, and 1 drop of methyl red TS.

Assemble all pieces of the apparatus, and check for leaks by blowing gently into the tubing attached to the neck of the separator. While blowing, close the stopcock of the separator. Let stand for a few min; if the liquid levels in the U-tubes equalize, reseal all connections and test again. If the system is airtight proceed as directed below.

Procedure

Disperse about 25 g of the sample, accurately weighed, in 300 ml of recently boiled and cooled water, and transfer the slurry to the flask with the aid of water, using a large-bore funnel. Dilute to about 400 ml with water, and reseal the separator. Add 90 ml of 4 N hydrochloric acid to the separator, and force the acid into the flask by blowing gently into the tube in the neck of the separator. Close the stopcock of the separator.

Unclamp the tubing on both sides of the gas-washing bottle, and start the nitrogen flow at a steady stream of bubbles. Heat the distilling flask with a heating mantle to cause refluxing in approximately 20 min. When steady refluxing is reached, apply the line voltage to the mantle and reflux for 1.75 h. Turn off the water in the condenser, and continue heating until the inlet joint of the first U-tube shows

condensation and slight warming. Remove the separator and turn off the heat.

When the joint at the top of the condenser cools, remove the connecting assembly and rinse it into the second U-tube, leaving the crossover tube attached to the exit joint of the first U-tube but disconnected from the entrance of the second U-tube. Rotate the crossover tube until the free end almost touches the entrance of the first U-tube. Add 1 drop of methyl red TS to the first U-tube, and titrate with 0.1 N sodium hydroxide just to a clear yellow colour, mixing with a gentle rocking motion. After titrating the first U-tube, remove the crossover tube, attach it to the second U-tube exit, and titrate similarly. Record the sum of the two titers as S, in ml.

Perform a blank determination, and record the volume of 0.1 N sodium hydroxide required as B. Calculate the percentage of sulfur dioxide in the sample by the formula:

$$SO_2\ \% = \frac{(S - B) \times 0.0032 \times 100}{W}$$

where

W is the weight of the sample taken, in g.

Ammonium nitrogen

Add 25 ml of 0.1 N sulfuric acid to a 500-ml receiving flask, and connect it to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser such that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask. Transfer about 2 g of caramel colour, accurately weighed, into an 800-ml long-neck Kjeldahl digestion flask, and to the flask add 2 g of magnesium oxide (carbonate-free), 200 ml of water, and several boiling chips. Swirl the digestion flask to mix the contents, and quickly connect it to the distillation apparatus. Heat the digestion flask to boiling, and collect about 100 ml of distillate in the receiving flask. Wash the tip of the delivery tube with a few ml of water, collecting the washings in the receiving flask, then add 4 or 5 drops of methyl red indicator (500 mg of methyl red in 100 ml of alcohol), and titrate with 0.1 N sodium hydroxide, recording the volume, in ml, required as S. Conduct a blank determination, and record the volume, in ml, of 0.1 N sodium hydroxide required to neutralize as B. Calculate the percentage of ammoniacal nitrogen in the sample by the formula:

$$\text{Ammoniacal nitrogen} = \frac{(B - S) \times 0.0014 \times 100}{W}$$

where

W is the weight of caramel colour taken, in g.

4-Methylimidazole

NB: Information on an improved method is sought.
The following materials and reagents are required (the reagents should be ACS grade or equivalent where applicable).

Materials

Pyrex glasswool, 22 x 300 mm chromatography column with PTFE stopcock (e.g. Kimax 17800); 150 ml polypropylene beaker (e.g.

Nalge 1201); 250 ml round-bottom flask (e.g., Pyrex 4320); 75 mm powder funnel; 5 cm spatula; rotary vacuum evaporator; hot plate; pan for water bath; disposable Pasteur pipets; 5 ml volumetric flask.

Reagents
Acetone; Celite 545; methylene chloride; sodium hydroxide; and tetrahydrofuran.

Procedure
After thoroughly mixing the caramel colour sample by shaking or stirring, weigh a 10.00 g aliquot into a 150 ml polypropylene beaker. Polypropylene is considered superior to glass because of its hydrophobic surface which facilitates quantitative sample transfer. A 5.0 g portion of 3.0 N NaOH is added and thoroughly mixed to ensure that the pH of the entire sample exceeds 12. A 20 g portion of Celite 545 is added to the beaker, and the contents are mixed until a semi-dry mixture is obtained. This normally requires approximately 2 to 3 min. With samples of unusually high water content, the resultant caramel colour-Celite 545 mixture may be overly wet. In such cases, a 5.00 g aliquot of caramel colour may be mixed with 2.5 g of 3.0 N NaOH and 15 g of Celite 545 and carried through the remainder of the analysis.

A plug of Pyrex glasswool is placed in the bottom of a 22 x 300 mm chromatographic column with PTFE stopcock. The caramel colour-Celite 545 mixture is placed in the column with the aid of a 75 mm powder funnel. The column contents are settled by repeatedly allowing the column to fall vertically about 10 cm to a padded surface. When properly settled, the caramel colour-Celite 545 mixture should occupy approximately the lower 250 mm of the column. Care should be exercised at this point to avoid a column bed which is either too loosely or too tightly packed. Loose packing will allow too rapid elution of the methylene chloride and possibly incomplete extraction. A too tightly packed column, e.g., the result of tamping down the column contents, can result in regions of the bed which are relatively inaccessible to the extraction solvent. This can also result in incomplete extraction.

With the stopcock open, the column is filled with methylene chloride poured from the sample beaker. When the solvent reaches the glasswool plug, the stopcock is closed and the solvent is allowed to stand in contact with the bed for 5 min. The stopcock is then opened and the column is further eluted with methylene chloride until 200 ml have been collected in a 250 ml round-bottom flask. A 1.00 ml aliquot of 2 MEI internal standard solution (50.0 mg of 2 MEI/50.0 ml of methylene chloride) is added to the collected eluate. The 2 MEI is well separated from the 4 MEI under the GLC conditions employed and has not been found in caramel colour.

The bulk of the solvent is then removed from the eluate on a rotary vacuum evaporator operated at 45-50 kPa and with the round-bottom flask maintained at 35° in a water bath. The extracted residue is transferred quantitatively to a 5 ml volumetric flask with a disposable Pasteur pipet, by rinsing the round-bottom flask several times with small (ca. 0.75 ml) portions of either tetrahydrofuran or acetone. Both solvents have been used with equal success. After mixing the contents thoroughly by several inversions of the flask, the extract is

ready for GLC analysis. The extracts should be analysed as soon as possible after their preparation, because stability problems have occasionally been encountered with extracts more than 1 day old. The GLC analysis is carried out using a gas chromatograph equipped with a hydrogen flame detector. The column is glass, 1 mm x 6 mm o.d. x 4 mm i.d., filled with 7.5% Carbowax 20M + 2% K0H on 90/100 mesh Anakrom ABS. The GLC parameters are as follows: carrier, nitrogen, 50 ml/min; hydrogen, 50 ml/min; oxygen, 80 ml/min; injection port, 200°; column isothermal, 180°; detector, 250°; sample size, 5 µl. All quantitation is done by using the internal standard technique.

2-Acetyl-4-tetrahydroxy-butylimidazole (THI)

NB: Information on an improved method is sought.
THI is converted into its 2,4-dinitrophenylhydrazone (THI-DNPH). This derivative is separated from excess reagent and carbonylic contaminants by HPLC on RP-8, then determined by its absorbance at 385 nm.

Procedure
Caramel colour (200-250 mg) is weighed accurately, then dissolved in water (3 ml). The solution is transferred quantitatively to the upper part of a Combination Column. Elution with water is started, and a total of about 100 ml of water is passed through the columns.

The upper column is then disconnected. The lower column is eluted with 0.5 N HCl. The first 10.0 ml of eluate are discarded, then a volume of 35 ml is collected.

The solution is concentrated to dryness at 40^{o} and 15 torr. The syrup residue is dissolved in carbonyl-free methanol (250 µl) and the 2,4-dinitrophenylhydrazine reagent (250 µl) is added. The reaction mixture is transferred to a septum-capped vial and stored for 5 h at room temperature.
A volume of 5 µl (but also from 1 to 25 µl) is injected onto a LiChrosorb RP-8 (10 µm) HPLC column. The mobile phase is MeOH/0.1 M H_3PO_4 50/50 (v/v). Adjustments in mobile phase composition may be needed as column characteristics vary, depending upon the manufacturer. (Use of LiChrosorb RP-8, 10 µm, 250 x 4 mm "Vertex" column manufactured by Knauer, Bad Homburg, F.R.G. is strongly recommended). At a mobile phase flow rate of 2 ml/min and column dimensions of 250 x 4.6 mm, THI-DNPH is eluted at about 6.3±0.1 min. It is detected at 385 nm and the peak height is measured. The amount is calculated from a calibration curve prepared with THI-DNPH in methanol.

Materials
- 2,4,-Dinitrophenylhydrazine hydrochloride reagent: Commercial 2,4-dinitrophenylhydrazine (5 g) is added to concentrated hydrochloric acid (10 ml) in a 100-ml Erlenmeyer flask, and the latter is gently shaken until the free base (red) is converted to the hydrochloride (yellow). Ethanol (100 ml) is added and the mixture is heated on a steam bath until all the solid has dissolved. After crystallization at room temperature, the hydrochloride is filtered off, washed with ether, dried at room temperature and stored in a desiccator. On storage the hydrochloride is slowly converted to the free base. The latter can be removed by washing with dimethoxyethane. The reagent is prepared

by mixing 0.5 g of 2,4-dinitrophenylhydrazine hydrochloride in 15 ml of 5% methanol in dimethoxyethane for 30 min. It should be stored in the refrigerator and be checked periodically by HPLC.
- Cation-exchange resin (strong): Dowex 50 AG x 8, H^+, 100-200 mesh.
- Cation-exchange resin (weak): Amberlite CG AG 50 I, H^+, (100-200 mesh). (Sediment two or three times prior to use).
- Methanol, carbonyl-free: Methanol is prepared after Y. Peleg and C.H. Mannheim, J. Agr. Fd. Chem, 18 (1970) 176, by treatment with Girard P reagent.
- Dimethoxyethane: If impure, dimethoxyethane is purified by distillation from 2,4-dinitrophenylhydrazine in the presence of acid and redistilled from sodium hydroxide. Immediately prior to use it is passed through a column of neutral aluminium to remove peroxides.

Instrumental

Combination Columns: Similar to the set-up described in J. Agr. Fd. Chem. 22 (1974) 110. The upper column (150 x 12.5 mm, filling height max. 9 cm, or 200 x 10 mm, filling height max. 14 cm, with capillary outlet of 1 mm i.d.) is filled with weakly acidic cation-exchanger; bed height, approx. 50-60, or 80-90 mm, respectively. The lower column (total length 175 mm, i.d. 10 mm, with capillary outlet and Teflon stopcock) is filled with strongly acidic cation-exchanger to a bed-height of 60 mm. As a solvent reservoir, a dropping funnel (100 ml) with Teflon stopcock is used. All parts are connected by standard ground-glass joints (14.5 mm).

HPLC: With the column specified above and an ultraviolet detector capable of reading at 385 nm.

Calibration: THI-DNPH is dissolved in absolute, carbonyl-free methanol (about 100 mg/l; calculated concentration of THI: 47.58 ng/µl). A portion of this solution is diluted tenfold with methanol (4.7 ng THI/µl). THI-DNPH standard solutions are stable for at least twenty weeks when stored in the refrigerator.

CARBON DIOXIDE

Prepared at the 49th JECFA (1997) , published in FNP 52 Add5 (1997) superseding specifications prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992) An ADI 'not specified' was established at the 29th JECFA (1985)

SYNONYMS INS No. 290

DEFINITION

C.A.S. number 124-38-9

Chemical formula CO_2

Formula weight 44.01

Assay Not less than 99% of CO_2, by volume

DESCRIPTION Colourless, odourless gas, 1 litre of which weighs about 1.98 g at 0^o and 760 mm of mercury. Under a pressure of about 59 atmospheres it may be condensed to a liquid, a portion of which forms a white solid (Dry Ice) upon rapid evaporation. Solid carbon dioxide evaporates without melting upon exposure to air. Commercial carbon dioxide is shipped and handled in pressurized cylinders or low pressure bulk liquid systems, or in solid blocks.

FUNCTIONAL USES Propellant, freezing agent, carbonating agent, preservative, extraction solvent

CHARACTERISTICS The following specifications apply to gaseous carbon dioxide as produced from its condensed liquid or solid phase by evolution to the gas phase at normal environmental conditions. Additional specifications may be applied to liquid or solid forms of carbon dioxide by vendors or by specific users of commercial carbon dioxide products.

IDENTIFICATION

Precipitate formation When a stream of the sample is passed through a solution of barium hydroxide, a white precipitate is produced which dissolves with effervescence in dilute acetic acid.

Detector tube test Passes test
See description under TEST

PURITY

Acidity Transfer 50 ml of water, previously boiled and cooled to room temperature, into a Nessler tube. Introduce 1,000 ml of the sample into the water through a tube (1 mm internal diameter) keeping the opening of the tube within 2 mm from the bottom of the vessel. Add 0.1 ml of methyl orange TS. The red colour produced is not darker than the colour of an identical control solution to which has been added 1.0 ml of 0.01 N hydrochloric

acid instead of the carbon dioxide.

Phosphine, hydrogen sulfide, and other organic reducing substances	Transfer 25 ml of silver ammonium nitrate TS and 3 ml of ammonia TS into a Nessler tube. In the absence of light, introduce 1,000 ml of the sample in the same manner as in the test of Acidity. No brown colour is produced.
Carbon monoxide	Not more than 10 µl/l See description under TESTS
Non-volatile hydrocarbons	Not more than 10 mg/kg See description under TESTS
Volatile hydrocarbons	Not more than 50 µl/l See description under TESTS
Water	Passes test See description under TESTS

TESTS

IDENTIFICATION TESTS

Detector tube test

Pass 100±5 ml, released from the vapour phase of the contents of the container, through a carbon dioxide detector tube (see below) at the rate specified for the tube: The indicator change extends throughout the entire indicating range of the tube.

Detector tube is a fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable adsorbing filters and support media for the indicators hydrazine and crystal violet. (The Draeger Reference Number is CH 30801, National Draeger Inc., P.O. Box 120, Pittsburgh, PA 15205-0120, USA; the measuring range is 0.01% to 0.30%).

PURITY TESTS

Carbon monoxide

Principle

Carry out the test on the first portion of gas issuing from the cylinder. Use 5.0 l of the sample mixed with an equal volume of carbon monoxide-free nitrogen and 10 l of carbon monoxide-free nitrogen as the control. The difference between the volumes of 0.002 N sodium thiosulfate used in the two titrations is not greater than 0.5 ml.

Apparatus

The apparatus consists of the following parts connected in series:
-U-tube containing anhydrous silica gel impregnated with chromium trioxide.
-Scrubber bottle (dreschel type) containing 100 ml of a 40% w/v solution of potassium hydroxide.
-U-tube containing phosphorus pentoxide dispersed on previously granulated, fused pumice.
-Tube containing recrystallized iodic anhydride (I_2O_5) in granules, previously dried at 200^{o} and kept at a temperature of 120^{o}. The iodic

anhydride is packed in the tube in 1-cm columns separated by 1-cm columns of glass wool to give an effective length of 5 cm.
-Flask containing 2.0 ml of potassium iodide TS and 3 drops of starch solution TS.

Procedure

Flush the apparatus with 5.0 l of carbon dioxide-free air and, if necessary, discharge the blue colour in the iodide solution by adding the smallest necessary quantity of freshly prepared 0.002 N sodium thiosulfate. Continue flushing until not more than 0.045 ml of 0.002 N sodium thiosulfate is required after passing 5.0 l of carbon dioxide-free air. Pass the gas from the cylinder through the apparatus.

Flush the last traces of liberated iodine into the reaction flask by passing through the apparatus 1.0 l of carbon monoxide-free air. Titrate the liberated iodine with 0.002 N sodium thiosulfate. Carry out a blank assay using 10 l of carbon monoxide-free nitrogen. The difference between the volumes of 0.002 N sodium thiosulfate solution used in the two titrations should not be more than 0.5 ml.

Non-volatile hydrocarbons

Pass a sample of liquid carbon dioxide from storage container or sample cylinder through a commercial carbon dioxide snow horn directly into an open, clean container. Weigh 500 g of this sample into a clean beaker. Allow the carbon dioxide solid to sublime completely, with a watch-glass placed over the beaker to prevent ambient contamination. Wash the beaker with a residue-free solvent, and transfer the solvent from the beaker to a clean, tared watch-glass or petri dish with two additional rinses of the beaker with the solvent. Allow the solvent to evaporate, using heating to 104^{o} until the watch-glass or petri dish is at a constant weight. Determine the weight of the residue by difference. The weight of the residue does not exceed 5 mg.

Volatile hydrocarbons

Standard preparation

Flush a 500-ml glass, septum-equipped sampling bulb with helium, and inject into the bulb a 5.00-ml sample of methane. Allow the bulb to stand for 15 min to permit the gases to mix, and then inject 2.50 ml of the mixture into a second 500-ml sampling bulb, also flushed with helium, and allow this tube to stand for 15 min to permit the gases to mix. This mixture is a nominal 50 ppm v/v standard. Determine the exact concentration from the exact volumes of the gas-sampling bulbs. To determine these volumes, weigh the empty tubes, fill them with water, and reweigh. From the weight of the water and its temperature, calculate the volumes of the tubes.

Chromatographic system

The gas chromatograph is equipped with the flame ionization detector and a 1.8-m x 3-mm o.d. metal column packed with 80- to 100-mesh Hayesep Q (or equivalent). The carrier gas is helium at a flow rate of 30 ml/min. The injector temperature and the detector temperatures both are maintained at 230^{o}. The column temperature is programmed according to the following steps: It is held at 70^{o} for 1 min, then increased to 200^{o} at a rate 20^{o}/min, and then held at 200^{o} for 10 min. The parameters for the detector are sensitivity range: 10^{-12} A/mV; attenuation: 32. The concentration of volatile

hydrocarbons is reported in methane equivalents. The various gas chromatographic responses, excluding the carbon dioxide response, are summed to yield the total volatile hydrocarbon concentration. The composition of hydrocarbons present will vary from sample to sample. Typical retention times are methane: 0.4 min; carbon dioxide: 0.8 min; hexane: 14.4 min.

Procedure

Inject in triplicate 1.00 ml of the standard preparation into the gas chromatograph, and average the peak area responses. The relative standard deviation should not exceed 5.0%. Similarly, inject in triplicate 1.00 ml of sample, sum the average peak areas of the individual peaks, except for the carbon dioxide peaks, and calculate the concentration v/v in the sample by formula:

$\mu l/L = S(A_U/A_S)$,

where
S is the calculated ppm of methane in the standard preparation (approximately 50 µl/l)
A_U is the sum of the averages of the individual peak area responses in the sample
A_S is the average area of the standard preparation area responses

Water

Water vapour detector tube. A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for the indicator, which consists of a selenium sol in suspension of sulphuric acid. (The Draeger Reference Number is CH 67 28531, National Draeger Inc., P.O. Box 120, Pittsburgh, PA 15205-0120, USA; the measuring range is 5 to 200 mg/m^3).

Pass 24,000 ml of the gas sample through a suitable water-absorption tube not less than 100 mm in length, which previously has been flushed with about 500 ml of the sample and weighed. Regulate the flow so that about 60 min will be required for passage of the gas. The gain in weight of the absorption tube does not exceed 1.0 mg.

METHOD OF ASSAY

Transfer a 1 in 3 potassium hydroxide solution into a gas pipette of adequate volume. Measure accurately about 1,000 ml of the sample into a gas burette containing a 1 in 10 sodium chloride solution. Transfer the sample into the gas pipette and shake well. When the volume of gas remaining unabsorbed is constant (V ml), the content of carbon dioxide is calculated by:

$$\text{Content v / v\%} = \frac{\text{Vol of sample ml} - \text{V ml}}{\text{Vol of sample ml}} \times 100$$

CARMINES

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding specifications prepared at the 44th JECFA (1995) and published in FNP 52 Add 3 (1995). Metals and arsenic specifications revised at the 59th JECFA (2002). A group ADI of 0-5 mg/kg bw for carmines, as ammonium carmine or the equivalent of Ca, K and Na salts was established at the 26th JECFA (1982) and maintained at the 55th JECFA (2000).

SYNONYMS Cochineal carmine, Carmine, CI Natural Red 4, CI (1975) No. 75470; INS No. 120

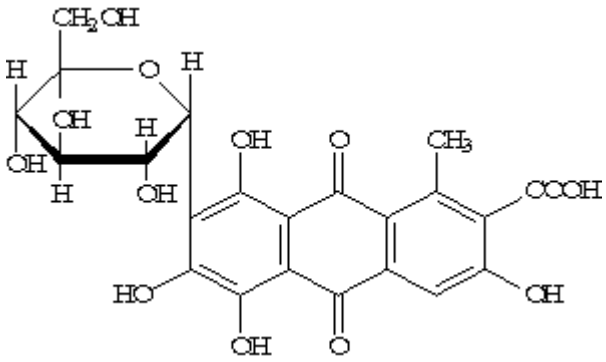
DEFINITION Obtained by aqueous extraction of cochineal, which consists of the dried bodies of the female insect *Dactylopius coccus* Costa; the colouring principle is a hydrated aluminium chelate of carminic acid in which aluminium and carminic acid are thought to be present in the molar ratio 1:2.
In commercial products the colouring principle is present in association with ammonium, calcium, potassium or sodium cations, singly or in combination, and these cations may also be present in excess. Products may also contain proteinaceous material derived from the source insect, and may also contain free carminate or a small excess of aluminium cations.

Chemical names Hydrated aluminium chelate of carminic acid (7-beta-D-glucopyranosyl-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-anthracene-2-carboxylic acid)

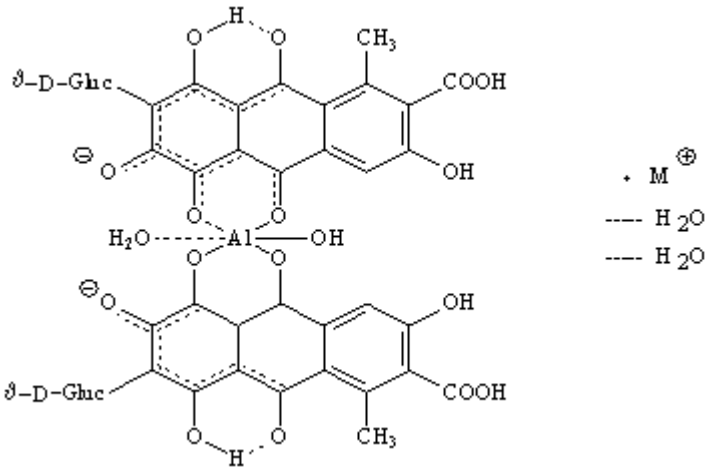
C.A.S. number 1390-65-4 (carmine)
1260-17-9 (carminic acid)

Chemical formula Carminic acid: $C_{22}H_{20}O_{13}$

Structural formula Carminic acid:



Possible structural formula for the aluminium complex of carminic acid:



M^+: cation ½ Ca^{++}, Na^+, K^+, NH_4^+

Formula weight — Carminic acid: 492.39

Assay — Not less than 50% of $C_{22}H_{20}O_{13}$ on the dry basis

DESCRIPTION

Red to dark red, crumbly solid or powder

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — The solubility of carmine preparations varies depending on the nature of the cations present. Products where the major cation is ammonium (ammonium carminate) are freely soluble in water at pH 3.0 and pH 8.5. Products where the major cation is calcium (calcium carminate) are very slightly soluble in water at pH 3.0 but freely soluble at pH 8.5.

Colour reactions — Make a solution of the sample slightly alkaline by adding 1 drop of 10% sodium hydroxide or potassium hydroxide solution. A violet colour is produced.

Add a small sodium dithionite ($Na_2S_2O_4$) crystal to acid, neutral or alkaline solutions of the sample. The solutions are not decolourized.

Dry a small quantity of the sample in a porcelain dish. Cool thoroughly and treat the dry residue with 1 or 2 drops of cold sulfuric acid TS. No colour change occurs.

Acidify a dispersion of the sample in water with 1/3 volume of hydrochloric acid TS and shake it with amyl alcohol. Wash the amyl alcohol solution 2-4 times with an equal volume of water to remove hydrochloric acid. Dilute the amyl alcohol solution with 1-2 volumes of petroleum ether (40-60^o) and

shake with a few small portions of water to remove colour. Add, dropwise, 5% uranium acetate, shaking thoroughly after each addition. A characteristic emerald-green colour is produced.

PURITY

Loss on drying (Vol. 4)	Not more than 20% (135 °, 3h)
Total ash (Vol. 4))	Not more than 12% Test 1 g of the sample as directed in the test for Ash (Total Ash)
Protein (Vol. 4)	Not more than 25% Proceed as directed under Nitrogen Determination (non-ammonia N x 6.25)
Matter insoluble in dilute ammonia (Vol. 4)	Not more than 1% Dissolve about 0.25 g of the sample, previously dried and accurately weighed, in 2.5 ml of dilute ammonia solution (160 ml of strong ammonia TS, made up to 500 ml) and dilute to 100 ml with water: the solution is clear. Filter through a sintered glass filter (British Standard Grade No. 3). Wash with a 0.1% ammonia solution and dry to constant weight at 105º.
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."
Microbiological criteria (Vol. 4)	*Salmonella*: Negative per test

METHOD OF ASSAY Weigh accurately about 100 mg of the sample, dissolve in 30 ml of boiling 2N hydrochloric acid and cool. Transfer quantitatively to a 1000-ml volumetric flask, dilute to volume with water, and mix. Determine the absorbance of the solution in a 1 cm cell at the wavelength of maximum absorbance (about 494 nm) using water as the blank. Calculate the percentage of carminic acid in the sample analysis using the formula:

$$\frac{100 \times A \times 100}{1.39 \times W}$$

where
A = absorbance of the sample solution;
W = weight, in mg, of the sample taken; and
1.39 = absorbance of a solution of carminic acid having a concentration of 100 mg per 1000 ml
If the measured absorbance of the solution is not within the range 0.650 to 0.750, prepare another sample and adjust the weight accordingly.

CARNAUBA WAX

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). ADI 0-7 mg/kg bw, established at the 39th JECFA in 1992.

SYNONYMS INS No. 903

DEFINITION The refined wax obtained from the fronds of the Brazilian tropical palm tree Copernicia cerifera (Arruda) Mart. [syn. C. purnifera (Muell.)]; a complex mixture of several chemical compounds, predominantly esters, e.g.,

- aliphatic esters (straight-chain acids with even-numbered carbon chains from C_{24} to C_{28} and straight-chain alcohols with even-numbered carbon chains from C_{30} to C_{34}),
- alpha-hydroxy esters (straight-chain hydroxy acids with even-numbered carbon chains from C_{22} to C_{28}, straight-chain acids with even-numbered carbon chains from C_{24} to C_{28}, straight-chain monohydric alcohols with even-numbered carbon chains from C_{24} to C_{34} and dihydric alcohols with even-numbered carbon chains from C_{24} to C_{34})
- cinnamic aliphatic diesters (p-methoxycinnamic acid and dihydric alcohols with even-numbered carbon chains from C_{24} to C_{34})

It also contains free acids (straight-chain acids with even-numbered carbon chains from C_{24} to C_{28}), free alcohols (straight-chain alcohols with even-numbered carbon chains from C_{30} to C_{34}), hydrocarbons (straight-chain odd-numbered carbon chains from C_{27} to C_{31}) and resins.

C.A.S. number 8015-86-9

DESCRIPTION A pale yellow to light brown, hard and brittle solid, having a clean fracture

FUNCTIONAL USES Glazing agent, bulking agent, acidity regulator, carrier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; partially soluble in boiling ethanol; soluble in ether

Melting range (Vol. 4) 80 - 86º

PURITY

Acid value (Vol. 4) Between 2 and 7

Saponification value (Vol. 4) Between 78 and 95

Ester value Between 71 and 93
Subtract the Acid value from the Saponification value to obtain the Ester value.

Sulfated ash (Vol. 4)

Not more than 0.25 % w/w
Heat a 2-g sample in a tared, open porcelain or platinum dish over an open flame. It volatilizes without emitting an acrid odour. Ignite as described in procedure for Ash (sulfated ash) Method I.

Unsaponifiable matter

Between 50 % and 55 %
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg
Prepare a sample solution as directed for organic compounds in the Limit Test and determine by *atomic absorption spectroscopy*, Volume 4

TESTS

PURITY TESTS

Unsaponifiable matter

Weigh accurately about 5 g of the sample into a 250-ml flask, add a solution of 2 g of potassium hydroxide in 40 ml ethanol, and boil gently under reflux for 1 h or until saponification is complete. Transfer the content of the flask to a glass-stoppered extraction cylinder (approximately 30 cm in length, 3.5 cm in diameter and graduated at 40, 80 and 130 ml).Wash the flask with sufficient alcohol to achieve a volume of 40 ml in the cylinder, and complete the transfer with warm and then cold water until the total volume is 80 ml. Finally wash the flask with a few ml of petroleum ether, add the washings to the cylinder, cool the contents of the cylinder to room temperature and add 50 ml of petroleum ether. Insert the stopper and shake the cylinder vigorously for at least 1 min, and allow both layers to become clear. Siphon the upper ether layer as completely as possible without removing any of the lower layer, collecting the ether fraction in a 500-ml separator. Repeat extraction and siphoning at least six times with 50-ml portions of petroleum ether, shaking vigorously each time. Wash the combined extracts, with vigorous shaking, with 25-ml portions of 10% ethanol until the wash water is neutral to phenolphthalein, and discard the washings. Transfer the ether extract to a tared beaker and rinse the separator with 10-ml of ether, adding the rinsings to the beaker. Evaporate the ether at a steam bath just to dryness, and dry the residue to constant weight, preferably at 75º to 80º under vacuum of not more than 200 mm of Hg, or at 100º for 30 min. Cool in a desiccator and weigh to obtain weight of unsaponifiable matter.

Dissolve the residue in 50 ml of warm neutral ethanol and titrate with 0.02N sodium hydroxide using phenolphthalein as indicator. Each ml of 0.02N sodium hydroxide is equivalent to 5.659 mg of fatty acids, calculated as oleic acid.

Subtract the calculated weight of fatty acids from the weight of the residue to obtain the corrected weight of unsaponifiable matter in the sample.

CAROB BEAN GUM

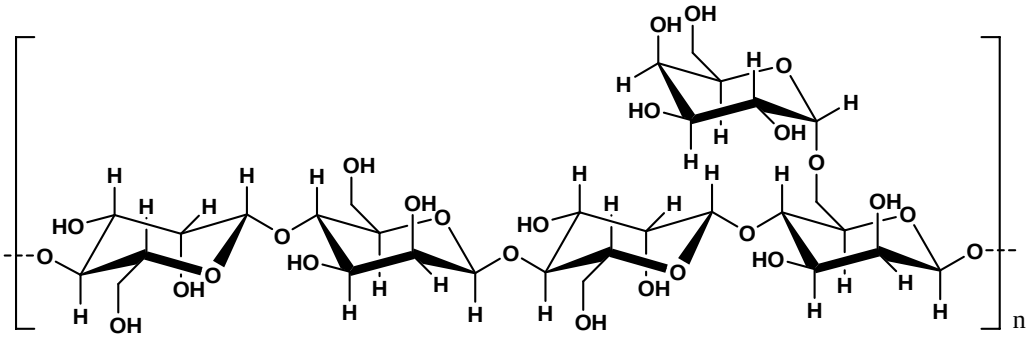
Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding tentative specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not specified" was established at the 25th JECFA (1981).

SYNONYMS Locust bean gum, INS No. 410

DEFINITION Primarily the ground endosperm of the seeds from *Ceratonia siliqua* (L.) Taub. (Fam. *Leguminosae*) mainly consisting of high molecular weight (approximately 50,000-3,000,000) polysaccharides composed of galactomannans; the mannose:galactose ratio is about 4:1. The seeds are dehusked by treating the kernels with dilute sulfuric acid or with thermal mechanical treatments, elimination of the germ followed by milling and screening of the endosperm to obtain native carob bean gum. The gum may be washed with ethanol or isopropanol to control the microbiological load (washed carob bean gum).

C.A.S. number 9000-40-2

Structural formula



DESCRIPTION White to yellowish white, nearly odourless powder

FUNCTIONAL USES Thickener, stabilizer, emulsifier, gelling agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol

Gel formation Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.

Viscosity Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add 200 ml of water with vigorous stirring until the

	gum is completely and uniformly dispersed. An opalescent, slightly viscous solution is formed. Transfer 100 ml of this solution into another 400-ml beaker. Heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is an appreciable increase in viscosity (differentiating carob bean gums from guar gums).
Gum constituents (Vol. 4)	Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 to 10 µl of the hydrolysate instead of 1 to 5 µl. Use galactose and mannose as reference standards. These constituents should be present.
Microscopic examination	Disperse a sample of the gum in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under a microscope. Carob bean gum contains long stretched tubiform cells, separated or slightly interspaced. Their brown contents are much less regularly formed than in Guar gum.
PURITY	
Loss on drying (Vol. 4)	Not more than 14% (105º, 5 h)
Total ash (Vol. 4)	Not more than 1.2% (800º, 3-4 h)
Acid-insoluble matter (Vol. 4)	Not more than 4.0%
Protein (Vol. 4)	Not more than 7.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method) in Volume 4 (under “General Methods, Inorganic components”). The percentage of nitrogen determined multiplied by 6.25 gives the percentage of protein in the sample.
Starch	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
Residual solvents	Not more than 1% of ethanol or isopropanol, singly or in combination See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenizing the mixture in a high-speed blender. Total (aerobic) plate count: Not more than 5,000 CFU/g *E. coli:* Negative in 1g

Salmonella: Negative in 25 g
Yeasts and moulds: Not more than 500 CFU/g

TESTS

PURITY TESTS

Residual solvents

Determine by gas chromatography in Volume 4 (under “Analytical Techniques, Chromatography”).

Chromatography conditions
Column: 25% Diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm i.d., 0.25 μm film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injector: 280°
- column: Hold for 6 min at 40°, then 40-110° at 4°/min, 110-250° at 25°/min, hold for 10 min at 250°
- detector: 250°

Standard solutions
Solvent standard solution: Transfer 100 mg each of chromatography grade ethanol and isopropanol into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
TBA standard solution: Transfer 100 mg of chromatography grade tertiary-butyl alcohol (TBA) into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
Mixed standard solutions: Transfer 1, 2, 3, 4 and 5 ml of Solvent standard solution into each of five 100-ml volumetric flasks. Add 4 ml of TBA standard solution to each flask and dilute to volume with water.

Sample preparation
Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 4 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distil about 95 ml, adjusting the heat so that foam does not enter the column. Add 4 ml of TBA standard solution to the distillate and make up to 100 ml with water to obtain the Sample solution.

Standard curves
Inject 1 μl of each Mixed standard solution into the chromatograph. Measure the peak areas for each solvent and TBA. Construct the standard curves by plotting the ratios of the peak areas of each of the solvents/TBA against the concentrations of each solvent (mg/ml) in the Mixed standard solutions.

Procedure
Inject 1 μl of the Sample solution into the chromatograph. Measure the peak areas for each solvent and TBA. Calculate the ratios of the peak areas of each solvent/TBA, and obtain the concentration of each solvent from the standard

curves.

Calculate the percentage of each solvent from:

$$\% \text{ Solvent} = (C \times 100/W \times 1000) \times 100$$

where C is the concentration of solvent (mg/ml)
W is weight of sample (g)

CAROB BEAN GUM (CLARIFIED)

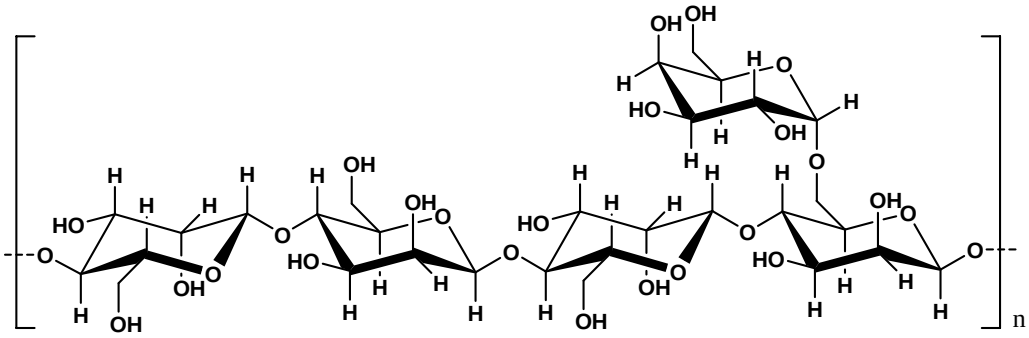
Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding tentative specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not specified" was established at the 25th JECFA (1981) for carob bean gum.

SYNONYMS Locust bean gum clarified, INS No. 410

DEFINITION Primarily the ground endosperm of the seeds from *Ceratonia siliqua* (L.) Taub. (Fam. *Leguminosae*) mainly consisting of high molecular weight (approximately 50,000-3,000,000) polysaccharides composed of galactomannans; the mannose:galactose ratio is about 4:1. The seeds are dehusked by treating the kernels with dilute sulfuric acid or with thermal mechanical treatments, elimination of the germ, followed by milling and screening of the endosperm to obtain native carob bean gum. The gum is clarified by dispersing in hot water, filtration and precipitation with ethanol or isopropanol, filtering, drying and milling. The clarified carob bean gum does not contain cell wall materials. Clarified carob bean gum in the market is normally standardized with sugars for viscosity and reactivity.

C.A.S. number 9000-40-2

Structural formula



DESCRIPTION White to yellowish white, nearly odourless powder

FUNCTIONAL USES Stabilizer, thickener, emulsifier, gelling agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol

Gel formation Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.

Viscosity	Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add 200 ml of water with vigorous stirring until the gum is completely and uniformly dissolved. An opalescent, slightly viscous solution is formed. Transfer 100 ml of this solution into another 400-ml beaker. Heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is an appreciable increase in viscosity (differentiating carob bean gums from guar gums).
Gum constituents (Vol. 4)	Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 to 10 µl of the hydrolysate instead of 1 to 5 µl. Use galactose and mannose as reference standards. These constituents should be present.

PURITY

Loss on drying (Vol. 4)	Not more than 14% (105º, 5 h)
Total ash (Vol. 4)	Not more than 1.2% (800º, 3-4 h)
Acid-insoluble matter (Vol. 4)	Not more than 3.5%
Protein (Vol. 4)	Not more than 1.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method) in Volume 4 (under "General Methods, Inorganic components"). The percentage of nitrogen determined multiplied by 6.25 gives the percentage of protein in the sample.
Starch	To a 1 in 10 solution of the sample add a few drops of iodine TS; no blue colour is produced
Residual solvents	Not more than 1% of ethanol or isopropanol, singly or in combination See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender. Total (aerobic) plate count: Not more than 5,000 CFU/g *E. coli:* Negative in 1 g *Salmonella*: Negative in 25 g Yeasts and moulds: Not more than 500 CFU/g

TESTS

PURITY TESTS

Residual solvents

Determine by gas chromatography in Volume 4 (under “Analytical Techniques, Chromatography”).

Chromatography conditions
Column: 25% Diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm i.d., 0.25 μm film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injector: 280°
- column: Hold for 6 min at 40°, then 40-110° at 4°/min, 110-250° at 25°/min, hold for 10 min at 250°
- detector: 250°

Standard solutions
Solvent standard solution: Transfer 100 mg each of chromatography grade ethanol and isopropanol into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
TBA standard solution: Transfer 100 mg of chromatography grade tertiary-butyl alcohol (TBA) into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
Mixed standard solutions: Transfer 1, 2, 3, 4 and 5 ml of Solvent standard solution into each of five 100-ml volumetric flasks. Add 4 ml of TBA standard solution to each flask and dilute to volume with water.

Sample preparation
Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 4 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distil about 95 ml, adjusting the heat so that foam does not enter the column. Add 4 ml of TBA standard solution to the distillate and make up to 100 ml with water to obtain the Sample solution.

Standard curves
Inject 1 μl of each Mixed standard solution into the chromatograph. Measure the peak areas for each solvent and TBA. Construct the standard curves by plotting the ratios of the peak areas of each of the solvents/TBA against the concentrations of each solvent (mg/ml) in the Mixed standard solutions.

Procedure
Inject 1 μl of the Sample solution into the chromatograph. Measure the peak areas for each solvent and TBA. Calculate the ratios of the peak areas of each solvent/TBA, and obtain the concentration of each solvent from the standard curves.

Calculate the percentage of each solvent from:

$$\% \text{ Solvent} = (C \times 100/W \times 1000) \times 100$$

where C is the concentration of solvent (mg/ml)
W is weight of sample (g)

β-CAROTENE, synthetic

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 31st JECFA (1987), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw for beta carotene, synthetic and from Blakeslea trispora, was established at the 57th JECFA (2001).

SYNONYMS CI Food Orange 5; INS No. 160a(i); CI (1975) No. 40800

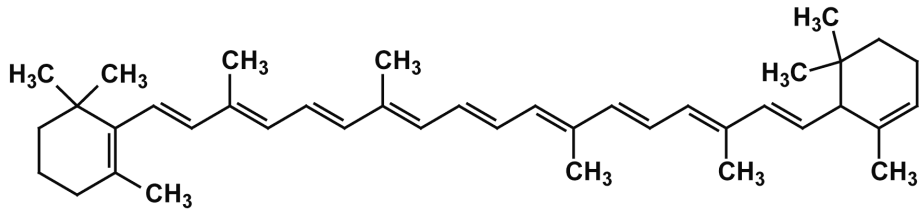
DEFINITION These specifications apply to synthetic β-carotene which consists predominantly of all-*trans*-β-carotene. Synthetic β-carotene may also contain minor amounts of *cis*-isomers and other carotenoids such as all-*trans*-retinal, β-apo-12'-carotenal, and β-apo-10'-carotenal. Commercial preparations of β-carotene intended for use in food are prepared from β-carotene meeting these specifications and are formulated as suspensions in edible oils or water-dispersible powders. These preparations may have different ratio of trans/cis isomers.

Chemical names ß-Carotene, ß,ß-carotene
1,1'-(3,7,12,16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene]

C.A.S. number 7235-40-7

Chemical formula $C_{40}H_{56}$

Structural formula All-*trans*-β-carotene (main compound)



Formula weight 536.88

Assay Not less than 96% total colouring matters, expressed as ß-carotene.

DESCRIPTION Red to brownish-red crystals or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water; practically insoluble in ethanol; slightly soluble in vegetable oils.
Test for carotenoids	The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 0.5 M sulfuric acid.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 483 nm. The ratio A_{455}/A_{483} is between 1.14 and 1.19. Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 340 nm. The ratio A_{455}/A_{340} is not lower than 15.

PURITY

Sulfated Ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Subsidiary colouring matters	Carotenoids other than ß-carotene: Not more than 3% of total colouring matters. See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, under “General Methods, Metallic Impurities.”

TESTS

PURITY TESTS

Subsidiary colouring matters

Carotenoids other than β-carotenes

Subsidiary colouring matters (carotenoids other than β-carotenes) are determined by high performance liquid chromatography (HPLC) using the following conditions:

Chromatographic system

- HPLC equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler and integrator
- Detector wavelength: 453 nm
- Column: Reverse phase C18, Suplex pkb-100 (250 x 4.6 mm, 5 µm) from Supelco or equivalent
- Mobile phase: In a 1000 ml volumetric flask, dissolve 50 mg BHT in 20 ml 2-propanol and add 0.2 ml N-ethyldiisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and approx. 450 ml methanol. Mixture cools and contracts. Allow to reach room temperature and dilute to volume with methanol. Discard after 2 days.
- Isocratic elution
- Column temperature: 30°

- Flow rate: 0.6 ml/min
- Injection volume: 10 µl
- Temperature of the autosampler: (approx. 15°)
- Run time: approx. 35 min

Reagents
- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyldiisopropyl-amine, reagent grade
- Ammonium acetate, reagent grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Ethanol, HPLC grade
- Tetrahydrofuran, HPLC grade

Sample solution
Weigh accurately (to ±0.1 mg) 0.010 g of the sample and dissolve in tetrahydrofuran (stabilized with 0.025% BHT). Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute to the ratio of 1:10 with ethanol.

Procedure
Inject the sample solution using the conditions detailed under *Chromatographic system*. The retention times for all-*trans*-β-carotene and cis-isomers are in the range of 20-25 min. The largest peak in the chromatogram corresponds to all-*trans*-β-carotene. The relative retention times of minor carotenoids and cis-isomers of β-carotene with respect to the retention time of all-*trans*-β-carotene are: all-*trans*-retinal (0.26), all-*trans*-β-apo-12'-carotenal (0.33), all-*trans*-β-apo-10'carotenal (0.34), all-*trans*-γ-carotene (0.85), all-*trans*-α-carotene (0.95), 9-cis-β-carotene (1.05), 13-cis-β-carotene (1.15) and 15-cis-β-carotene (1.18).
Integrate the areas of the peaks in the chromatogram.

Calculation
Calculate the percentage of carotenoids other than β-carotenes (%, w/w) using the following formula:

$$\text{Carotenoids other than } \beta\text{-carotenes (\%, w/w)} = \left(\frac{A_{\text{total}} - A_{\beta-\text{carotenes}}}{A_{\text{total}}} \right) \times 100$$

where

$A_{\text{total}'}$ is the sum of the area of all the peaks in the chromatogram, excluding the solvent peak (area units); and

$A_{\beta\text{-carotene}}$ is the sum of the areas of the peaks of all β-carotenes (all-*trans*-β-carotene, 9-cis-β-carotene, 13-cis-β-carotene and 15-cis-β-carotene) in the chromatogram (area units).

METHOD OF ASSAY (Vol. 4)

Total colouring matters content by spectrophotometry

Proceed as directed under Total Colouring Matters Content – Colouring Matters Content by Spectrophotometry, Procedure 2, using the following conditions:
Sample weight (W): 0.08 g (±0.01 g);
Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml;

Volume of the two pipets: $v_1 = v_2 = 5$ ml;
Specific absorbance of the standard: $A^{1\%}_{1\ cm} = 2500$;
Wavelength of maximum absorption: λ_{max} about 455 nm.

Calculation

Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, w/w) == \frac{A \times V_1 \times D}{A^{1\%}_{1cm} \times W}$$

where

A is the absorbance of the twice-diluted sample solution at 455 nm; and

D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.

β-CAROTENE from *BLAKESLEA TRISPORA*

Prepared at the 61st JECFA (2003), published in FNP 52 Add 11 (2003), superseding specifications prepared at the 57th JECFA (2001), published in FNP 52 Add 9 (2001). A group ADI with β-carotene (synthetic) of 0 - 5 mg/kg bw was established at the 57th JECFA (2001).

SYNONYMS CI Food Orange 5; INS No. 160a(iii)

DEFINITION Obtained by a fermentation process using the two sexual mating types (+) and (-) of the fungus *Blakeslea trispora*. The colour is isolated from the biomass by solvent extraction and crystallised. The colouring principle consists predominantly of trans β-carotene together with variable amounts of cis isomers of β-carotene. Minor amounts of other carotenoids of which γ-carotene accounts for the major part may also be present. The only organic solvents used in the extraction and purification are ethanol, isopropanol, ethyl acetate and isobutyl acetate. The main articles of commerce are suspensions in food grade vegetable/plant oil and water dispersible powders. This is for ease of the use and to improve stability as carotenes easily oxidise.

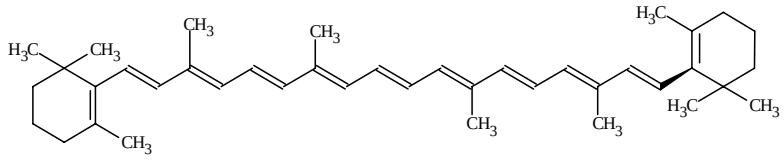
Class: Carotenoid

Chemical names: β-carotene, β,β-carotene

C.A.S. number: 7235-40-7

Chemical formula: $C_{40}H_{56}$

Structural formula



Formula weight: 536.88

Assay: Not less than 96.0% of total colouring matter (expressed as β-carotene)

DESCRIPTION Red to brownish-red crystals or crystalline powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water; practically insoluble in ethanol, slightly soluble in vegetable oil.

UV/VIS absorption

Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 483 nm. The ratio is between 1.14 and 1.19.

Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 340 nm. The ratio is not lower than 0.75.

Carotenoid

The colour of a solution of the sample in acetone disappears after successive addition of a 5% solution of sodium nitrite and 0.5 M of sulfuric acid.

PURITY

Sulfated ash (Vol. 4)

Not more than 0.2%

Carotenoids other than β-carotene

Not more than 3.0% of total colouring matters
See description under TESTS

Residual solvent (Vol. 4)

Ethanol:
Ethyl acetate: } Not more than 0.8% singly or in combination
Isopropanol: Not more than 0.1%
Isobutyl acetate: Not more than 1.0%

See description in Volume 4

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental methods".

TESTS

PURITY TESTS

Carotenoids other than β-carotene

Determine by liquid chromatography (see Volume 4) using the following procedure:

Apparatus:
HPLC system equipped with
- UV/VIS detector (445 nm)
- Column heater (30°)
- Refrigerated autosampler (0-10°)
- Column: 250 mm x 4.6 mm, Vydac 218 TP54, 5 μm, or equivalent
- Solvent system: 99% methanol and 1% tetrahydrofuran containing 50 mg/l of L-ascorbic acid.
-

Working conditions:
- Flow rate: 0.6 ml/min
- Injection 10 μl
- Run time: approximately 25 min

Sample preparation: Weigh 25 mg of the sample and dissolve in tetrahydrofuran. Make up to 100 ml in a volumetric flask. Dilute 1 ml of the solution to 25 ml in a volumetric flask with a ethanol:tetrahydrofuran (9:1) solution.

Results: The retention time for β-carotene (all trans isomer) is about 19 minutes corresponding to the largest peak in the chromatogram. The retention time for γ-carotene is about 20 minutes and the peak at about 22 minutes corresponds to the 13-cis isomer.
γ-carotene as a % of total β-carotene equals:

$$\frac{A_1 \times 100}{A_1 + A_2 + A_3}$$

where

A_1 is the area of the γ-carotene peak
A_2 is the area of the all-trans β-carotene peak
A_3 is the combined area of the peaks from the isomers of all-trans β-carotene

METHOD OF ASSAY Proceed as directed in Colouring matters, Total Content by Spectrophotometry (Volume 4), procedure 2 using the following conditions:

W = 0.08 g
$V_1 = V_2 = V_3 = 100$ ml
$v_1 = v_2 = 5$ ml
$A^{1\%}_{1\,cm} = 2500$
A_{max} = about 455 nm

ß-apo-8'-CAROTENAL

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw expressed as the sum of carotenoids including β-carotene, β-apo-8'-carotenal, and the methyl and ethyl esters of β-apo-8'-carotenoic acid was established at the 18th JECFA (1974).

SYNONYMS CI Food Orange 6; CI (1975) No. 40820; INS No. 160e

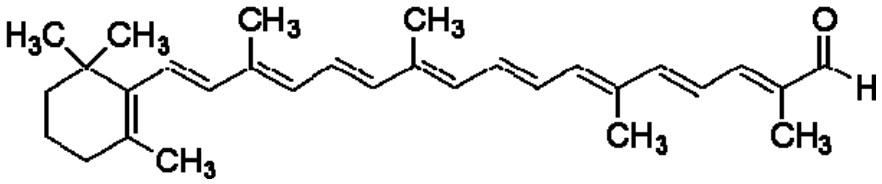
DEFINITION These specifications apply to β-apo-8'-carotenal which consists predominantly of all-trans- β-apo-8'-carotenal and may also contain minor quantities of other carotenoids such as all-trans-crocetindialdehyde, all-trans-β-apo-12'-carotenal and all-trans-β-carotene. Commercial preparations of β-apo-8'-carotenal intended for use in food are prepared from β-apo-8'-carotenal meeting these specifications and are formulated as suspensions in edible oil, emulsions and water dispersible powders. These preparations may also contain cis isomers.

Chemical names ß-Apo-8'-carotenal, 8'-apo-ß-carotene-al
2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethyl-1-cyclohexenyl)heptadeca-2,4,6,8,10,12,14,16-octaenal

C.A.S. number *1107-26-2*

Chemical formula $C_{30}H_{40}O$

Structural formula All-*trans*- β-apo-8'-carotenal (main compound)



Formula weight 416.65

Assay Not less than 96% of total colouring matters

DESCRIPTION Deep violet crystals with metallic lustre or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, slightly soluble in ethanol, sparingly soluble in vegetable oils.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 461 nm and 488 nm. The ratio A_{488}/A_{461} is between 0.80 and 0.84.
Test for carotenoid	The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 0.5 M sulfuric acid.

PURITY

Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Subsidiary colouring matters	Carotenoids other than ß-apo-8'-carotenal: Not more than 3% of total colouring matters. See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, under “General Methods, Metallic Impurities.”

TESTS

PURITY TESTS

Subsidiary colouring matters

Carotenoids other than ß-apo-8'-carotenal

Subsidiary colouring matters (carotenoids other than ß-apo-8'-carotenal) are determined by high performance liquid chromatography (HPLC) using the following conditions:

Chromatographic system

- HPLC equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler and integrator
- Detector wavelength: 463 nm
- Column: reverse phase C18, Suplex pkb-100 (250 x 4.6 mm, 5 µm) from Supelco or equivalent
- Mobile phase: In a 1000 ml volumetric flask, dissolve 50 mg BHT in 20 ml 2-propanol and add 0.2 ml N-ethyldiisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and approx. 450 ml methanol. Mixture cools and contracts. Allow to reach room temperature and dilute to volume with methanol. Discard after 2 days.
- Isocratic elution

- Column temperature: 30°
- Flow rate: 0.6 ml/min
- Injection volume: 10 µl
- Temperature of the autosampler: (approx. 15°)
- Run time: approx. 35 min

Reagents
- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyldiisopropyl-amine, reagent grade
- Ammonium acetate, reagent grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Ethanol, HPLC grade
- Tetrahydrofuran, HPLC grade

Sample solution
Weigh accurately (to ±0.1 mg) 0.010 g of the sample and dissolve in tetrahydrofuran (stabilized with 0.025% BHT). Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute to the ratio of 1:10 with ethanol.

Procedure
Inject the sample solution using the conditions detailed under *Chromatographic system*. The retention time for all-*trans*-ß-apo-8'-carotenal is in the range of 7-9 min and corresponds to the largest peak in the chromatogram. The relative retention times of minor carotenoids with respect to the retention time of all-*trans*-ß-apo-8'-carotenal are: all-*trans*-crocetindialdehyde (0.54); all-*trans*-β-apo-12'-carotenal (0.84); all-*trans*-β-carotene (2.55). Integrate the areas of the peaks in the chromatogram.

Calculation
Calculate the percentage of carotenoids other than ß-apo-8'-carotenal (%, w/w) using the following formula:

$$\text{Carotenoids other than ß - apo - 8'-carotenal (\%, w/w)} = \left(\frac{A_{\text{total}} - A_{\text{β-apo-8'-carotenal}}}{A_{\text{total}}} \right) \times 100$$

where

A_{total}, is the sum of the area of all the peaks in the chromatogram, excluding the solvent peak (area units); and

$A_{\text{ß-apo-8'-carotenal}}$ is the area of the peak of ß-apo-8'-carotenal in the chromatogram (area units).

METHOD OF ASSAY (Vol. 4)

Total colouring matters content by spectrophotometry

Proceed as directed under Total Colouring Matters Content – Colouring Matters Contents by Spectrophotometry, Procedure 2, using the following conditions:

Sample weight (W): 0.08 g (±0.01 g)

Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml

Volume of the two pipets: $v_1 = v_2 = 5$ ml

Specific absorbance of the standard: $A^{1\%}_{1\ cm} = 2640$

Wavelength of maximum absorption: λ_{max} about 461nm

Calculation

Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, w/w) = \frac{A \times V_1 \times D}{A^{1\%}_{1cm} \times W}$$

where

A is the absorbance of the twice-diluted sample solution at 461 nm; and

D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.

ß-apo-8'-CAROTENOIC ACID ETHYL ESTER

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw expressed as the sum of carotenoids including β-carotene, β-apo-8'-carotenal, and the methyl and ethyl esters of β-apo-8'-carotenoic acid was established at the 18th JECFA (1974).

SYNONYMS CI Food Orange 7; CI (1975) No. 40825; INS No. 160f

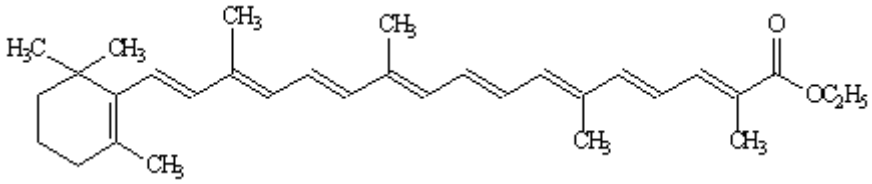
DEFINITION These specifications apply to β-apo-8'-carotenoic acid ethyl ester which consists predominantly of all-*trans*-β-apo-8'-carotenoic acid ethyl ester and may also contain minor quantities of all-*trans*- β-apo-12'-carotenal, methyl-all-*trans*-β-apo-8'-carotenoate, all-*trans*-ethyl 4'-apo-β-carotenate and all-*trans*-β-carotene. Commercial preparations of β-apo-8'-carotenoic acid ethyl ester intended for use in food are prepared from β-apo-8'-carotenoic acid ethyl ester meeting these specifications and are formulated as suspensions in edible oil, emulsions and water dispersible powders. These preparations may also contain cis isomers.

Chemical names All-*trans*-ß-apo-8'-carotenoic acid ethyl ester, ethyl 8'-apo-ß-caroten-8'-oate, ethyl (2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohexen-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaenoate

C.A.S. number 1109-11-1

Chemical formula $C_{32}H_{44}O_2$

Structural formula All-*trans*- ß-Apo-8'-carotenoic acid ethyl ester (main compound)



Formula weight 460.70

Assay Not less than 96% of total colouring matters

DESCRIPTION Red to violet-red crystals or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USES
Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, very slightly soluble in ethanol, slightly soluble in vegetable oils.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 449 nm and 475 nm. The ratio A_{475}/A_{449} is between 0.82 and 0.86.
Test for carotenoid	The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 0.5 M sulfuric acid.

PURITY

Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Subsidiary colouring matters	Not more than 3% of total colouring matters See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, under “General Methods, Metallic Impurities.”

TESTS

PURITY TESTS

Subsidiary colouring matters

Carotenoids other than ß-apo-8'-carotenoic acid ethyl ester

Subsidiary colouring matters (carotenoids other than ß-apo-8'-carotenoic acid ethyl ester) are determined by high performance liquid chromatography (HPLC) using the following conditions:

Chromatographic system

- HPLC system equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler
- Detector wavelength: 446 nm
- Column: reverse phase C18, Suplex pkb-100 (250 x 4.6 mm, 5 µm) from Supelco or equivalent
- Mobile phase: In a 1000 ml volumetric flask, dissolve 50 mg BHT in 20 ml 2-propanol and add 0.2 ml N-ethyldiisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and approx. 450 ml methanol. Mixture cools and contracts. Allow to reach room temperature and dilute to volume with methanol. Discard after 2 days.

- Isocratic elution
- Column temperature: 30°
- Flow rate: 0.6 ml/min
- Injection volume: 10 µl
- Temperature of the autosampler: (approx. 15°)
- Run time: approx. 35 min

Reagents
- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyldiisopropyl-amine, reagent grade
- Ammonium acetate, reagent grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Ethanol, HPLC grade
- Tetrahydrofuran, HPLC grade

Sample solution
Weigh accurately (to ±0.1 mg) 0.010 g of the sample and dissolve in tetrahydrofuran (stabilized with 0.025% BHT). Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute to the ratio of 1:10 with ethanol.

Procedure
Inject the sample solution using the conditions detailed under *Chromatographic system*. The retention time for all-*trans*-apo-8'-carotenoic acid ethyl ester is in the range of 9-11 min and corresponds to the largest peak in the chromatogram. The relative retention times of carotenoids with respect to the retention time of all-*trans*-ß-apo-8'-carotenoic acid ethyl ester are: all-*trans*- β-apo-12'-carotenal (0.73); methyl all-*trans*-β-apo-8'-carotenoate (0.97); all-*trans*-ethyl 4'-apo-β-carotenate (1.22), all-*trans*-β-carotene (2.23).
Integrate the areas of the peaks in the chromatogram.

Calculation
Calculate the percentage of carotenoids other than ß-apo-8'-carotenoic acid ethyl ester (%, w/w) using the following formula:

$$\text{Carotenoids other than ß-apo-8'-carotenoic acid ethyl ester (\%, w/w)} = \left(\frac{A_{\text{total}} - A_{\beta\text{-apo ester}}}{A_{\text{total}}} \right) \times 100$$

where
A_{total} is the sum of the area of all the peaks in the chromatogram, excluding the solvent peak (area units); and
$A_{ß\text{-apo-ester}}$ is the area of the peak of ß-apo-8'-carotenoic acid ethyl ester in the chromatogram (area units).

METHOD OF ASSAY (Vol. 4)

Total colouring matters content by spectrophotometry
Proceed as directed under Total Colouring Matters Content – Colouring Matters Contents by Spectrophotometry, Procedure 2, using the following conditions:
Sample weight (W): 0.08 g (±0.01 g)
Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml

Volume of the two pipets: $v_1 = v_2 = 5$ ml
Specific absorbance of the standard: $A^{1\%}_{1\ cm} = 2550$
Wavelength of maximum absorption: λ_{max} about 449 nm

Calculation
Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, w/w) = \frac{A \times V_1 \times D}{A^{1\%}_{1cm} \times W}$$

where

A is the absorbance of the twice-diluted sample solution at 449 nm; and

D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.

CARRAGEENAN

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 57th JECFA (2001), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI "not specified" for carrageenan and processed Eucheuma *seaweed was established at the 57th JECFA (2001).*

SYNONYMS

Irish moss gelose (from *Chondrus* spp.); Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); INS No. 407.

DEFINITION

A substance with hydrocolloid properties obtained from certain members of the class *Rhodophyceae* (red seaweeds).

The principal commercial sources of carrageenans are the following families and genera of the class of *Rhodophyceae*:

Furcellariacaea such as *Furcellaria*
Gigartinaceae such as *Chondrus*, *Gigartina*, *Iridaea*
Hypnaeceae such as *Hypnea*
Phyllophoraceae such as *Phyllophora*, *Gynmogongrus*, *Ahnfeltia*
Solieriaceae such as *Eucheuma*, *Anatheca*, *Meristotheca*.

Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α-1,3 and β-1,4 in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent polysaccharides in carrageenan are designated as kappa-, iota-, and lambda-carrageenan. Kappa-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; iota-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between kappa-carrageenan and iota-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In lambda-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).

Carrageenan is obtained by extraction from seaweed into water or aqueous dilute alkali. Carrageenan may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol.
Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers

carried over from drum drying processes.

C.A.S. number 9000-07-1

DESCRIPTION

Yellowish or tan to white, coarse to fine powder that is practically odourless.

FUNCTIONAL USES

Thickener, gelling agent, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in ethanol; soluble in water at a temperature of about 80°, forming a viscous clear or slightly opalescent solution that flows readily; disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.

Test for sulfate: Dissolve a 100-mg sample in 20 ml of water (with heating if necessary), and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS; filter if a precipitate forms. Boil the solution or the filtrate for 5 min. A white, crystalline precipitate appears.

Test for galactose and anhydrogalactose (Vol. 4): Proceed as directed in Vol.4 (under "General Methods, Organic Components, Gum Constituents Identification") using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.

Identification of hydrocolloid and predominant type of copolymer: Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel. To 50 ml of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa type, and a compliant ("elastic") gel indicates a predominantly iota type. If the solution does not gel, the carrageenan is of a predominantly lambda type.

Infrared absorption: Passes test
See description under TESTS

PURITY

Loss on drying (Vol. 4): Not more than 12% (105° to constant weight)

pH (Vol. 4): Between 8 and 11 (1 in 100 suspension)

Viscosity: Not less than 5 cp at 75° (1.5% solution)
See description under TESTS

Sulfate	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis See description under TESTS
Total ash	Not less than 15% and not more than 40% on the dried basis See description under TESTS.
Acid-insoluble ash (Vol. 4)	Not more than 1% Use the ash from the Total ash test
Acid-insoluble matter (Vol. 4)	Not more than 2% Use 2 g of sample obtained from part (a) of the procedure for sulfate determination.
Residual solvents (Vol. 4)	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination See description under TESTS
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50-g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender. Total (aerobic) plate count: Not more than 5000 cfu/g *Salmonella* spp.: Negative per test *E. coli*: Negative in 1 g
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine by the atomic absorption hydride technique. Use Method II for sample preparation.
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
Cadmium (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
Mercury (Vol.4)	Not more than 1 mg/kg Determine by the cold vapour atomic absorption technique

TESTS

IDENTIFICATION TESTS

Infrared absorption	Obtain infrared absorption spectra on the gelling and non-gelling fractions of the sample by the following procedure:

Disperse 2 g of the sample in 200 ml of 2.5% potassium chloride solution, and stir for 1 h. Let stand overnight, stir again for 1 h, and transfer into a centrifuge tube. (If the transfer cannot be made because the dispersion is too viscous, dilute with up to 200 ml of the potassium chloride solution.) Centrifuge for 15 min at approximately 1000 x g.

Remove the clear supernatant, resuspend the residue in 200 ml of 2.5% potassium chloride solution, and centrifuge again. Coagulate the combined supernatants by adding 2 volumes of 85% ethanol or isopropanol (NOTE: Retain the sediment for use as directed below). Recover the coagulum, and wash it with 250 ml of the alcohol. Press the excess liquid from the coagulum, and dry it at 60° for 2 h. The product obtained is the non-gelling fraction (lambda-carrageenan).

Disperse the sediment (retained above) in 250 ml of cold water, heat at 90° for 10 min, and cool to 60°. Coagulate the mixture, and then recover, wash, and dry the coagulum as described above. The product obtained is the gelling fraction (kappa- and iota-carrageenan).

Prepare a 0.2% aqueous solution of each fraction, cast films 0.5 mm thick (when dry) on a suitable non-sticking surface such as Teflon, and obtain the infrared absorption spectrum of each film. (Alternatively, the spectra may be obtained using films cast on potassium bromide plates, if care is taken to avoid moisture).

Carrageenan has strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. Absorption maxima are 1065 and 1020 cm^{-1} for gelling and non-gelling types, respectively. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave number (cm^{-1})	**Molecular Assignment**	**Absorbance relative to 1050 (cm^{-1})**		
		Kappa	**Iota**	**Lambda**
1220-1260	ester sulfate	0.3-1.4	1.2-1.7	1.4-2.0
928-933	3,6-anhydrogalactose	0.2-0.7	0.2-0.4	0-0.2
840-850	galactose-4-sulfate	0.2-0.5	0.2-0.4	-
825-830	galactose-2-sulfate	-	-	0.2-0.4
810-820	galactose-6-sulfate	-	-	0.1-0.3
800-805	3,6-anhydrogalactose-2-sulfate	0-0.2	0.2-0.4	-

PURITY TESTS

Sulfate

Principle

Hydrolysed sulfate groups are precipitated as barium sulfate.

Procedure

(a) Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60% w/w isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the

material remaining on the filter paper with two 15-ml portions of 60% isopropanol/water. Dry the material at 105° to constant weight. Approximately 1 g of the dried matter is to be used for part (b). The remainder should be retained for determination of Total ash, Acid-insoluble matter, and viscosity.

(b) Accurately weigh a 1 g sample (W_1) obtained from part (a). Transfer the sample to a 100-ml long-neck round-bottom flask. Add 50 ml of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 ml of a 10% (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear.

Transfer the solution to a 600-ml beaker, bring to a boil, and add dropwise 10 ml of a 10% barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash-free slow-filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800° in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator.

Weigh the crucible containing the ash. Calculate the percentage sulfate from the weight in g (W_2) of the ash (barium sulfate) using the formula:

$(W_2/W_1) \times 100 \times 0.4116$

Total ash

Accurately weigh 2 g of the dried sample (W_1) obtained from part (a) under the procedure for sulfate determination above. Transfer to a previously ignited, tared silica or platinum crucible. Heat the sample with a suitable infrared lamp, increasing the intensity gradually, until the sample is completely charred; continue heating for an additional 30 min. Transfer the crucible with the charred sample into a muffle furnace and ignite at about 550º for 1 h. Cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight (W_2) is obtained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 1-in-10 solution of ammonium nitrate and dry under an infrared lamp. Repeat the ignition step.

Calculate the percentage of total ash of the sample:

$(W_2/W_1) \times 100$

Retain the ash for the Acid-insoluble ash test.

Viscosity

Transfer 7.5 g of the dried sample obtained from part (a) under the procedure for sulfate determination into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in a water bath with continuous agitation, until a temperature of 80^o is reached (20 - 30 min). Add water to adjust for loss by evaporation, cool to 76-77^o, and heat in a constant temperature bath at 75^o.

Pre-heat the bob and guard of a Brookfield LVF or LVT viscometer to approximately 75º in water. Dry the bob and guard, and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent. (Note. Samples of some types of carrageenan may be too viscous to read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle and take the reading on the 0-100 scale or on the 0-500 scale.)

Record the results in centipoises, obtained by multiplying the reading on the scale by the factor given by the Brookfield manufacturer.

Residual solvents (Vol.4)

See Method 1 under Vol. 4. General Methods, Organic Components, Residual Solvents.

Prepare standard, blank, and calibration solutions as directed under Method 1.

Sample Preparation

Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column and distil about 100 ml, adjusting the heat so that the foam does not enter the column. Quantitatively transfer the distillate to a 200-ml volumetric flask, fill to the mark with water and shake the flask to mix. Weigh accurately 8.0 g of this solution into an injection vial. Add 1.0 ml of the internal standard solution. Heat at 60° for 10 min and shake vigorously for 10 sec.

CASTOR OIL

Prepared at the 27th JECFA (1983), published in FNP 28 (1983) and in FNP 52 (1992) Metals and arsenic specifications revised at the 63rd JECFA (2004) An ADI of 0-0.7 mg/kg bw established at the 23rd JECFA (1979)

SYNONYMS Ricinus oil; INS No. 1503

DEFINITION The fixed oil obtained from the seed of *Ricinus Communis* L. (family *Euphorbiaceae*) is essentially the triglyceride of ricinoleic acid.

C.A.S. number 8001-79-4

DESCRIPTION Pale yellow or almost colourless, clear, viscous liquid, with a faint, mild odour

FUNCTIONAL USES Carrier solvent, release agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in 95% ethanol; miscible with absolute ethanol; slightly soluble in light petroleum
Specific gravity (Vol. 4)	0.952 - 0.966
Refractive index (Vol. 4)	n (20, D): 1.477 - 1.481

PURITY

Acid value (Vol. 4)	Not more than 2
Hydroxyl value (Vol. 4)	160 - 168
Saponification value (Vol. 4)	176 - 185
Iodine value (Vol. 4)	83 - 88
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

CHLORINE

Prepared at the 28th JECFA (1984), published in FNP 31/2 (1984) and in FNP 52 (1992) Metals and arsenic specifications revised at the 63rd JECFA (2004). A treatment level of 2.5g Chlorine /kg flour was established at the 29th JECFA (1985)

SYNONYMS INS No. 925

DEFINITION

Chemical names: Chlorine

C.A.S. number: 7782-50-5

Chemical formula: Cl_2

Formula weight: 70.91

Assay: The content of the vapourized liquid shall be not less than 99.5% of Cl_2 (v/v)

DESCRIPTION Liquid chlorine is a clear yellow liquid which on vapourizing yields a greenish yellow gas. It is gaseous at atmospheric temperatures and pressures but normally supplied under pressure in containers in which it is present in both liquid and gaseous phases. Such containers should meet appropriate requirements for the transportation and storage of hazardous gases.
Caution: Chlorine gas is a respiratory irritant. Large amounts cause coughing, laboured breathing and irritation of the eyes. In extreme cases the difficulty in breathing may cause death due to suffocation. Liquid chlorine causes skin and eye burns on contact. Safety precautions to be observed in handling the material are available, e.g., in the Chlorine Manual obtainable from the Chlorine Institute, 342 Madison Avenue, New York, N.Y. 10017, USA.

FUNCTIONAL USES Flour treatment agent, bleaching agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Very soluble in water

Test for chlorine: Cautiously pass a few ml of chlorine gas through 10 ml of sodium hydroxide TS that has been previously chilled in an ice bath. The resulting solution gives positive tests for *Chloride* and it darkens starch iodide paper.

PURITY

Moisture: Not more than 150 mg/kg
Determine by ASTM method E 410-70 "Moisture and Residue in Liquid Chlorine"

Residue on evaporation	Not more than 150 mg/kg Determine by ASTM method E 410-70 "Moisture and Residue in Liquid Chlorine"
Mercury (Vol. 4)	Not more than 1 mg/kg Dissolve the residue obtained in the Test for Residue on evaporation in 2.5 ml of freshly prepared aqua regia and dilute with water to a volume in ml equivalent to the weight in g of the initial chlorine sample, so that 1 ml of the final dilution is equivalent to 1 g of chlorine. Transfer 2.0 ml of the Sample Solution into a 50 ml beaker, add 10 ml of water, 1 ml of dilute sulfuric acid (1 in 5), and 1 ml of potassium permanganate solution (1 in 25), cover with a watch glass, boil for a few sec, and cool. Use the resulting solution as the Sample Preparation for the Limit Test.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine by ASTM Method E 412-70, "Assay of Liquid Chlorine (Zinc Amalgam Method)".

CHLOROPHYLLS

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not limited' was established at the 13th JECFA (1969)

SYNONYMS Magnesium chlorophyll, magnesium phaeophytin, CI Natural Green 3; C.I. (1975) No. 75810; INS No. 140

DEFINITION Obtained by solvent extraction of grass, lucerne, nettle and other plant material; during the subsequent removal of solvent, the naturally present co-ordinated magnesium may be wholly or partly removed from the chlorophylls to give the corresponding phaeophytins; the principal colouring matters are the phaeophytins and magnesium chlorophylls; the extracted product, from which the solvent has been removed, contains other pigments such as carotenoids as well as oils, fats and waxes derived from the source material.
Only the following solvents may be used for the extraction: acetone, dichloromethane, methanol, ethanol, propan-2-ol and hexane.

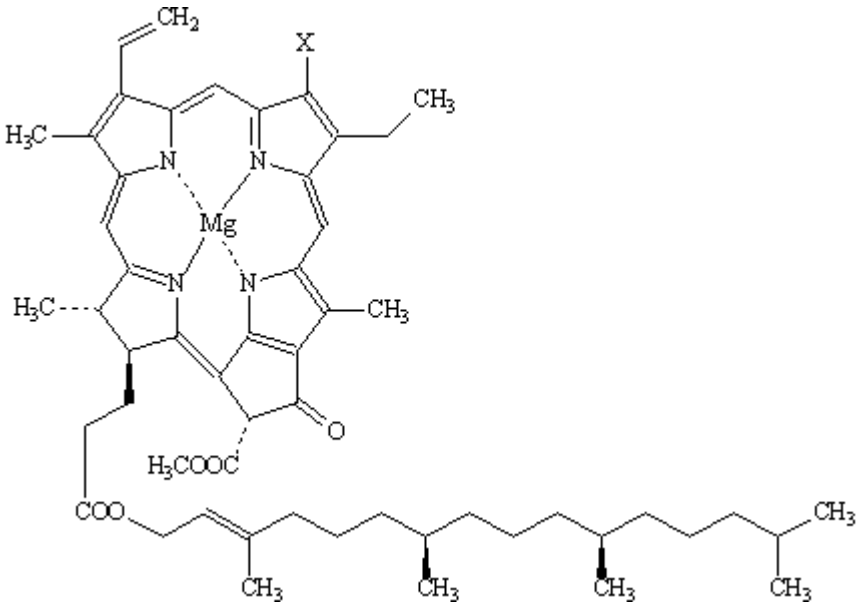
Chemical names The major colouring principles are:

Phytyl (13^2R,17S,18S)-3-(8-ethyl-13^2-methoxycarbonyl-2,7,12,18-tetramethyl-13'-oxo-3-vinyl-13^1-13^2-17,18-tetrahydrocyclopenta [at]-prophylrin-17-yl)propionate, (Pheophytin a), or as the magnesium complex (Chlorophyll a).
Phytyl (13^2R,17S,18S)-3-(8-ethyl-7-formyl-13^2-methoxycarbonyl-2,12,18-trimethyl-13'-oxo-3-vinyl-13^1-13^2-17,18-tetrahydro-cyclopenta [at]-prophylrin-17-yl)propionate, (Pheophytin b), or as the magnesium complex (Chlorophyll b).

C.A.S. number Phaeophytin a, Magnesium complex: 479-61-8
Phaeophytin b, Magnesium complex: 519-62-0

Chemical formula Phaeophytin a Magnesium complex (Chlorophyll a): $C_{55}H_{72}MgN_40_5$
Phaeophytin a: $C_{55}H_{74}N_40_5$
Phaeophytin b Magnesium complex (Chlorophyll b): $C_{55}H_{70}MgN_40_6$
Phaeophytin b: $C_{55}H_{72}N_40_6$

Structural formula



where
X = CH_3 for the "a" series
X = CHO for the "b" series
Loss of Mg converts chlorophyll into the corresponding phaeophytin.

Formula weight

Phaeophytin a magnesium complex (Chlorophyll a) 893.51
Phaeophytin a 871.22
Phaeophytin b magnesium complex (Chlorophyll b) 907.49
Phaeophytin b 885.20

Assay

Content of total combined phaeophytins and their magnesium complexes is not less than 10%.

DESCRIPTION

Waxy solid ranging in colour from olive green to dark green depending on the content of co-ordinated magnesium.

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water; soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed oils

Thin-layer chromatography

Apply a 1 in 20 solution of the sample in chloroform as a band of the length of 2 cm to a Silica 60 TLC plate. After drying, develop the plate by a mixture of 50% hexane, 45% chloroform and 5% ethanol (General purpose reagent grade chloroform is supplied with 2% of added ethanol as a stabilizer. The 5% ethanol in the solvent mixture is in addition to this), until the solvent ascends to a point 15 cm above the initial spots. Allow the solvent to evaporate, then visually examine the separated spots and identify the components of interests by their R_f values and colours. Approximate R_f values and colour of the spots are as follows:

Phaeophytin a: 0.77, grey/brown
Phaeophytin b: 0.75, yellow/brown
Chlorophyll a: 0.50, blue/green
Chlorophyll b: 0.63, yellow/green

In addition spots may be visible for ß-carotene at R_f 0.81 and xanthophyll at R_f 0.47 and 0.23.

PURITY

Residual solvents (Vol. 4)

Acetone, methanol, ethanol, propan-2-ol, hexane: Not more than 50 mg/kg, singly or in combination
Dichloromethane: Not more than 10 mg/kg
Determine by *gas chromatographically* using either the method of entrainment distillation (*Determination of Residual Solvents*) or headspace analysis (*Limit Test for Solvent Residues*).

Arsenic (Vol. 4)

Not more than 3 mg/kg (Method II)

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Accurately weigh about 100 mg of the sample and dissolve in diethyl ether, making the volume to 100 ml. Dilute 2 ml of this solution to 25 ml with diethyl ether. The concentration of the sample should not give an absorbance at 660.4 nm that is in excess of the working range for Absorbance measurements, i.e., not in excess of 0.7.

Measure the absorbances of the solution in a 1 cm cell against a diethyl ether blank at 660.4 nm, 642.0 nm, 667.2 nm and 654.4 nm. (These being the absorbance maxima in diethyl ether for chlorophyll a, chlorophyll b, phaeophytin a, and phaeophytin b, respectively). In addition measure at 649.8 nm and 628.2 nm. To the remaining diluted solution add one crystal of oxalic acid and after dissolution and mixing, remeasure the absorbances at the same wavelengths. "delta A" is the difference between the absorbances between the absorbance at the respective wavelengths, before and after addition of oxalic acid.

Calculate the concentration of the individual compounds in micromoles per litre from the following equations:

Chlorophyll a = 17.7 delta A (660.4 nm) + 7.15 delta A (642.0 nm)
Chlorophyll b = 19.4 delta A (642.0 nm) - 2.92 delta A (660.4 nm)
Phaeophytin a = -4.89 A (649.8 nm) + 0.0549 A (628.2 nm)
+18.7 A (667.2 nm) + 0.0575 A (654.4 nm) - chlorophyll a
Phaeophytin b = -71.0 A (649.8 nm) + 2.51 A (628.2 nm)
- 13.5 A (667.2 nm) + 84.3 A (654.4 nm) - chlorophyll b
Convert the figures in micro moles per litre to percentages using the following equations:

$$\%\ chlorophyll\ a\ =\ \frac{micromoles\ x\ 0.8935\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ chlorophyll\ b\ =\ \frac{micromoles\ x\ 0.9075\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ Phaephytin\ a\ =\ \frac{micromoles\ x\ 0.8712\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ Phaeophytin\ b\ =\ \frac{micromoles\ x\ 0.8852\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

CHLOROPHYLLS, COPPER COMPLEXES

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-15 mg/kg bw was established at the 13th JECFA (1969)

SYNONYMS Copper chlorophyll, copper phaeophytin, CI Natural Green 3; C.I. (1975) No. 75810, INS No. 141(i)

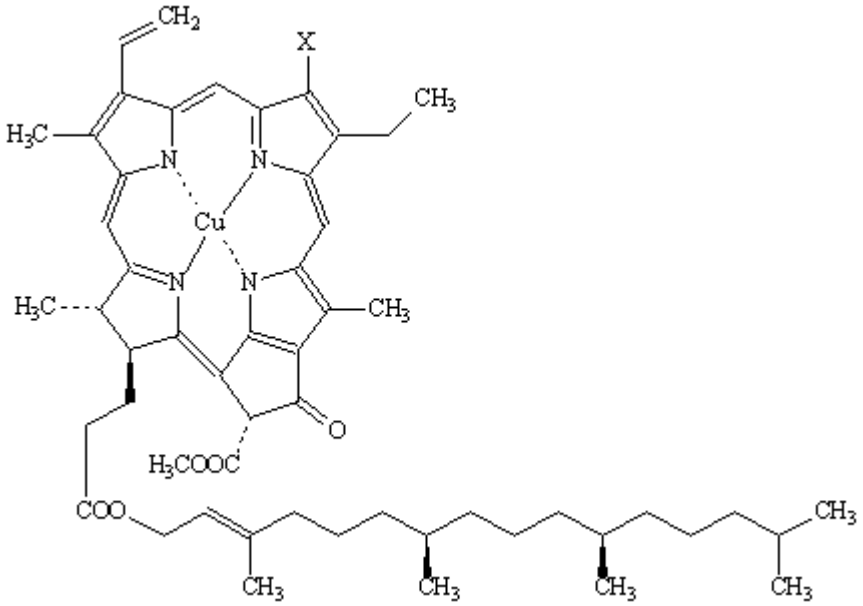
DEFINITION Obtained by addition of an organic salt of copper to the substance obtained by solvent extraction of grass, lucerne, nettle and other plant material; the product, from which the solvent has been removed, contains other pigments such as carotenoids as well as fats and waxes derived from the source material; the principal colouring matters are the copper phaeophytins.
Only the following solvents may be used for the extraction: Acetone, dichloromethane, methanol, ethanol, propan-2-ol and hexane.

Chemical names [Phytyl (13^2R,17S,18S)-3-(8-ethyl-13^2-methoxycarbonyl-2,7,12,18-tetramethyl-13^1-oxo-3-vinyl-13^1,13^2,17,18-tetra-hydrocyclopenta [at]-prophyrin-17-yl)propionate] copper (II) (Copper chlorophyll a)
[Phytyl (13^2R,17S,18S)-3-(8-ethyl-7-formyl-13^2-methoxycarbonyl-2,12,18-trimethyl-13^1-oxo-3-vinyl-13^1,13^2,17,18-tetrahydro-cyclopenta [at]prophyrin-17-yl)propionate] copper (II) (Copper chlorophyll b)

C.A.S. number 65963-40-8

Chemical formula Copper phaeophytin a: C_{55} H_{72}Cu $N_4 0_5$
Copper phaeophytin b: C_{55} H_{70}Cu $N_4 0_6$

Structural formula



where
X = CH_3 for the "a" compound
X = CHO for the "b" compound

Formula weight: Copper phaeophytin a: 932.75
Copper phaeophytin b: 946.73

Assay: Not less than 10% of total copper phaeophytins

DESCRIPTION Waxy solid ranging in colour from blue green to dark green depending on the source material.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water; soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed oils

Spectrophotometry (Vol. 4): A (1%, 1 cm) at 422 nm in chloroform is not less than 54.

Thin-layer chromatography: Apply a 1 in 20 solution of the sample in chloroform as a band of the length of 2 cm to a Silica 60C plate. After drying, develop the plate by a mixture of 50% hexane, 45% chloroform and 5% ethanol (general purpose reagent grade chloroform is supplied with 2% of added ethanol as a stabilizer. The 5% ethanol in the solvent mixture is in addition to this), until the solvent ascends to a point 15 cm above the initial spots. Allow the solvent to evaporate, then visually chromatography examine the separated spots and identify the components of interests by their R_f values and colours. Approximate R_f values and colour of the spots are as follows:

Copper phaeophytin a: 0.5, green
Copper phaeophytin b: 0.73, yellow/green

In addition spots may be visible for ß-carotene at Rf 0.81 and xanthophyll at R_f 0.47 and 0.23.

PURITY

Residual solvents (Vol. 4): Acetone, methanol, ethanol, propan-2-ol, hexane: Not more than 50 mg/kg, singly or in combination
Dichloromethane: Not more than 10 mg/kg
Determine *gas chromatographically* using either the method of entrainment distillation (*Determination of Residual Solvents*) or headspace analysis (*Limit Test for Solvent Residues*).

Free ionizable copper: Not more than 200 mg/kg
Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil, with the aid of gentle heat. Add exactly 200 ml of water, stir mechanically, and adjust to pH 3.0 by careful addition of 0.5 N hydrochloric acid (avoid over-shooting). Allow the mixture to stand for 10 min. If necessary readjust to pH 3.0 by careful addition of 0.5 N hydrochloric acid. Transfer to a separating

funnel and allow to stand for about 20 min. Filter the aqueous phase through a No. 50 Whatman filter paper, rejecting the first 10 ml. Subject this solution to analysis for copper by *atomic absorption spectrometry* (see Volume 4).

Total copper

Not more than 8% of the total copper phaeophytins
Ignite about 0.1 g, accurately weighed, of the sample contained in a silica dish, at a temperature not exceeding 500º, until all carbon is removed; moisten with one or two drops of concentrated sulphuric acid and re-ash. Dissolve the ash by boiling with 3 portions (each of 5 ml) of 10% (w/w) hydrochloric acid, filtering each addition through the same small filter paper into a 100 ml volumetric flask. Cool, and make up to volume with purified water. Subject this solution to analysis for copper by *atomic absorption spectrometry* (see Volume 4).

Arsenic (Vol. 4)

Not more than 3 mg/kg (Method II)

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Accurately weigh about 100 mg of the sample and dissolve in diethyl ether, making the volume to 100 ml. Dilute 2 ml of this solution to 25 ml with diethyl ether. The concentration of the sample should not give an absorbance at 660.4 nm that is in excess of the working range for Absorbance measurements, i.e., not in excess of 0.7.

Measure the absorbances (A) of the solution in a 1 cm cell against a diethyl ether blank at 667.2 nm, 654.4 nm, 649.8 nm and 628.2 nm. (The latter two wavelengths being the absorbance maxima in diethyl ether for copper phaeophytin a and copper phaeophytin b respectively).

Calculate the concentration of the individual compounds in micromoles per liter from the following equations:
Copper phaeophytin a = 45.6 A (649.8nm) - 2.75 A (628.2nm) + 3.10 A (667.2nm)- 35.4 A (654.4nm)
Copper phaeophytin b = -8.46 A (649.8nm)+ 20.7 A (628.2nm) - 1.69 A (667.2nm)+ 5.13 A (654.4nm)
Convert the figures in micromoles per liter to percentages using the following equations:

$$\%\ copper\ phaephytin\ a\ =\ \frac{micromoles\ x\ 0.9327\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ copper\ phaeophytin\ b\ =\ \frac{micromoles\ x\ 0.9467\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

CHLOROPHYLLINS, COPPER COMPLEXES SODIUM and POTASSIUM SALTS

Prepared at the 31st JECFA (1987), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). Corrected at the 69th JECFA (2008). An ADI of 0-15 mg/kg bw was established at the 22nd JECFA (1978).

SYNONYMS

Sodium copper chlorophyllin, potassium copper chlorophyllin, C.I. (1975) No. 75815, INS No. 141(ii)

DEFINITION

The alkali salts of Copper Chlorophyllins are obtained by the addition copper to the product obtained by the saponification of a solvent extraction of grass, lucerne, nettle and other plant material; the saponification removes the methyl and cyclophytol ester groups and may partially cleave the pentenyl ring; after addition of copper to the purified chlorophyllins, the acid groups are neutralised to form the salts of potassium and/or sodium; the commercial products may be presented as aqueous solutions or dried powders.
Only the following solvents may be used for the extraction: acetone, dichloromethane, methanol, ethanol, propan-2-ol and hexane.

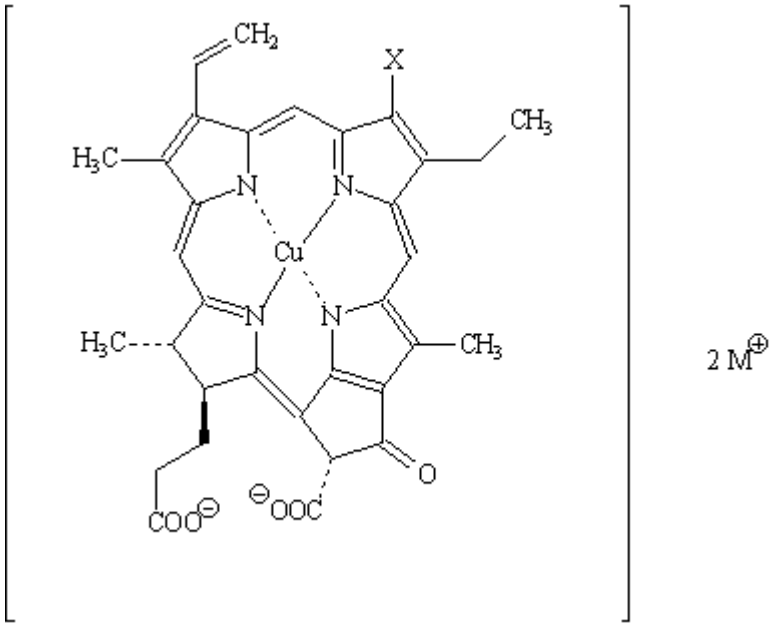
Chemical names

The major colouring principles in their acid forms are
3-(10-Carboxylato-4-ethyl-1,3,5,8-tetramethyl-9-oxo-2-vinylphorbin-7-yl)propionate, copper complex (Copper chlorophyllin a)
3-(10-carboxylato-4-ethyl-3-formyl-1,5,8-trimethyl-9-oxo-2-vinylphorbin-7-yl)propionate, copper complex (Copper chlorophyllin b)
Depending on the degree of hydrolysis the cyclopentenyl ring may be cleaved with the resultant production of a third carboxyl function.

Chemical formula

Copper chlorophyllin a (acid form): $C_{34}H_{32}CuN_4O_5$
Copper chlorophyllin b (acid form): $C_{34}H_{30}CuN_4O_6$

Structural formula



where
X = CH_3 for the "a" compound
X = CHO for the "b" compound
M = Potassium and/or sodium

Formula weight

Copper chlorophyllin a: 640.20
Copper chlorophyllin b: 654.18
Each may be increased by a 18 Daltons if the cyclopentenyl ring is cleaved.

Assay

Not less than 95% total copper chlorophyllins after drying (100º, 1 h).

DESCRIPTION

Dark green to blue/black powder or dark green solution.

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Soluble in water; very slightly soluble in lower alcohols and ketones and diethyl ether; insoluble in chloroalkanes, hydrocarbons and fixed oils

Spectrophotometry (Vol. 4)

A (1%, 1 cm) of the sample, dried at 100º for 1 h, at 405 nm in pH 7.5 phosphate buffer is not less than 540.

Test for copper

Dissolve the sulfated ash of the sample (using 1 g of sample, Method I) in 10 ml of dilute hydrochloric acid TS by heating on a water bath. Filter if the solution is not clear, and dilute to 10 ml with water. Use this solution as the test preparation for the following tests.

To 5 ml of the test preparation add ammonia TS to make the solution alkaline. Blue colour should appear.

To 5 ml of the above test preparation add 0.5 ml of a 1 in 1000 sodium diethyldithiocarbamate solution. A brown precipitate should be formed.

Test for sodium (Vol. 4)

Passes test
Test the solution described under TESTS, IDENTIFICATION TESTS Test for copper

Test for potassium (Vol. 4)

Passes test
Test the solution described under TESTS, IDENTIFICATION TESTS Test for copper

PURITY

Basic dyes

To 5 ml of a 0.5% aqueous solution of the sample in a test- tube add 1 ml of 1 N hydrochloric acid and add 5 ml of diethyl ether. Mix thoroughly and allow to separate. The ether layer should be no darker than pale green.

Residual solvents (Vol. 4)

Acetone, methanol, ethanol, propan-2-ol, hexane: Not more than 50 mg/kg, singly or in combination
Dichloromethane: Not more than 10 mg/kg
Determine *gas chromatographically* using either the method of entrainment distillation (*Determination of Residual Solvents*) or headspace analysis (*Limit Test for Solvent Residues*).

Free ionizable copper

Not more than 200 mg/kg
Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil, with the aid of gentle heat. Add exactly 200 ml of water, stir mechanically, and adjust to pH 3.0 by careful addition of 0.5 N hydrochloric acid (avoid over-shooting). Allow the mixture to stand for 10 min. If necessary readjust to pH 3.0 by careful addition of 0.5 N hydrochloric acid. Transfer to a separating funnel and allow to stand for about 20 min. Filter the aqueous phase through a No. 50 Whatman filter paper, rejecting the first 10 ml. Subject this solution to analysis for copper by *atomic absorption spectrometry* (see Volume 4).

Total copper

Not more than 8% of the total copper phaeophytins
Ignite about 0.1 g, accurately weighed, of the sample contained in a silica dish, at a temperature not exceeding 500º, until all carbon is removed; moisten with one or two drops of concentrated sulphuric acid and re-ash. Dissolve the ash by boiling with 3 portions (each of 5 ml) of 10% (w/w) hydrochloric acid, filtering each addition through the same small filter paper into a 100 ml volumetric flask. Cool, and make up to volume with purified water. Subject this solution to analysis for copper by *atomic absorption spectrometry* (see Volume 4).

Arsenic (Vol.4)

Not more than 3 mg/kg (Method II)

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 1 g of the sample, dried previously at 100º for 1 h, then dissolve in 20 ml Phosphate Buffer Solution (pH 7.5) and dilute to 1000 ml with distilled water.
Dilute 10 ml of this solution to 100 ml with Phosphate Buffer Solution (pH 7.5). Measure the optical density of the final solution (0.001% w/v) in a suitable spectrophotometer, using a 1 cm cell and slit width of 0.10 mm at 403-406 nm, recording the maximum within this range.
The percentage of sodium copper chlorophyllin is given by the expression:

$$\frac{\text{optical density} \times 10^{4}}{565 \times \text{weight of sample (g)}}$$

This formula was derived on the assumption that 100% pure sodium copper chlorophyllin has a specific absorbance of 565.

CITRIC ACID

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI "Not limited" for citric acid and its calcium, potassium, sodium and ammonium salts established at the 17th JECFA in 1973.

SYNONYMS INS No. 330

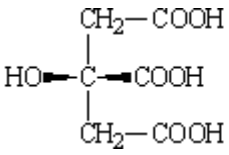
DEFINITION Citric acid may be produced by recovery from sources such as lemon or pineapple juice or fermentation of carbohydrate solutions or other suitable media using *Candida* spp. or non-toxicogenic strains of *Aspergillus niger*

Chemical names 2-hydroxy-1,2,3-propanetricarboxylic acid

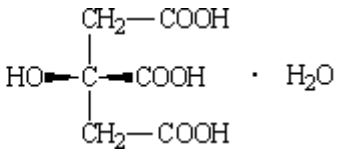
C.A.S. number 77-92-9 (anhydrous)
5949-29-1 (monohydrate)

Chemical formula $C_6H_8O_7$ (anhydrous)
$C_6H_8O_7 \cdot H_2O$ (monohydrate)

Structural formula



Anhydrous



Monohydrate

Formula weight 192.13 (anhydrous)
210.14 (monohydrate)

Assay Not less than 99.5% and not more than 100.5% on the anhydrous basis

DESCRIPTION White or colourless, odourless, crystalline solid; the monohydrate form effloresces in dry air

FUNCTIONAL USES Acidulant; sequestrant; antioxidant synergist; flavouring agent (see "Flavouring agents" monograph)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Very soluble in water; freely soluble in ethanol; slightly soluble in ether

Test for citrate (Vol. 4) Passes test

PURITY

Water (Vol. 4) Anhydrous: Not more than 0.5% (Karl Fischer Method)
Monohydrate: Not less than 7.5% and not more than 8.8% (Karl Fischer

	Method)
Sulfated ash (Vol. 4)	Not more than 0.05%
Oxalate (Vol. 4)	Not more than 100 mg/kg Test 1.0 g of the sample by the Oxalate Limit Test (Volume 4). Measure absorbance at 520 nm in a 10 mm cell. The test solution should have less than 0.023 absorbance units.
Sulfates (Vol. 4)	Not more than 150 mg/kg Test 20 g of the sample by the Sulfates Limit Test (Volume 4) using 6.0 ml of 0.01N sulfuric acid in the standard
Readily carbonizable substances	Heat 1.0 g of sample with 10 ml of 98% sulfuric acid in a water bath at 90±1° for 60 min. No colour darker than *MatchingFluid K* (25°) should be produced (not more than 0.5 absorbance units at 470 nm in a 10 mm cell).
Lead (Vol. 4)	Not more than 0.5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh, to the nearest mg, 2.5 g of the sample and place in a tared flask. Dissolve in 40 ml of water and titrate with 1 N sodium hydroxide, using phenolphthalein TS as the indicator. Each ml of 1 N sodium hydroxide is equivalent to 64.04 mg of $C_6H_8O_7$.

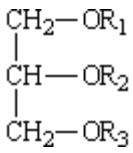
CITRIC and FATTY ACID ESTERS of GLYCEROL

Prepared at the 35th JECFA (1989), published in FNP 49 (1990) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS Citric acid esters of mono- and di-glycerides, citroglycerides, CITREM; INS No. 472c

DEFINITION Obtained by esterification of glycerol with citric acid and edible fatty acids or by reaction of a mixture of mono- and diglycerides of edible fatty acid with citric acid; consists of mixed esters of citric acid and edible fatty acids with glycerol; may contain minor parts of free fatty acids, free glycerol, free citric acid and mono- and diglycerides; may be wholly or partially neutralized with sodium hydroxide or potassium hydroxide (as declared on the label).

Structural formula



Where at least one of R_1, R_2 or R_3 represents a citric acid moiety, one represents a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen.

DESCRIPTION White to ivory coloured, oily to waxy material.

FUNCTIONAL USES Stabilizer, emulsifier, dough conditioner, antioxidant synergist

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water; dispersible in hot water; soluble in oils and fats; insoluble in cold ethanol

Tests for fatty acids (Vol. 4) Passes tests

Test for citric acid (Vol. 4) Passes tests

Test for glycerol (Vol. 4) Passes tests

PURITY

Sulfated ash (Vol. 4) Not neutralized products: not more than 0.5%
Partially or wholly neutralized products: not more than 10%
Test 2 g of the sample (Method I)

Free glycerol (Vol. 4) Not more than 4%

Total glycerol 8-33%
See description under TESTS

Total citric acid

13-50%
See description under TESTS

Total fatty acid

37-81%
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Total glycerol

Weigh accurately about 2 g of the sample into a saponification flask, add 50 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min.
To a 1-liter volumetric flask add exactly 99 ml (from a buret) of chloroform and 25 ml of glacial acetic acid. Quantitatively transfer the content of the saponification flask to the 1-liter volumetric flask, using three 25 ml portions of water. Add about 500 ml of water, and shake vigorously for about 1 min. Dilute to volume with water, mix thoroughly, and set aside for separation of layers.

Pipet 50 ml of acetic periodic acid TS into a series of 400-ml beakers. Prepare two blanks by adding 50 ml of water to each. Pipet 50 ml of the aqueous sample solution into one of the beakers containing 50 ml of acetic periodic acid TS, shake gently to mix, cover with watch glass, and allow to stand 30 min. but not longer than 1.5 h. Add 20 ml of 15% potassium iodide solution, shake gently to mix, and allow to stand at least 1 min. but not more than 5 min. Do not allow to stand in bright or direct sunlight. Add 100 ml of water and titrate with 0.1 N sodium thiosulfate. Use a variable speed electric stirrer to keep the solution thoroughly mixed. Continue the titration to the disappearance of the brown iodine colour from the aqueous layer. Add 2 ml of starch TS and continue the titration to the disappearance of iodine from the tiny chloroform layer separated during titration and the disappearance of the blue iodo-starch colour from the aqueous layer.

Calculation
% total glycerol = [(B - S) x N x 2.302]/W

where
B = titration of blank containing 50 ml of water
S = titration of sample
N = exact normality of 0.1 N thiosulfate
W = weight of sample represented by aliquot pipetted for test
i.e. W = [a x 50]/900
where a = weight in g of sample

Total citric acid

Principle
The sample is saponified with alcoholic potassium hydroxide solution and the fatty acids removed by extraction. The citric acid is converted to trimethylsilyl

(TMS) derivatives and analyzed by *gas liquid chromatography.*

Saponification
Weigh accurately about 1 g of the sample into a round bottomed flask, add 25 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min. Acidify the mixture with hydrochloric acid and evaporate in rotary evaporator or by other suitable method.

Extraction
Quantitatively transfer the content of the flask to a separator, using not more than 50 ml of water and extract with three 50-ml portions of heptane, discarding the extracts. Transfer the aqueous layer to a 100-ml volumetric flask, neutralize, dilute to volume with water, and mix.

Derivatization
Pipette 1 ml of this solution, and 1 ml of tartaric acid solution (1 mg/ml in water) into a 10 ml cappable round bottom flask and evaporate to dryness. Add 1 ml of pyridine, 0,2 ml of trimethyl-chlorosilane (TMCS), 0.4 ml of hexamethyl- disilazane (HMDS), 0.1 ml of N-methyl-N-trimethylsilyl-tri-fluoroacetamide (MSTFA). Cap the flask tight and swirl carefully to obtain total dissolution. Heat the flask in an oven at 60º for 1 h.

Gas chromatography
Any suitable gas chromatograph may be used fitted with a flame ionization detector and a column (glass 1.8 m x 2 mm i.d.) packed with 10% DC-200 on chromosorb Q (80/100 mesh). Recommended conditions are: oven temperature, 165º; injection block temperature, 240º; detector block temperature, 240º; nitrogen carrier gas flow rate, 24 ml/min.

Procedure
Inject a 5 µl sample of the TMS derivatives. The retention time for tartaric acid is about 12 min. and the relative retention time citric acid/tartaric acid is about 2.3.

Repeat the procedure as described above under Derivatization and Gas chromatography using 1 ml of a reference citric acid - solution (3 mg/ml in water) instead of 1 ml of sample solution.

Calculation
Measure each peak area by a suitable method.
% total citric acid = $[A_{CS} \times A_{TR} \times W_{CR} \times 100 \times 100]/[A_{TS} \times A_{CR} \times W]$

where
A_{CS} = peak area of citric acid (sample solution)
A_{TS} = peak area of tartaric acid (sample solution)
A_{TR} = peak area of tartaric acid (reference solution)
A_{CR} = peak area of citric acid (reference solution)
W_{CR} = weight (g) of citric acid in 1 ml of the reference solution
W = weight (g) of sample of citric and fatty acid esters of glycerol

Total fatty acid

Weigh accurately about 5 g of the sample into a 250-ml round-bottomed flask, add 50 ml of 1 N ethanolic potassium hydroxide, and reflux for 1 h on a water bath.

Quantitatively transfer the content of the saponification flask to a 1,000-ml separating funnel, using three 25-ml portions of water, and add 5 drops of methyl orange TS.

Cautiously add concentrated hydrochloric acid until the colour of solution changes clearly red, and shake well to separate fatty acids.

Extract the separated fatty acids with three 100-ml portions of diethyl ether. Combine the extracts, and wash with 50-ml portions of 10% sodium chloride solution until the washed sodium chloride solution becomes neutral.

Dry the ether solution with anhydrous sodium sulfate. Then evaporate off ether on a steam bath, leave additional 10 min on the steam bath, and weigh the residue.

CROSS-LINKED SODIUM CARBOXYMETHYL CELLULOSE

New specifications prepared at the 59th JECFA (2002), published in FNP 52 Add 10 (2002), re-published with editorial corrections in FNP 52 Add 11(2003). This substance was included at the 59th meeting in the group ADI "not specified" for modified celluloses, established at the 35th JECFA in 1989.

SYNONYMS Cross-linked carboxymethyl cellulose, cross-linked sodium CMC, cross-linked CMC, croscarmellose sodium, cross-linked cellulose gum; INS No. 468

DEFINITION Cross-linked sodium carboxymethyl cellulose is the sodium salt of thermally cross-linked partly O-carboxymethylated cellulose. It is manufactured by acidifying an aqueous suspension of sodium carboxymethyl cellulose and heating the suspension to achieve cross-linking. The product is then washed and dried. It is also produced during the manufacture of sodium carboxymethyl cellulose by lowering the pH and heating to achieve cross-linking.

C.A.S. number 74811-65-7

Chemical formula The polymers contain substituted anhydroglucose units with the general formula: $C_6H_7O_2(OR_1)(OR_2)(OR_3)$
where R_1, R_2, and R_3 represent the following groups, present in varying proportions

-H
$-CH_2COONa$
$-CH_2COOH$

DESCRIPTION A slightly hygroscopic, white to greyish-white, odourless powder

FUNCTIONAL USES Tabletting agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in acetone, in ethanol and in toluene

Colour reaction Add 1 g of the powdered sample to 50 ml water, while stirring to produce a uniform dispersion. Dilute 1 ml of this mixture with 1 ml of water in a small test tube and add 5 drops of 1-naphthol TS . Incline the test tube, and carefully introduce down the side of the tube 2 ml of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface.

Precipitate formation Mix 1 g of the powdered sample with 100 ml of solution containing 4 mg/kg of methylene blue in water and allow to settle. The substance absorbs methylene blue and settles as a blue, fibrous mass.

Test for sodium (Vol. 4) Passes test

PURITY

pH (vol.4)	Not less than 5.0 and not more than 7.0 (1 in 100 suspension in water)
Loss on drying (Vol. 4)	Not more than 6% (105°, 3h)
Sulfated ash (Vol. 4)	Not less than 14.0 % and not more than 28.0 % on the dried basis (2 g of sample)
Water-soluble substances	Not more than 10% See description under TESTS
Degree of substitution	Not less than 0.2 and not more than 1.5 carboxymethyl groups (-CH_2COOH) per anhydroglucose unit on the dried basis. See description under TESTS
Sodium chloride and sodium glycolate	Not more than 0.5% (sum of sodium chloride and sodium glycolate) on the dried basis See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, "Instrumental Methods."

TESTS

PURITY TESTS

Water soluble substances

Weigh accurately about 10 g of sample and disperse in 800 ml of water. Stir for 1 min at the beginning, at 10 min, 20 min and 30 min. Allow to stand for 1 h and centrifuge, if necessary. Filter under vacuum using a fast filter paper and collect 150 ml of the filtrate. Evaporate 100 ml to dryness and dry the residue at 100 – 105° for 4 h. Weigh the residue and calculate percentage of water soluble substances using the formula:

$$\%\ \text{water soluble substances} = \frac{\text{M x } 800}{\text{W}}$$

where:
M = weight of residue, in g
W = weight of sample, in g

Degree of substitution

Degree of substitution is the sum of the degree of acid carboxymethyl substitution and degree of sodium carboxymethyl substitution.

Weigh accurately about 1 g of sample into a 500 ml Erlenmeyer flask and add 300 ml sodium chloride solution (10 % in water). Pipette 25.0 ml of 0.1M sodium hydroxide into the flask, stopper flask and allow to stand for 5 min shaking occasionally. Add 5 drops of m-cresol purple solution (dissolve 0.1g in minimum volume of alcohol and dilute to 100 ml with water), 15 ml of 0.1M hydrochloric acid from a burette, stopper the flask and shake. If the solution is violet, add 0.1M hydrochloric acid in 1 ml portions until the solution becomes yellow, shaking after each addition. Titrate with 0.1M sodium hydroxide until the colour turns to violet.

Calculate the number of milliequivalents of the base required for the neutralisation equivalent to 1 g of dried substance.
Calculate the degree of acid carboxymethyl substitution from the expression:

$$\frac{1150M}{(7102 - 412M - 80C)}$$

where:
M = milliequivalents of sodium hydroxide required for neutralisation of 1 g of dried substance
C = percentage of sulfated ash

Calculate the sodium carboxymethyl substitution from the expression:

$$\frac{(162 + 58A)C}{(7102) - (80xC)}$$

where:
A = acid carboxymethyl substitution
C = percentage of sulfated ash

Sodium chloride and sodium glycolate

Determine sodium chloride and sodium glycolate separately and calculate their sum.

Sodium chloride

Weigh accurately about 5 g of sample (previously dried to constant weight) to a 250 ml conical flask, add 50 ml of water and 5 ml of hydrogen peroxide solution (30%) and heat on a water-bath for 20 min, stirring occasionally to ensure total hydration. Cool, add 100 ml of water and 10 ml of nitric acid. Titrate with 0.05 M silver nitrate determining the end point potentiometrically using a silver electrode and a double-junction reference electrode containing a 100 g/l solution of potassium nitrate in the outer jacket and a standard filling solution in the inner jacket, and stirring constantly. 1 ml of 0.05 M silver nitrate is equivalent to 2.922 mg of NaCl.

Sodium glycolate

Weigh 0.5 g of the sample to the nearest 0.1 mg and transfer to a 100 ml beaker. Moisten the sample thoroughly with 5 ml of glacial acetic acid, 5 ml of water and stir for about 15 min to ensure total hydration. Slowly add 50 ml of acetone and 1 g of sodium chloride. Continue the stirring for several minutes to ensure complete precipitation of the carboxymethyl cellulose. Filter through a fast filter paper previously washed with acetone into a 100 ml volumetric flask, rinse the beaker and filter with 30 ml of acetone and make up to volume with acetone. Allow to stand for 24 h without shaking. Use the clear supernatant to prepare the test solution.
Prepare a blank solution containing 5 ml water, 5 ml of glacial acetic acid and acetone in 100 ml volumetric flask. Pipette 2 ml each of the test and blank solutions into separate 25 ml volumetric flasks. Remove the acetone by heating the uncovered flasks upright in a boiling water bath for exactly 20 min. Cool to room temperature and add 5 ml of 2,7-dihydroxynaphthalene TS and mix thoroughly, then add 15 ml more of the 2,7-dihydroxynaphthalene TS and mix. Cover the mouth of the flask with a small piece of aluminium foil and heat on a water-bath for 20 min. Cool to room temperature and make up to volume with 2,7-dihydroxynaphthalene TS. Measure the absorbance of the test solution against blank solution at 540 nm using 1 cm cells. Read the corresponding mg of glycolic acid from

the calibration curve obtained as follows.

Weigh accurately 0.100 g of glycolic acid standard (previously dried in vacuum over phosphorus pentoxide), dissolve in water in a 100 ml volumetric flask and make up to volume with water (use the solution within 30 days). Pipette 0.0, 1.0, 2.0, 3.0 and 4.0 ml aliquots of standard glycolic acid solution into a series of 100 ml volumetric flasks, add water to each flask to a volume of 5 ml, then add 5 ml of glacial acetic acid, dilute to volume with acetone and mix. Pipette 2 ml of each solution (containing, respectively, 0, 1, 2, 3 and 4 mg of glycolic acid per 100 ml) into a series of 25 ml volumetric flasks and proceed in the same manner as described for the test solution. Plot the mg of glycolic acid in the original 100 ml solution against absorbance to give a calibration curve.
Calculate the sodium glycolate content from the following formula:

$$\%\ \text{sodium glycolate} = \frac{\text{a x } 1.29}{\text{b}}$$

where
1.29 = Factor for converting glycolic acid into sodium glycolate
a = mg of glycolic acid read from the calibration curve
b = dry weight of sample, in g

CURDLAN

Prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001), superseding specifications prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999). An ADI "not specified" was established at the 57th JECFA (2001).

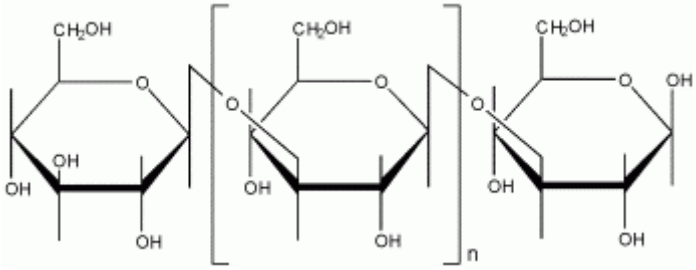
SYNONYMS Beta-1,3-glucan; INS No. 424

DEFINITION Curdlan is a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, produced by pure-culture fermentation from a non-pathogenic and non-toxicogenic strain of *Agrobacterium* biovar 1 (identified as *Alcaligenes faecalis* var. *myxogenes* at the time of discovery) or *Agrobacterium radiobacter*. Curdlan consists of β-(1,3)-linked glucose residues and has the unusual property of forming an elastic gel upon heating its aqueous suspension.

C.A.S. number 54724-00-4

Chemical formula $(C_6H_{10}O_5)_n$

Structural formula



Assay Not less than 80% (calculated as anhydrous glucose)

DESCRIPTION Odourless or almost odourless, white to nearly white powder.

FUNCTIONAL USES Firming agent, gelling agent, stabilizer, thickener.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol.

Solubility in alkali Passes test
Suspend 0.2 g of the sample in 5 ml of water, add 1 ml of 3 N sodium hydroxide, and shake. The sample dissolves.

Gel formation Heat a 2% aqueous suspension of the sample in a boiling water bath for 10 min and cool. A firm gel forms.

Precipitate formation with cupric tartrate Passes test
See description under TESTS

PURITY

Gel strength	Not less than 600 g/cm² (2% aqueous suspension) See description under TESTS	
pH (Vol. 4)	6.0 - 7.5 (1% aqueous suspension)	
Loss on drying (Vol. 4)	Not more than 10% (60º for 5 h, in vacuum)	
Sulfated ash (Vol. 4)	Not more than 6% Test 1 g of the sample (Method I)	
Nitrogen (Vol. 4)	Not more than 0.3% Test 1 g of the sample (Method II)	
Lead (Vol. 4)	Not more than 0.5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."	
Microbiological criteria (Vol. 4)	Total plate count:	Not more than 1,000 cfu/g
	E. coli:	Negative in 1 g

TESTS

IDENTIFICATION TESTS

Precipitate formation with cupric tartrate	Add 5 ml of sulfuric acid TS to 10 ml of a 2% aqueous suspension of the sample, heat in a boiling water bath for 30 min and cool. Neutralize the mixture with barium carbonate. Centrifuge the mixture at 900xg for 10 min. Add 1 ml of the supernatant to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide is formed.
Gel strength	Place 200 mg of the sample into the tube of a Potter homogenizer, add 10 ml of water and homogenize at about 3,500 rpm for 5 min. Transfer the suspension into a 16 mm × 150 mm test tube, deaerate in vacuum for 3 min and heat in a boiling water bath for 10 min to form a gel. Cool the tube under running water, let stand for 30 min, then remove the gel from the tube. Cut the gel accurately at distances of 20 mm and 30 mm from the bottom to obtain a piece 10 mm long. Determine gel strength with a Rheo Meter Model CR-200D (Sun Scientific Co., Ltd., Japan; Load cell: 1,000 g) or an equivalent instrument, under the following conditions: Measurement mode: 4 Velocity of moving plate: 250 mm/min Plunger: cylindrical type, 0.5 cm diameter Read the breaking point of the gel (B). Calculate the gel strength using the following formula: Gel strength (g/cm²) = $1{,}000B/\pi r^2$ where r = the radius of the plunger (cm)

METHOD OF ASSAY Transfer about 100 mg of the sample, accurately weighed, into a 100-ml volumetric flask and dissolve in about 90 ml of 0.1 N sodium hydroxide. Add 0.1 N sodium hydroxide to volume and mix well. Transfer 5 ml of the solution into a 100-ml volumetric flask, add water to volume and mix well. Quantitatively transfer 1 ml of the solution to a small flask or test tube, add 1 ml of a 5% (w/v) solution of reagent grade phenol in water and 5 ml of sulfuric acid TS. Shake vigorously and cool in ice-cold water. Prepare a blank and a reference standard solution in the same manner, using 0.1 ml of water and 100 mg of reagent grade glucose, respectively. Determine the absorbances of the sample solution and the reference standard solution in 1-cm cells at 490 nm with a suitable spectrophotometer, using the blank solution as the blank.

Calculate the content (%) of curdlan in the sample using the following formula:

$$\text{Curdlan content (\%)} = (A/A_R) \times (0.9 \times W_R/W) \times 100$$

where
A = the absorbance of the sample solution
A_R = the absorbance of the reference standard solution
0.9 = the molecular weight of anhydrous glucose divided by the molecular weight of glucose
W = the weight of the sample (mg)
W_R = the weight of the glucose standard used as reference (mg)

CYCLOHEXYLSULFAMIC ACID

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 24th JECFA (1980), published in FNP 17 (1980) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI of 0-11 mg/kg bw for cyclamic acid and its calcium and sodium salts (as cyclamic acid) was established at the 26th JECFA (1982)

SYNONYMS Cyclamic acid, INS No. 952

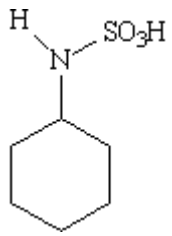
DEFINITION

Chemical names Cyclohexylsulfamic acid, cyclohexanesulfamic acid

C.A.S. number 100-88-9

Chemical formula $C_6H_{13}NO_3S$

Structural formula



Formula weight 179.24

Assay Not less than 98.0% and not more than 102.0% on the dried basis

DESCRIPTION Practically colourless, white crystalline powder

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, and in ethanol

Precipitate formation Acidify a 2% solution with hydrochloric acid, add 1 ml of barium chloride TS and filter if any haze or precipitate forms. To the clear solution add 1 ml of a 10% solution of sodium nitrite. A white precipitate forms.

PURITY

Loss on drying (Vol. 4) Not more than 1.0% (105º, 1 h)

Cyclohexylamine (Vol. 4) Not more than 10 mg/kg
Transfer 25 g of the sample into a 100 ml volumetric flask containing 20 ml of water, mix, and add 60 ml of sodium hydroxide TS. Add sodium hydroxide TS, if necessary, to make the solution alkaline to litmus. Dilute to

volume with water, and mix. Use this solution as the test preparation.

Dicyclohexylamine (Vol. 4)	Not more than 1 mg/kg
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 350 mg, accurately weighed, into a 250 ml flask. Dissolve the sample in 50 ml of water, add phenolphthalein TS, and titrate with 0.1N sodium hydroxide. Each ml of 0.1N sodium hydroxide is equivalent to 17.82 mg of $C_6H_{13}NO_3S$.

CALCIUM CYCLAMATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 24th JECFA (1980), published in FNP 17 (1980). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI of 0-11 mg/kg bw for cyclamic acid and its calcium and sodium salts (as cyclamic acid) was established at the 26th JECFA (1982)

SYNONYMS INS No. 952(ii)

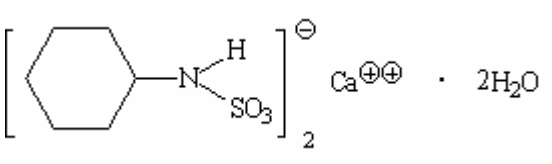
DEFINITION

Chemical names: Calcium cyclohexylsulfamate, calcium cyclohexanesulfamate

C.A.S. number: 139-06-0

Chemical formula: $C_{12}H_{24}CaN_2O_6S_2 \cdot 2H_2O$

Structural formula:



Formula weight: 432.57

Assay: Not less than 98.0% and not more than 101.0% on the anhydrous basis

DESCRIPTION White colourless crystals or crystalline powder. Approximately 30 times sweeter than sucrose

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, sparingly soluble in ethanol

Precipitate formation: To 10 ml of a 1 in 100 solution of the sample add 1 ml of hydrochloric acid TS, mix, add 1 ml of barium chloride TS. The solution remains clear, but upon the addition of 1 ml sodium nitrite TS, a white precipitate is formed

Test for calcium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not less than 6.0% and not more than 9.0% (140°, 2 h)

Cyclohexylamine (Vol. 4)	Not more than 10 mg/kg Transfer 25 g of the sample into a 100 ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Use this solution as the test preparation.
Dicyclohexylamine (Vol. 4)	Not more than 1 mg/kg
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 0.4 g of the sample accurately weighed, in a mixture of 50 ml of water and 5 ml of dilute hydrochloric acid TS and titrate with 0.1M sodium nitrite. Add the last ml of titrant dropwise until a blue colour is produced immediately when a glass rod dipped into the titrated solution is streaked on a piece of starch iodide test paper or alternatively the end point may be detected electro-metrically. When the titration is complete, the end-point is reproducible after the mixture has been allowed to stand for 1 min. Each ml of 0.1M sodium nitrite is equivalent to 19.83 mg of $C_{12}H_{24}CaN_2O_6S_2$, calculated on the anhydrous basis.

SODIUM CYCLAMATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 24th JECFA (1980), published in FNP 17 (1980). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI of 0-11 mg/kg bw for cyclamic acid and its calcium and sodium salts (as cyclamic acid) was established at the 26th JECFA (1982)

SYNONYMS INS No. 952(iv)

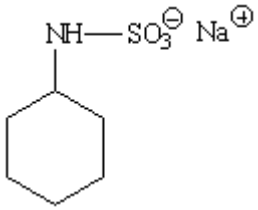
DEFINITION

Chemical names: Sodium cyclohexylsulfamate, sodium cyclohexanesulfamate

C.A.S. number: 139-05-9

Chemical formula: $C_6H_{12}NNaO_3S$

Structural formula:



Formula weight: 201.22

Assay: Not less than 98.0% and not more than 101.0% on the dried basis

DESCRIPTION White colourless crystals or crystalline powder

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, practically insoluble in ethanol

Precipitate formation: To 10 ml of a 1 in 100 solution of the sample add 1 ml of hydrochloric acid TS, mix, add 1 ml of barium chloride TS. The solution remains clear, but upon the addition of 1 ml sodium nitrite TS, a white precipitate is formed

Test for sodium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 1.0% (105^{o}, 1 h)
Cyclohexylamine (Vol. 4)	Not more than 10 mg/kg Transfer 25 g of the sample into a 100 ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Use this solution as the test preparation.
Dicyclohexylamine (Vol. 4)	Not more than 1 mg/kg
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 0.4 g of the sample accurately weighed, in a mixture of 50 ml of water and 5 ml of dilute hydrochloric acid TS and titrate with 0.1M sodium nitrite. Add the last ml of titrant dropwise until a blue colour is produced immediately when a glass rod dipped into the titrated solution is streaked on a piece of starch iodide test paper or alternatively the end point may be detected electrometrically. When the titration is complete, the end-point is reproducible after the mixture has been allowed to stand for 1 min.

Each ml of 0.1M sodium nitrite is equivalent to 20.12 mg of $C_6H_{12}NNaO_3S$, calculated on the dried basis.

ß-CYCLODEXTRIN

Prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995) superseding specifications prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-5 mg/kg bw was established at the 44th JECFA (1995)

SYNONYMS Beta-cyclodextrin, ßCD, BCD, ß-Schardinger dextrin, cyclodextrin B, INS No. 459

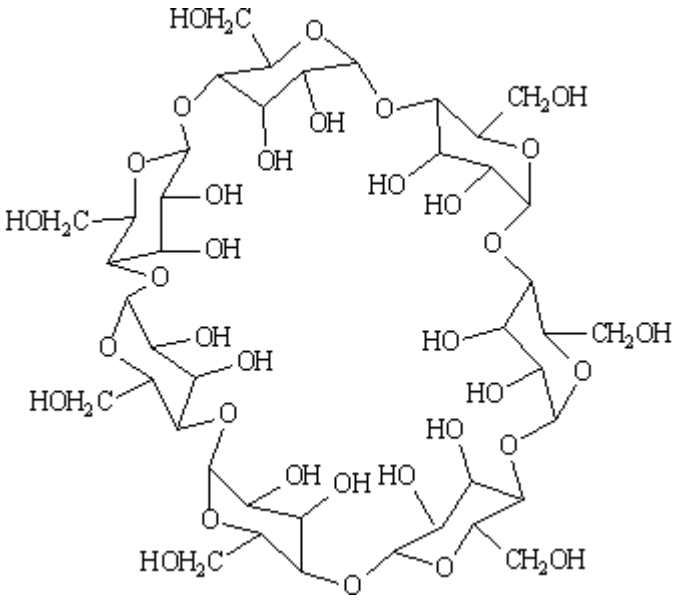
DEFINITION A non-reducing cyclic saccharide consisting of seven alpha-1,4-linked D-glucopyranosyl units manufactured by the action of cyclodextrin transglycolase on hydrolysed starch followed by purification of the ß-cyclodextrin; purification is by preparation of a ß-cyclodextrin/solvent inclusion compound followed by steam-stripping of the solvent before final purification.

Chemical names Cycloheptaamylose

C.A.S. number 7585-39-9

Chemical formula $(C_6H_{10}O_5)_7$

Structural formula



Formula weight 1135.00

Assay Not less than 98.0% of $(C_6H_{10}O_5)_7$ on an anhydrous basis

DESCRIPTION Virtually odourless, slightly sweet tasting white or almost white crystalline solid

FUNCTIONAL USES Encapsulation agent for food additives, flavouring and vitamins

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Sparingly soluble in water; freely soluble in hot water; slightly soluble in ethanol
Specific rotation (Vol. 4)	[alpha] 25, D: Between +160 and +164° (1% solution)
Infrared absorption	The infrared spectrum of the sample corresponds with that of a reference standard.
Chromatography	The retention time for the major peak in the liquid chromatogram of the sample solution corresponds to that for ß-cyclodextrin in the chromatograms of the standard solutions prepared as directed in the Method of Assay.

PURITY

Water (Vol. 4)	Not more than 14% (Karl Fischer Method)
Other cyclodextrins	Not more than 2% on an anhydrous basis See description under TESTS
Residual solvents	Not more than 1 mg/kg of each of toluene and trichloroethylene See description under TESTS
Reducing substances (Vol. 4)	Not more than 1% (as glucose)
Sulfated ash (Vol. 4)	Not more than 0.1%
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

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TESTS

PURITY TESTS

Other cyclodextrins	Analyses of alpha- and gamma-cyclodextrins are included in the Method of Assay. Adjust the attenuation of the instrument or adjust sample size to obtain a chromatogram in which the ß-cyclodextrin peak height nearly reaches the top of the recording chart. Measure peak heights or peak areas of the alpha-, ß- and gamma-cyclodextrin responses. Calculate % other cyclodextrins (CX) using the formula: $$\%\ Other\ CX = \frac{\alpha peaks + \gamma peaks}{\alpha peaks + \beta peaks + \gamma peaks} \times 100$$
Residual solvents	A dynamic-headspace *gas chromatographic technique* is used for the following procedure. The organic volatile impurities are trapped on an absorbent trap and the purge gas is vented. The trapped organic volatile

impurities are desorbed from the trap by heating the trap, and carried into the gas chromatograph by back flushing the trap with the carrier gas. Quantitate each solvent by the technique of standard additions.

Purge and Trap Apparatus

(The apparatus is based on that described in the US Environmental Protection Agency Test Method for Purgeable Halocarbons - Method 601): The apparatus consists of three separate sections: the sample purge; the trap; and the desorber. The sample purge is designed to accept 5 ml samples with a water column at least 3 cm deep. The gaseous headspace between the water column and the trap has a total volume of less than 15 ml. The purge gas is passed through the water column as finely-divided bubbles with a diameter of less than 3 mm at the origin. The purge gas is introduced not more than 5 mm from the base of the water column.
The trap is not less than 25 cm long and has an inside diameter of not less than 2.67 mm. The trap is packed to contain the indicated minimum lengths of adsorbents in the following order, beginning at the trap inlet:

7.7 cm of 2,6-diphenylene oxide polymer (the 2,6-diphenylene oxide polymer is commercially available as TENAX TA), 7.7 cm of silica gel, and 7.7 cm of coconut charcoal.

The desorber is capable of rapidly heating the trap at 2500. The trap should not be heated higher than 2500.

Condition the assembled trap, prior to initial use, at 2250 overnight with an inert gas at a flow rate of not less than 20 ml per min. Prior to use daily, condition the trap for 15 min at 225º.

Standard Solution

Accurately weigh 50 mg of trichloroethylene and 50 mg of toluene in a 50 ml volumetric flask. Dilute with methanol.

Calibration Solutions

Into five 50 ml volumetric flasks, accurately add 0.5, 1.0, 2.0, 3.0, and 5.0 ml of the Standard Solution and dilute with water. These calibration solutions correspond to the concentrations 10.2, 20.4, 40.8, 61.2 and 102 ng per for each solvent.

Chromatographic system

The purge and trap apparatus is connected to a gas chromatograph with a flame-ionisation detector.

Column: capillary column, 30 m, 0.32 mm diameter, 1 micron film thickness of dimethylpolysiloxane oil (such as DB-1, OV-1).
Temperature programme: 40º for 3 min, then raise to 220º at 40 per min.
Detector: 280º
Carrier gas: Helium
Purge gas: Nitrogen
Flow rate: 40 ml/min

Calibration

Introduce precisely 20 µl of each calibration solution on the wall (inner side)

of the sample purge. Desorb according to equipment instructions. Record the peak areas. Prepare calibration graphs of peak areas versus weight of each solvent introduced into the purge.

Procedure
Introduce on the fritted sparger of the sample purge an accurately weighed amount of sample (W), about 250 mg. Purge and desorb according to equipment instructions. Record the peak area of each solvent and read the corresponding weight (X) from the respective calibration curve.

Calculation
Calculate the amount of each residual solvent by the formula:

$$Residual\ solvent\ (mg/kg) = \frac{X\ (ng)}{W\ (mg)}$$

METHOD OF ASSAY

Principle
ß-Cyclodextrin is identified by *liquid chromatography* and quantified by comparison to reference standards containing standard cyclodextrins.

Preparation of sample solution
Weigh accurately about 500 mg of sample. Add 50 ml of twice-distilled water. Heat and stir until the sample has completely dissolved. Cool, adjust the total volume to 100 ml. Filter on a Millex HA 0.45 µm filter.

Preparation of standard solutions
Use USP grade alpha- and ß-cyclodextrin. Samples of gamma-cyclodextrin can be obtained from commercial suppliers such as Aldrich Chemical Co. or Sigma Chemical Co. Prepare three standard solutions (S_1, S_2 and S_3) containing increasing amounts (mg/kg) of alpha-cyclodextrin, ß-cyclodextrin and gamma-cyclodextrin as follows:

S_1 : 2.0 mg/kg A + 3.0 mg/kg B + 2.0 mg/kg G
S_2 : 3.5 mg/kg A + 5.0 mg/kg B + 3.5 mg/kg G
S_3 : 5.0 mg/kg A + 8.0 mg/kg B + 5.0 mg/kg G

where
A = alpha- cyclodextrin
B = ß-cyclodextrin
G = gamma-cyclodextrin

Apparatus
Liquid chromatograph maintained at a constant temperature of 25^o and equipped with a refractive index detector.

Conditions
Column
- length: 25 cm
- diameter: 4.6 mm
- packing: 5 µm octadecylsilane bonded to silica (Silica Cl8) with a guard column containing the same packing
Solvent: Water: methanol (94:6)
Flow rate: 0.7 ml/min

Procedure

Inject 10 µl of each of the 3 standard solutions. For each cyclodextrin draw a graph by plotting on the x axis the concentration in g/l and on the y axis the areas of the peaks. Inject 10 µl of the sample solution and determine the area of the eluted ß-cyclodextrin peak. The concentration of ß-cyclodextrin (L g/l) in the sample solution is then read from the graphs.

Calculation

Calculate the content of ß-cyclodextrin in the sample using the formula:

$$B = \frac{L}{C} x\ 100$$

where

B = percentage of ß-cyclodextrin in the sample

L = the concentration of ß-cyclodextrin in the sample solution as determined under "Procedure"

C = the concentration of sample in the sample solution in g/l.

DIACETYLTARTARIC and FATTY ACID ESTERS of GLYCEROL

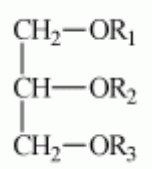
Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009) superseding specifications prepared at the 57th JECFA (2001) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-50 mg/kg bw was established at the 61st JECFA (2003).

SYNONYMS Diacetyltartaric acid esters of mono- and diglycerides; DATEM; Tartaric, acetic and fatty acid esters of glycerol, mixed; Mixed acetic and tartaric acid esters of mono and diglycerides of fatty acids; INS No. 472e

DEFINITION The product consists of mixed glycerol esters of mono- and diacetyltartaric acid and fatty acids from edible fats and oils. It is made by the interaction of diacetyltartaric anhydride and mono- and diglycerides of fatty acids in the presence of acetic acid, or by interaction of acetic anhydride and mono- and diglycerides of fatty acids in the presence of tartaric acid.
Because of inter- and intramolecular acyl-group exchange, both methods of production lead to the same essential components, the distribution of which depends on the relative proportions of the basic raw materials, on temperature, and on reaction time. The product may contain small amounts of free glycerol, free fatty acids, and free tartaric and acetic acids.

C.A.S. numbers 308068-42-0
100085-39-0

Structural formula



in which one or two of the R groups is a fatty acid moiety and the other R groups are either:

- diacetylated tartaric acid moiety
- monoacetylated tartaric acid moiety
- tartaric acid moiety
- acetic acid moiety
- hydrogen

DESCRIPTION Liquid, paste, or wax-like solid

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Dispersible in cold and hot water; soluble in methanol, ethanol, acetone, and ethyl acetate.

1,2-diols To a solution of 500 mg in 10 ml methanol, add dropwise, lead

	acetate TS. A white, flocculent, insoluble precipitate is formed.
Fatty acids (Vol. 4)	Passes test (See under “Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups; Test A: Methyl Esters of Fatty Acids”)
Acetic acid (Vol. 4)	Passes test (See under “Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups”)
Tartaric acid (Vol.4)	Passes test (See under “Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups”)
Glycerol (Vol. 4)	Passes test (See under “Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups”)
PURITY	
Acids (Vol. 4)	Acids other than acetic, tartaric and fatty acids, shall not be detectable (See under “Specific methods, Fats, Oils and Hydrocarbons; Identification Tests for Functional Groups”)
Sulfated ash (Vol. 4)	Not more than 0.5% determined at 800±25º Test 5 g of sample (Method I for solids; Method II for liquids) (See under, “General methods, Inorganic Components; Ash”)
Acid value (Vol. 4)	Not less than 40 and not more than 130 (See under, “Specific methods, Fats, Oils and Hydrocarbons”)
Total acetic acid	Not less than 8% and not more than 32% after hydrolysis See description under TESTS
Total tartaric acid	Not less than 10% and not more than 40% after saponification See description under TESTS
Total glycerol	Not less than 11% and not more than 28 % after saponification See description under TESTS
Free glycerol (Vol. 4)	Not more than 2.0% Prepare the aqueous extracts as directed under the "Procedure for 1-Monoglyceride (see under, "Specific methods, Fats, Oils and Hydrocarbons; 1-Monoglyceride and Free Glycerol Content") and proceed as directed under the "Procedure for Glycerol".
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

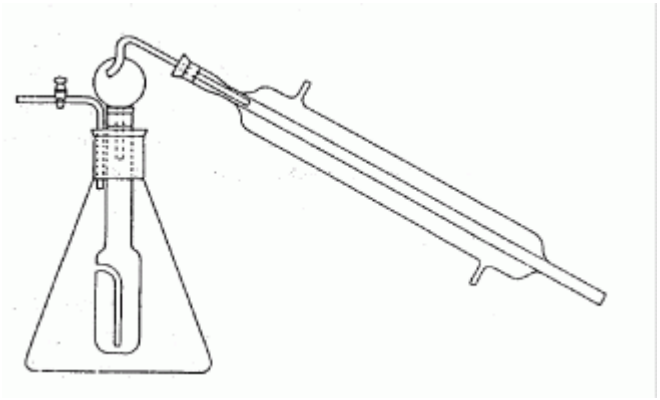
TESTS

PURITY TESTS

Total acetic acid

Apparatus

Assemble a modified Hortvet-Sellier distillation apparatus as shown in the figure, using a sufficiently large (approximately 38- x 203-mm) inner Sellier tube and large distillation trap.



Modified Hortvet-Sellier Distillation Apparatus

Procedure

Transfer 4 g of sample, accurately weighed, into the inner tube of the assembly, and insert the tube in the outer flask containing about 300 ml of recently boiled hot water. To the sample cautiously add 10 ml of approximately 4N perchloric acid [35 ml (60 g) of 70% perchloric acid in 100 ml of water], and connect the inner tube to a water-cooled condenser through the distillation trap. Distil by heating the outer flask so that 100 ml of distillate is collected within 20 to 25 min. Collect the distillate in 100-ml portions, add phenolphthalein TS to each portion, and titrate with 0.5N sodium hydroxide. Continue the distillation until a 100-ml portion of the distillate requires no more than 0.5 ml of 0.5N sodium hydroxide for neutralization. (CAUTION: Do not distil to dryness.) Calculate the weight, in mg, of volatile acids in the sample taken by the formula V x *e*, in which V is the total volume, in ml, of 0.5N sodium hydroxide consumed in the series of titrations and *e* is the equivalence factor 30.03.

Total tartaric acid

Sample Preparation

Transfer about 4 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, and add 80 ml of approximately 0.5N potassium hydroxide and 0.5 ml of phenolphthalein TS. Connect an air condenser at least 65 cm in length to the flask, and heat the mixture on a hot plate for about 2.5 h. Add to the hot mixture approximately 10% phosphoric acid until it is definitely acid to congo red test paper. Reconnect the air condenser, and heat until the fatty acids are liquefied and clear. Cool and then transfer the mixture into a 250-ml separator with the aid of small portions of water and hexane. Extract the liberated fatty acids with three

successive 25-ml portions of hexane and collect the extracts in a second separatory funnel. Wash the combined hexane extracts with two 25-ml portions of water and add the washings to the first separatory funnel containing the water layer. Transfer the contents of the first funnel to a 250-ml beaker, heat on a steam bath to remove traces of hexane, filter through acid-washed, fine-texture filter paper into a 500-ml volumetric flask, and finally dilute to volume with water (Solution I). Pipet 25.0 ml of this solution into a 100-ml volumetric flask, and dilute to volume with water (Solution II). Retain the rest of Solution I for the determination of Total glycerol.

Standard solutions and blank

Transfer 100 mg of reagent-grade tartaric acid, accurately weighed, into a 100-ml volumetric flask, dissolve it in about 90 ml of water, add water to volume, and mix well. Transfer 3.0-. 4.0-, 5.0-, and 6.0-ml portions into separate 19- x 150-mm matched cuvettes, and add sufficient water to make 10.0 ml. To each cuvette add 4.0 ml of a freshly prepared 1 in 20 solution of sodium metavanadate and 1.0 ml of acetic acid. (NOTE: Use these solutions within 10 min after colour development.) Prepare a blank in the same manner, using 10 ml of water in place of the tartaric acid solutions.

Sample solution

Transfer 10.0 ml of Solution II into a 19- x 150-mm cuvette and add 4.0 ml of a freshly prepared 1 in 20 solution of sodium metavanadate and 1.0 ml of acetic acid. (NOTE: Use this solution within 10 min after colour development.)

Analysis

Set the suitable spectrophotometer at zero with the blank. Then determine the absorbance of the four Standard solutions of tartaric acid and the Sample solution at 520 nm. From the data thus obtained, prepare a standard curve by plotting the absorbances on the ordinate against the corresponding quantities, in mg, of the tartaric acid on the abscissa. Then from the curve, determine the weight, in mg, of tartaric acid in the final dilution, multiply this by 20, and divide the result by the weight of the original sample to give the percentage of tartaric acid.

Total glycerol

Transfer 5.0 ml of Solution I prepared in the test for Total tartaric acid into a 250-ml glass-stoppered Erlenmeyer or iodine flask. Add to the flask 15 ml of glacial acetic acid and 25.0 ml of periodic acid solution, prepared by dissolving 2.7 g of periodic acid (H_5IO_6) in 50 ml of water, adding 950 ml of glacial acetic acid, and mixing thoroughly; protect this solution from light. Shake the mixture for 1 or 2 min, allow it to stand for 15 min, add 15 ml of potassium iodide solution (150 mg/ml) and 15 ml of water, swirl, and let stand 1 min. Titrate the liberated iodine with 0.1N sodium thiosulfate, using starch TS as the indicator. Perform a residual blank titration using water in place of the sample. The corrected volume is the number of ml of 0.1N sodium thiosulfate required for the glycerol and the tartaric acid in the sample represented by the 5 ml of Solution I. From the percentage of the tartaric acid determined in the test for Total tartaric acid, calculate the volume of 0.1N sodium thiosulfate required for the tartaric acid in the titration. The difference between

the corrected volume and the calculated volume required for the tartaric acid is the number of ml of 0.1N sodium thiosulfate consumed due to the glycerol in the sample. One ml of 0.1N sodium thiosulfate is equivalent to 2.303 mg of glycerol and to 7.505 mg of tartaric acid.

DIMETHYL DICARBONATE

Prepared at the 37th JECFA (1990), published in FNP 52 (1990). Metals and arsenic specifications revised at the 63rd JECFA (2004). Considered to be acceptable in accordance with GMP (max 250mg/l) at the 37th JECFA (1990)

SYNONYMS DMDC, dimethyl pyrocarbonate; INS No. 242

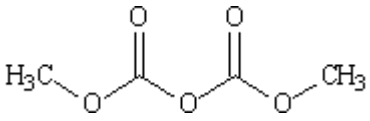
DEFINITION

Chemical names: Dimethyl dicarbonate

C.A.S. number: 004-525-33-1

Chemical formula: $C_4H_6O_5$

Structural formula:



Formula weight: 139.09

Assay: Not less than 99.8%

DESCRIPTION Colourless liquid.
Caution: Corrosive to eyes and skin and toxic by inhalation and ingestion; must be kept in a tightly sealed container to exclude moisture.

FUNCTIONAL USES Preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water with decomposition; miscible with toluene

Infrared absorption: The infrared spectrum of the sample corresponds with the reference infrared spectrum below

PURITY

Dimethyl carbonate: Not more than 0.2%
See description under TESTS

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Dimethyl carbonate

Apparatus

Gas chromatograph with a flame ionization detector, equipped for capillary gas chromatography, and inlet part for the "on column" technique (e.g., Carlo Erba Fraktovap 4160, or Hewlett-Packard 5880 or 5792).
- Capillary: 50 m SE 30-D (internal diameter: 0,3 mm).
- Potentiometric recorder, 1 mV.
- Microliter syringes with quartz needles, contents 0,010 ml, suitable for the "on column" technique (e.g., microliter syringe from SGE, type 5 A- SOC), (See Remark 5 below). Rolled rim glass vial, 10 ml content.
- Seal cap for the rolled rim vial with teflon coating.
- Closure tongs for the rolled rim vial
- Auxiliary for the peak area determination (e.g. HP calculator 3353).

Reagents

Methylisobutylketone, purest grade.

Operating conditions

Temperatures:
- Injection block: Room temperature (approximately 25^{o})
- Detector: 200^{o}
- Capillary Tube: Initial temperature 30^{o}; Preliminary phase 5 min, Rate of ascent 40^{o}/min; Final temperature 120^{o}, Final phase 5 min

Carrier gas:
- Preliminary pressure: 3.0 bar (Helium)
- Capillary tubes: Approx. 11 ml/min
- Make-up gas: 28 ml/min

Hydrogen: 35 ml/min
Air: 300 ml/min
Recorder feed: 1 cm/min

Procedure

Accurately weigh about 10 g of the sample to the nearest mg (W_1 mg) into a rolled rim vial. Add in a quantity of dimethyl-isobutylketone (W_2 mg), corresponding to the anticipated dimethylcarbonate content. Seal the rolled rim vial, mix well and inject 0.002 ml.

Retention times:
- Dimethylcarbonate: Approx. 2 min
- Methylisobutylketone: Approx. 4.5 min
- Dimethyldicarbonate: Approx. 8 min

Typical Chromatogram: See Figure below. Determine the peak areas of the standard (F2) and dimethylcarbonate (F1).

Calculation

Calculate the % of dimethyl carbonate from:

$$\frac{W2 \times F1 \times K \times 100}{F2 \times W1}$$

where
K = is a Correction Factor for Dimethylcarbonate
(See Remark 3, below)

Remarks
1. It should be noted the DMDC is sensitive to moisture and heat.
2. If so-called peak splits occur to some extent at the given gaschromatography conditions, then the peak areas are best determined by summation of the areas under both peaks.
3. The correction factor for dimethylcarbonate should be determined with corresponding test solutions in DMDC which is to the greatest extent possible free of dimethylcarbonate.
4. The specimen mixed with the standard must be measured immediately.
5. Hamilton syringes with Metal needles can result in a partial decomposition of the DMDC.

METHOD OF ASSAY

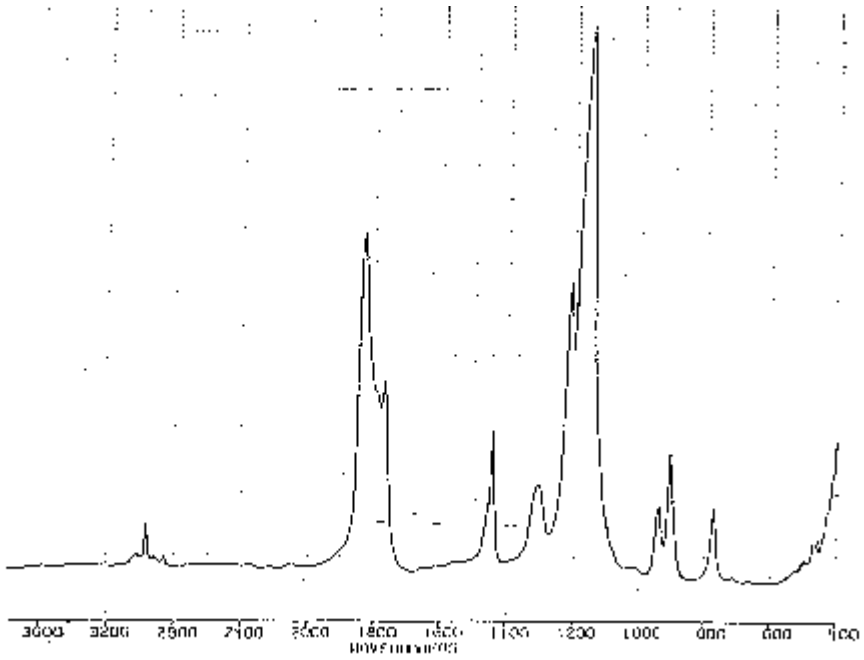
Introduce about 70 ml of pure acetone into a 150-ml glass beaker. Using a disposable 2 ml syringe weigh 1.0-1.3 g of the sample to an accuracy of ±0.1 mg into the glass beaker. Pipette exactly 20 ml dibutyl amine solution (add chlorobenzene to 120 g dibutyl amine until the 1 L mark is reached) while stirring. Titrate the solution potentiometrically with 1N hydrochloric acid. Run a blank test.

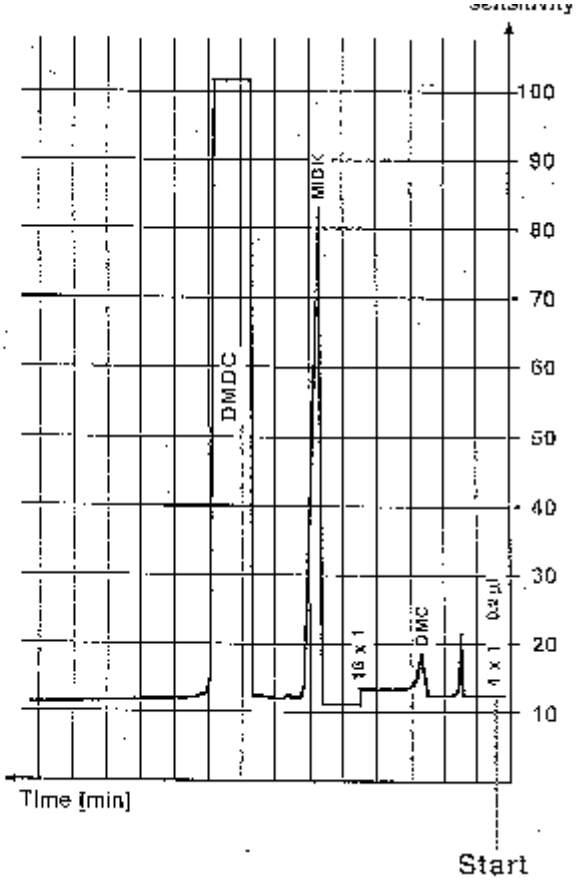
Calculate the % of dimethyldicarbonate from:

$$\frac{(V_2 - V_1) \times t \times 134.1 \times 100}{1000 \times W} = \frac{(V_2 - V_1) \times t \times 13.41}{M}$$

where
V_1 = amount of hydrochloric acid used for titration of the sample (ml)
V_2 = amount of hydrochloric acid used for titration of the blank (ml)
t = normality of hydrochloric acid
W = weight of sample (g)

Infrared spectrum Dimethyl dicarbonate





Typical chromatogram Dimethyl dicarbonate

DIPOTASSIUM 5'-GUANYLATE

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for guanylic acid and its Ca, K & Na salts, was established at the 29th JECFA (1985)

SYNONYMS Potassium guanylate, potassium 5'-guanylate, INS No. 628

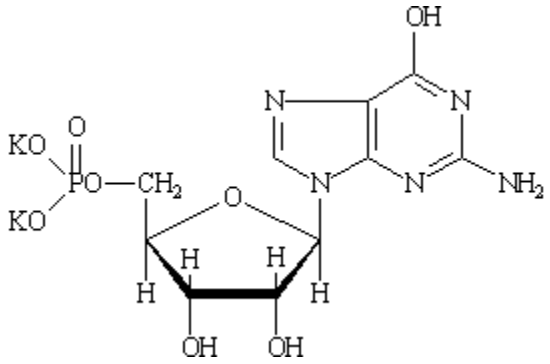
DEFINITION

Chemical names: Dipotassium guanosine-5'-monophosphate

C.A.S. number: 3254-39-5

Chemical formula: $C_{10}H_{12}K_2N_5O_8P$

Structural formula



Formula weight: 439.40

Assay: Not less than 97.0% and not more than 102.0% on the dried basis

DESCRIPTION Odourless, colourless or white crystals, or a white crystalline powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; practically insoluble in ethanol

Spectrophotometry (Vol. 4): A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 256±2nm. The ratio A250/A260 is between 0.95 and 1.03, and the ratio A280/260 is between 0.63 and 0.71.

Test for potassium (Vol. 4): Passes test

Test for ribose (Vol. 4): Passes test

Test for organic phosphate (Vol. 4): Passes test
Test 5 ml of a 1 in 20 solution

PURITY

Loss on drying (Vol. 4)	Not more than 5% (120°, 4 h)
pH (Vol. 4)	7.0 - 8.5 (1 in 20 soln)
Related foreign substances (Vol. 4)	Chromatographically not detectable Test 1 µl of a 1 in 200 solution
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 260 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of $C_{10}H_{12}K_2N_5O_8P$, in % by the formula:

$$\frac{A}{268.6} \times \frac{250,000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{loss on drying (\%)}} \times 100$$

DISODIUM 5'-GUANYLATE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for 5'guanylic acid and its Ca & Na salts was established at the 18th JECFA (1974)

SYNONYMS Sodium 5'-guanylate, sodium guanylate, GMP, INS No. 627

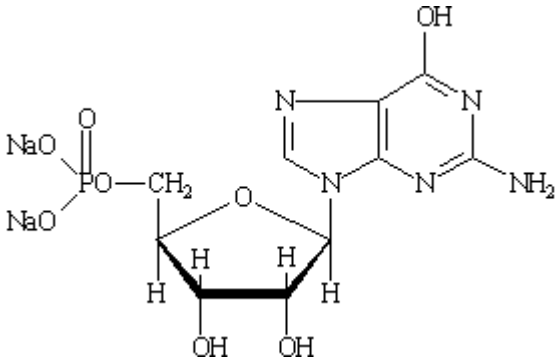
DEFINITION

Chemical names: Disodium guanosine-5'-monophosphate

C.A.S. number: 5550-12-9

Chemical formula: $C_{10}H_{12}N_5Na_2O_8P \cdot x\ H_2O$ (x = approximately 7)

Structural formula:



Formula weight: 407.19 (anhydrous)

Assay: Not less than 97.0% and not more than 102.0% calculated on the dried basis

DESCRIPTION Odourless, colourless or white crystals, or a white crystalline powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, sparingly soluble in ethanol, practically insoluble in ether

Spectrophotometry (Vol. 4): A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 256±2nm. The ratio A250/A260 is between 0.95 and 1.03, and the ratio A280/260 is between 0.63 and 0.71.

Test for sodium (Vol. 4): Passes test

Test for ribose (Vol. 4): Passes test

Test for organic: Passes test

phosphate (Vol. 4)	Test 5 ml of a 1 in 100 soln
PURITY	
Loss on drying (Vol. 4)	Not more than 25% (120º, 4 h)
pH (Vol. 4)	7.0 - 8.5 (1 in 20 soln)
Amino acids	Not detectable by the following test: To 5 ml of a 1 in 1,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.
Related foreign substances (Vol. 4)	Chromatographically not detectable Test 1 µl of a 1 in 200 soln
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 500 mg of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. To 10.0 ml of this solution add 0.01 N hydrochloric acid to make to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 260 nm using 0.01 N hydrochloric acid as the reference blank. Calculate the content of $C_{10}H_{12}N_5Na_2O_8P$, in % in the sample by the formula:

$$\frac{A}{289.8} \times \frac{250{,}000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{loss on drying \%}} \times 100$$

DISODIUM 5'-INOSINATE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for inosinic acid and its Ca, K & Na salts, was established at the 29th JECFA (1985)

SYNONYMS Sodium 5'-inosinate, sodium inosinate, IMP, INS No. 631

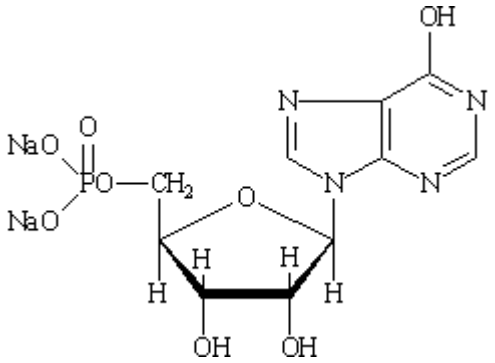
DEFINITION

Chemical names: Disodium inosine-5'-monophosphate

C.A.S. number: 4691-65-0

Chemical formula: $C_{10}H_{11}N_4Na_2O_8P \cdot x\ H_2O$ (x = approximately 7)

Structural formula:



Formula weight: 392.17 (anhydrous)

Assay: Not less than 97.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION Odourless, colourless or white crystals, or a white crystalline powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, sparingly soluble in ethanol, practically insoluble in ether

Spectrophotometry (Vol. 4): A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 250±2nm. The ratio A250/A260 is between 1.55 and 1.65, and the ratio A280/260 is between 0.20 and 0.30.

Test for sodium (Vol. 4): Passes test

Test for ribose (Vol. 4): Passes test

Test for organic phosphate (Vol. 4)	Passes test Test 5 ml of a 1 in 20 soln
PURITY	
Water (Vol. 4)	Not more than 29% (Karl Fischer Method)
pH (Vol. 4)	7.0 - 8.5 (1 in 20 soln)
Amino acids	Not detectable by the following test: To 5 ml of a 1 in 1,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.
Related foreign substances (Vol. 4)	Chromatographically not detectable Test 1 µl of a 1 in 200 soln
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 500 mg of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10.0 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 250 nm using 0.01 N hydrochloric acid as the reference blank. Calculate the content of $C_{10}H_{11}N_4Na_2O_8P$, in % in the sample by the formula:

$$\frac{A}{310} \times \frac{250{,}000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{water \%}} \times 100$$

DISODIUM 5'-RIBONUCLEOTIDES

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 18th JECFA (1974)

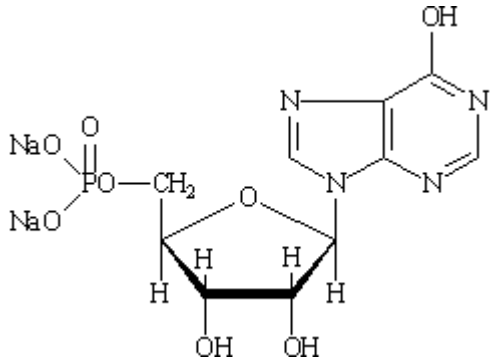
SYNONYMS Sodium 5'-ribonucleotides, sodium ribonucleotides, INS No. 635

DEFINITION

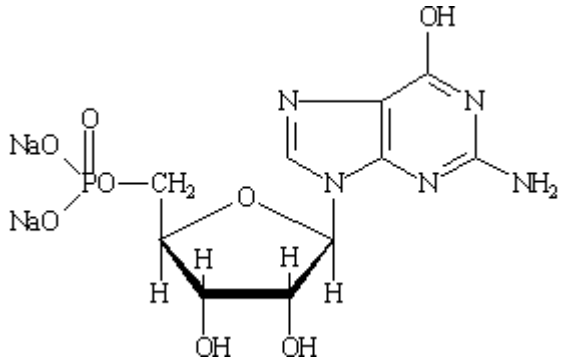
Chemical names (Mixture of) disodium inosine-5'-monophosphate and disodium guanosine-5'-monophosphate

Chemical formula $C_{10}H_{11}N_4Na_2O_8P \cdot x\ H_2O$ and
$C_{10}H_{12}N_5Na_2O_8P \cdot x\ H_2O$

Structural formula



Disodium inosine-5'-monophosphate



Disodium guanosine-5'-monophosphate

Assay Not less than 97% and not more than the equivalent of 102% of $C_{10}H_{11}N_4Na_2O_8P$ and $C_{10}H_{12}N_5Na_2O_8P$, calculated on the anhydrous basis. The proportion of $C_{10}H_{11}N_4Na_2O_8P$ or $C_{10}H_{12}N_5Na_2O_8P$ to the sum of them is between 47% and 53%.

DESCRIPTION Odourless, white or off-white crystals or powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol; practically insoluble in ether
Test for ribose (Vol. 4)	Passes test Test 1 ml of a 1 in 2,000 soln
Test for organic phosphate (Vol. 4)	Passes test Test 5 ml of a 1 in 20 soln
Test for inosinic acid	To 1 ml of a 1 in 1,000 solution add 2 ml of 10% hydrochloric acid and 0.1 g of zinc powder, heat in a water bath for 10 min, and filter. Cool the filtrate in ice water, add 1 ml of a 3 in 1,000 sodium nitrite solution, shake well, and allow to stand for 10 min. Add 1 ml of a 1 in 700 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution. A violet red colour is produced.
Test for guanylic acid	To 1 ml of a 1 in 5,000 solution add 1 ml of 10% hydrochloric acid, heat in a water bath for 10 min, cool, and add 0.5 ml of Folin-Ciocalteu TS and 2 ml of saturated sodium carbonate solution. A blue colour is produced.
Test for sodium (Vol. 4)	Passes test

PURITY

Water (Vol. 4)	Not more than 27% (Karl Fischer Method)
pH (Vol. 4)	7.0 - 8.5 (1 in 20 soln)
Amino acids	Not detectable by the following test: To 5 ml of a 1 in 1,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.
Related foreign substances (Vol. 4)	Chromatographically not detectable Test 1 µl of a 1 in 200 soln. Only inosinic acid and 5'-guanylic acid are detected
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Calculate the contents of disodium inosine-5'-monophosphate (I) and disodium guanosine-5'-monophosphate (G) in the sample by the following equation, using values for I and G obtained as described below.

$$Content\ (\%) = \frac{I + G}{100 - water\ (\%)} \times 100$$

Weigh accurately about 650 mg of the sample, and dissolve in water to make 500 ml (Solution A).

To determine I (disodium inosine-5'-monophosphate), take a 1-ml portion

of Solution A, add 4 ml of 6 N hydrochloric acid and water to make to 10 ml. Heat in a water bath for 40 min, cool, add 0.4 g of zinc powder, allow to stand for 50 min, shaking occasionally and vigorously, and add water to make to 20 ml. Filter through filter paper. To a 10-ml portion of the filtrate add 1 ml of 6 N hydrochloric acid, and add 1 ml of a 1 in 1,000 sodium nitrite solution, cooling in an ice-water bath. Shake well, allow to stand for 10 min, add 1 ml of a 1 in 200 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution, shake well, allow to stand for 15 min at room temperature, and add water to make to 20 ml (Sample solution). For the control, prepare in the same manner as the sample, using 1 ml of water instead of Solution A.

Determine the absorbance of the sample solution at 515 nm against the control solution.

To prepare calibration curves, weigh accurately about 3 mg each of disodium 5'-inosinate and disodium 5'-guanylate, and dissolve respectively in 100 ml of 0.01 N hydrochloric acid. Determine the absorbance at 250 nm on the solution of disodium 5'-inosinate and at 260 nm on the solution of disodium 5'-guanylate, using 0.01 N hydrochloric acid as the control. Determine the molecular extinction coefficients E_I and E_G, and calculate the contents of (I) disodium inosine-5'-monophosphate and (G) disodium guanosine-5'-monophosphate by the equations:

$$Content\ (\%)\ of\ I = \frac{E_I}{12,160} \times 100$$

$$Content\ (\%)\ of\ G = \frac{E_G}{11,800} \times 100$$

Weigh accurately a quantity of each which is equivalent to about 50 mg, combine and dissolve in water to make 200 ml (Solution B). To 1-ml, 2-ml and 3-ml portions of Solution B add 4 ml of 6 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed for preparing Sample Solution from Solution A. Determine the absorbance of each Standard Solution at 515 nm and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of I (disodium inosine-5'-monophosphate) from the calibration curve and the absorbance of Sample Solution.

To determine G (disodium guanosine-5'-monophosphate), take 1 ml of Solution A, add 4 ml of 2 N hydrochloric acid and water to make to 10 ml. Heat in a water bath for 30 min, cool, add 2 ml of Folin-Ciocalteu TS and 5 ml of a 4 in 5 sodium carbonate solution. Allow to stand for 15 min, and add water to make to 50 ml. Centrifuge if necessary, and use the supernatant for the test (Sample Solution).

Prepare the control in the same manner as the Sample Solution, using 1 ml of water instead of Solution A. Determine the absorbance of the Sample Solution at 750 nm.

To 1-ml, 2-ml and 3-ml portions of Solution B, add 4 ml of 2 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed in preparing Sample Solution. Determine the absorbance of each Standard Solution at 750 nm, and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of G (disodium guanosine-5'-monophosphate) from the calibration curve and the absorbance of Sample Solution.

ERYTHORBIC ACID

Prepared at the 37th JECFA (1990), published in FNP 52 (1992) superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI 'not specified' was established at the 37th JECFA (1990)

SYNONYMS Isoascorbic acid, D-araboascorbic acid, INS No. 315

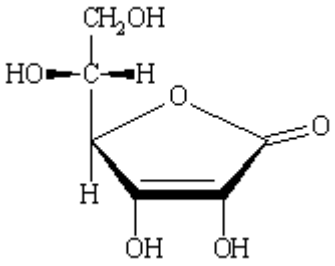
DEFINITION

Chemical names D-Erythro-hex-2-enoic acid delta-lactone, isoascorbic acid, D-isoascorbic acid

C.A.S. number 89-65-6

Chemical formula $C_6H_8O_6$

Structural formula



Formula weight 176.13

Assay Not less than 99% on the dried basis

DESCRIPTION White to slightly yellow crystalline solid which darkens gradually on exposure to light

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water, soluble in ethanol

Melting range (Vol. 4) About 164 - 172° with decomposition

Test for ascorbate (Vol. 4) Passes test

Reducing reaction	A solution of the sample in water immediately reduces potassium permanganate TS without heating, producing a brown precipitate A solution of the sample in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol TS

PURITY

Loss on drying (Vol. 4)	Not more than 0.4% (reduced pressure, silica gel, 3 h)
Specific rotation (Vol. 4)	[alpha] 25, D: Between -16.5 and -18^{o}
Sulfated ash (Vol. 4)	Not more than 0.3% Test 1 g of the sample (Method I)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.4 g of the sample, previously dried, and dissolve in a mixture of 100 ml of water, recently boiled and cooled, and 25 ml of diluted sulfuric acid TS. Titrate the solution immediately with 0.1 N iodine, adding starch TS near the end point. Each ml of 0.1 N iodine is equivalent to 8.806 mg of $C_6H_8O_6$.

ERYTHRITOL

New specification prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7. ADI "not specified", established at the 53rd JECFA in 1999.

SYNONYMS Meso-erythritol; tetrahydroxybutane; erythrite.

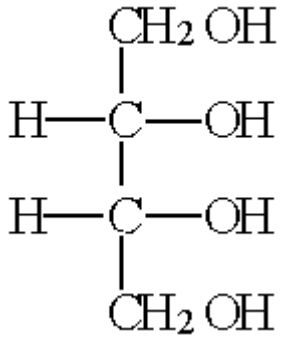
DEFINITION Obtained by fermentation of starch enzyme hydrolysate (from starches such as wheat and corn) by safe and suitable food grade osmophilic yeasts such as *Moniliella pollinis* or *Trichosporonoides megachilensis.* The heat-sterilized broth is filtered, purified by ion exchange resin, activated charcoal and ultrafiltration, crystallised washed and dried.

Chemical names 1,2,3,4-Butanetetrol

C.A.S. number 149-32-6

Chemical formula $C_4H_{10}O_4$

Structural formula



Formula weight 122.12

Assay Not less than 99% after drying

DESCRIPTION White, odourless, non-hygroscopic, heat-stable crystals. It has a sweetness approximately 60-80% that of sucrose.

FUNCTIONAL USES Flavour enhancer, humectant, carrier, sweetener.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water, slightly soluble in ethanol, insoluble in diethyl ether

Melting range (Vol. 4) Between 119 and 123º

Main Peak in HPLC The retention time of the major peak in the chromatogram of the Assay Solution corresponds to that in the chromatogram of the Standard Solution obtained in the Assay.

PURITY

Loss on drying (Vol. 4)	Not more than 0.2% (70°, 6 h, in a vacuum desiccator)
Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Reducing substances (Vol.4)	Not more than 0.3% calculated as D-glucose (Method I)
Ribitol and glycerol	Not more than 0.1% See description under TESTS
Lead (Vol. 4)	Not more than 0.5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

PURITY TESTS

Ribitol and glycerol

In the chromatogram obtained from the *Assay Solution* as directed in the *Procedure* under *Method of Assay*, the elution pattern may include individual minor peaks representing glycerol and ribitol. The retention times for ribitol and glycerol, relative to erythritol (1.0), are approximately 0.93 and 1.10, respectively. Measure the peak responses for erythritol (*E*), glycerol (*G*), and ribitol (*R*), and calculate the total area (*T*). Calculate the percentage of glycerol in the sample by the formula % glycerol = 100*G/T*, and the percentage of ribitol by the formula % ribitol = 100*R/T*. The sum of the % glycerol and the % ribitol is not greater than 0.1%.

METHOD OF ASSAY

Determine the erythritol content of the sample by liquid chromatography.

Mobile phase
Deionized water

Standard Solution
Transfer about 2 g of Standard Erythritol, previously dried in a vacuum desiccator at 70° for 6 hr and accurately weighed to the nearest 0.1 mg (*W*), into a 50 ml volumetric flask, dissolve in and dilute to volume with deionized water and mix. Filter the solution through a disposable 0.45 µm filter before use in the 'Procedure'.

(Standard Erythritol may be obtained from Cerestar, EBS Vilvoorde R&D Centre, Centre of Fermentation Expertise, 84 Havenstraat, 1800 Vilvoorde, Belgium; Mitsubishi Chemical Corporation, Speciality Chemicals Company, Intermediate Chemicals Department, 5-2 Marunonchi 2-chome, Chiyodaq-ku, Tokyo 100-0005, Japan; or Nikken Chemicals Co., Ltd., Development Department, Sumitomo-Tsukiji Bldg., No. 4-414, kTsukiji 5-chome, Chuo-ku, Tokyo, 104-0045, Japan.

Assay

Prepare as directed for 'Standard Solution', using about 2 g of the sample, previously dried in a vacuum desiccator at 70º for 6 h and accurately weighed to the nearest 0.1 mg (*w*).

Chromatographic System

Use a high-pressure liquid chromatograph equipped with a constant-flow pulseless pump and fitted with a sensitive differential refractive index detector such as the RID-6A or equivalent. The column is packed with a strong cation exchange resin in the hydrogen form, such as MCI Gel-CK08EH, Shodex KC-811 or equivalent, consisting of a macroreticular sulfonated polystyrene-divinylbenzene copolymer, 8% crosslinked, 9 µm particle size. The column temperature is 60ºC. . The sample injector is preferably of the fixed-loop type (manual or automatic), capable of accurately injecting 30 µl. The integrator can be any modern data acquisition system with recording and processing capabilities. The operating flow rate is about 0.5 ml/min. The maximum pressure of the total system is about 50 kgf/cm^2.

System Start-up

Connect the injector outlet to the column inlet, and connect the column outlet directly to waste. Activate the pump and elute the system at a flow rate of 0.1 ml/min. Set the pressure limit control to about 15 kgf/cm^2 above the normal operating pressure. Increase the flow rate by increments of 0.1 ml/min up to the operating rate, and elute the column for 2 hours. Connect the column outlet to the detector tube, flush both the reference and sample cells for 30 min, and then zero the refractometer and adjust the sensitivity

System Suitability Test

The area responses of triplicate 30-µl injections of the *Standard Solution* show a relative standard deviation (100 × standard deviation/mean peak area) of not more than 1.0%.

Procedure

Chromatograph triplicate 30-µl portions of the *Standard Solution* and record the mean of the erythritol peak areas as *A*. In a similar manner, chromatograph triplicate 30-µl portions of the *Assay Solution* and record the mean of the erythritol peak areas as *a*.

Calculate the percentage of erythritol in the sample by the formula:
% Erythritol = 100(*W*/*w*)(*a*/*A*).

ERYTHROSINE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-0.1 mg/kg bw was established at the 37th JECFA (1991)

SYNONYMS CI Food Red 14, FD&C Red No. 3; C.I. (1975) No. 45430 INS No. 127

DEFINITION Consists essentially of disodium salt of 9-(o-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone monohydrate and subsidiary colouring matters together with water, sodium chloride and/or sodium sulfate as the principal uncoloured components.

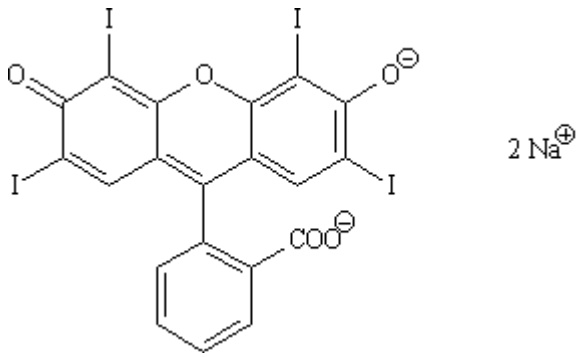
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* shall apply.

Chemical names Disodium salt of 9-(o-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone monohydrate

C.A.S. number 16423-68-0

Chemical formula $C_{20}H_6I_4Na_2O_5 \cdot H_2O$

Structural formula



Formula weight 897.88

Assay Not less than 87% total colouring matters

DESCRIPTION Red powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water and in ethanol

Identification of colouring matters (Vol. 4) Passes test

PURITY

Loss on drying at 135° (Vol. 4)	Not more than 13% together with chloride and sulfate calculated as sodium salts
Inorganic iodides	Not more than 0.1% calculated as sodium iodide See description under TESTS
Water insoluble matter (Vol. 4)	Not more than 0.2%
Zinc (Vol. 4)	Not more than 50 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."
Subsidiary colouring matters (Vol. 4)	Not more than 4% (except fluorescein) Use the following conditions: Developing solvent: No. 5 Height of ascent of solvent front: 17 cm Note: Take special care not to allow the chromatograms to be exposed to direct sunlight.
Fluorescein	Not more than 20 mg/kg See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Tri-iodoresorcinol: Not more than 0.2% 2-(2,4-dihydroxy-3,5-di-iodobenzoyl) benzoic acid: Not more than 0.2% Proceed as directed under *Column chromatography*, using, for example, the following absorptivities: 2(2,4-dihydroxy-3,5-di-iodobenzoyl)benzoic acid: 0.047 mg $L^{-1}cm^{-1}$ at 348 nm (alkaline) Tri-iodoresorcinol: 0.079 mg $L^{-1}cm^{-1}$ at 223 nm (acidic).
Ether extractable matter (Vol. 4)	From a solution of pH not less than 7, not more than 0.2%

TESTS

PURITY TESTS

Inorganic iodides	Weigh 1.0 g of the sample into a 100-ml beaker. Add 75 ml distilled water and the magnetic follower. Stir to dissolve. Immerse a iodide specific electrode and a reference electrode in the solution and set a suitable millivoltmeter to read the potential of the system in millivolts. Add 0.001 M silver nitrate solution from a burette initially in 0.5 ml aliquots, reducing these to 0.1 ml as the end-point approaches as indicated by an

increasing change in potential for each addition. After allowing time for the reading to stabilize, record the millivolt readings after each addition. Continue the titration until further additions make little change in the potential.

Plot the millivolt readings against the volume of silver nitrate solution added. The equivalent point is the volume corresponding to the maximum slope of the curve. The percentage of sodium iodide in sample is: Titre x 0.015%

Fluorescein

Principle
The fluorescein is separated from the sample by TLC and compared with a standard chromatogram prepared from fluorescein at the concentration corresponding to the limit figure.

Solvent
Methanol+water+ammonia (s.g. 0.890) (500 ml+400 ml+100 ml)

Sample
Weigh 1.0 g of the sample, dissolve in about 50 ml solvent and dilute to 100 ml in a volumetric flask.

Standard
Weigh an amount of fluorescein, previously purified by recrystallisation from ethanol, equal to 1 g x the colouring matter content of the sample as determined under Assay. Dissolve in water (or in water with 10 ml ammonia s.g. 0.890 if fluorescein-free acid is being used) and dilute to 100 ml. Make further sequential dilutions as follows:
- 1 ml to 100 ml with water
- 1 ml to 100 ml with water
- 20 ml to 100 ml with solvent

Chromatography solvent
n-Butanol+water+ammonia (s.g. 0.890)+ethanol (100 ml+44 ml+1 ml+ 22.5 ml)

Procedure
Spot 25 µl of the sample and standard solutions side by side on a cellulose plate. Develop for 16 h in the chromatography solvent. Allow the plate to dry. View under a UV light source and compare the fluorescence of the standard with the fluorescence of the corresponding area on the chromatogram of the sample. The intensity of the latter shall not be greater than that of the former.

Note**:** Take special care not to allow the chromatograms to be exposed to direct sunlight.

METHOD OF ASSAY

Dissolve about 1 g of the sample, accurately weighed, in 250 ml of water, transfer to a clean 500-ml beaker, add 8.0 ml of 1.5 N nitric acid and stir well. Filter through a sintered glass crucible (porosity 3, diameter 5 cm) which has been weighed containing a small glass stirring rod. Wash thoroughly with 0.5% nitric acid until the filtrate gives no turbidity with silver nitrate TS, and then wash with 30 ml water. Dry to constant weight at 135±5º, carefully breaking up the precipitate by means of the glass rod.

Cool in a desiccator and weigh.

$$Total\ colouring\ matters = \frac{weight\ of\ residue\ x\ 107.4}{weight\ of\ sample}\ \%$$

Determination of Hydrochloric Acid-insoluble Matter in Erythrosine Lake

Reagents
- Concentrated hydrochloric acid
- Hydrochloric acid 0.5% v/v
- Dilute ammonia solution (dilute 10 ml ammonia, s.g. 0.890 to 100 ml with water).

Procedure
Accurately weigh approximately 5 g of the lake into a 500-ml beaker. Add 250 ml water and 60 ml concentrated hydrochloric acid. Boil to dissolve the alumina while the Erythrosine converts to its "free acid" form, which is insoluble in acid. Filter through a tared No. 4 sintered glass crucible. Wash the crucible with a small amount of hot 0.5% hydrochloric acid and then with some hot distilled water. Remove the acid filtrate from the filter flask, replace the crucible and wash with hot dilute ammonia solution until the washings are colourless. Dry the crucible to constant weight at 135º. Express the residue as a percentage of the weight taken.

CALCIUM DISODIUM ETHYLENEDIAMINETETRAACETATE

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-2.5 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Calcium disodium EDTA, calcium disodium edetate; INS No. 385

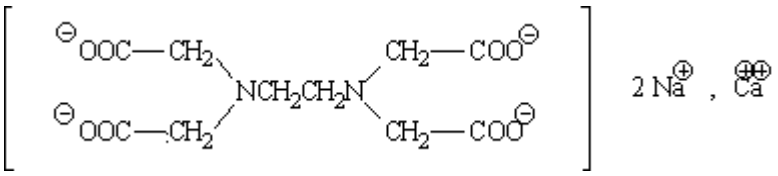
DEFINITION

Chemical names: N,N'-1,2-Ethanediylbis[N-(carboxymethyl)-glycinate](4-)-N,N',O,O',O^N,O^N]calciate(2-)disodium; Calcium disodium ethylenediaminetetraacetate; Calcium disodium (ethylene-dinitrilo)-tetraacetate.

C.A.S. number: 662-33-9

Chemical formula: $C_{10}H_{12}CaN_2Na_2O_8 \cdot 2H_2O$

Structural formula:



Formula weight: 410.31

Assay: Not less than 97% and not more than the equivalent of 102% calculated on the anhydrous basis

DESCRIPTION White, odourless crystalline granules or a white to nearly white powder; slightly hygroscopic

FUNCTIONAL USES Sequestrant, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, practically insoluble in ethanol.

Test for calcium (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

Chelating activity to metal ions: To 5 ml of water in a test tube add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS. A deep red solution develops. Add about 50 mg of the sample and mix. The deep red colour disappears.

PURITY

pH (Vol. 4): 6.5 - 7.5 (1 in 100 soln).

Magnesium chelating substances

Transfer 1 g of the sample, accurately weighed, to a small beaker, and dissolve it in 5 ml of water. Add 5 ml of buffer solution prepared by dissolving 67.5 g of ammonium chloride in 200 ml of water, adding 570 ml of strong ammonia TS, and diluting with water to 1000 ml. To the buffered solution add 5 drops of eriochrome black TS, and titrate with 0.1 M magnesium acetate to the appearance of a deep wine-red colour. Not more than 2.0 ml should be required.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Transfer about 1.2 g of the sample, accurately weighed, into a 250-ml beaker, and dissolve in 75 ml of water. Add 25 ml of dilute acetic acid TS and 1.0 ml of diphenylcarbazone solution (1 g in 100 ml ethanol). Titrate slowly with 0.1 M mercuric nitrate (see below) to the first appearance of a purplish colour. Each ml of 0.1 M mercuric nitrate is equivalent to 37.43 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

Mercuric nitrate solution:
Dissolve about 35 g of mercuric nitrate $Hg(NO_3)_2 \cdot H_2O$ in a mixture of 5 ml of nitric acid and 500 ml of water and dilute with water to 1000 ml. Standardize the solution as follows: Transfer an accurately measured volume of about 20 ml of the solution into an Erlenmeyer flask and add 2 ml of nitric acid and 2 ml of ferric ammonium sulfuric TS. Cool to below 20^o and titrate with 0.1 N ammonium thiocyanate to the first appearance of a permanent brownish colour. Calculate the molarity. (0.1 M = 32.46 g $Hg(NO_3)_2$ per litre).

DISODIUM ETHYLENEDIAMINETETRAACETATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-2.5 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Disodium EDTA, disodium edetate; INS No. 386

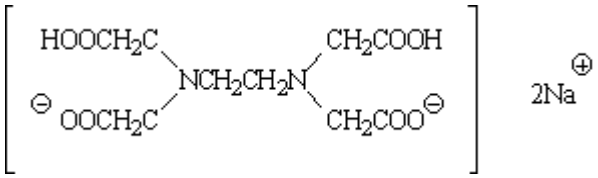
DEFINITION

Chemical names: Disodium salt of N,N'-1,2-Ethanediylbis[N-(carboxymethyl)glycine]; disodium dihydrogen ethylenediaminetetraacetate; disodium dihydrogen (ethylene-dinitrilo)-tetraacetate

C.A.S. number: 139-33-3

Chemical formula: $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$

Structural formula:



Formula weight: 372.24

Assay: Not less than 99%

DESCRIPTION White, odourless crystalline granules or a white to nearly white powder

FUNCTIONAL USES Sequestrant, antioxidant synergist, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, practically insoluble in ethanol

Infrared absorption: The infrared spectrum of the sample corresponds with that of a reference standard (a Reference standard may be obtained from the U.S. Pharmacopeia, 12601 Twin Brook Parkway, Rockville, Maryland 20852)

Test for sodium (Vol. 4): Passes test

Chelating activity to metal ions: To 5 ml of water in a test tube add 2 drops of ammonium to thiocyanate TS and 2 drops of ferric chloride TS. A deep red solution develops. Add about 50 mg of the sample and mix. The deep red colour disappears.

PURITY

pH (Vol. 4): 4.3 - 4.7 (1 in 100 soln)

Nitrilotriacetic acid: Passes test
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Nitrilotriacetic acid

Stock test solution
Transfer 10 g of the sample into a 100-ml volumetric flask, dissolve in 40 ml of a (1 in 10) potassium hydroxide solution, dilute to volume with water, and mix.

Diluted stock test solution
Pipette 10 ml of the "stock test solution" into a 100-ml volumetric flask, dilute to volume with water, and mix.

Test preparation
Pipette 20 ml of the "diluted stock test solution" into a 150-ml beaker, add 1 ml of a (1 in 10) potassium hydroxide solution, 2 ml of a (1 in 10) ammonium nitrate solution, and about 50 mg of eriochrome black T indicator, and titrate with a (3 in 100) cadmium nitrate solution to a red end-point. Record the volume, in ml, of the titrant required as V, and discard the solution.

Pipette 20 ml of the "diluted stock test solution" into a 100-ml volumetric flask, and add the volume V of the (3 in 100) cadmium nitrate solution required in the initial titration, plus 0.05 ml in excess. Add 1.5 ml of a (1 in 10) potassium hydroxide solution, 10 ml of a (1 in 10) ammonium nitrate solution, and 0.5 ml of methyl red TS, then dilute to volume with water and mix.

Stock standard solution
Transfer 1.0 g of nitrilotriacetic acid into a 100-ml volumetric flask, dissolve in 10 ml of a (1 in 10) potassium hydroxide solution, dilute to volume with water, and mix.

Dilute stock standard solution
Pipette 1 ml of the "stock standard solution" and 10 ml of the "stock test solution" into a 100-ml volumetric flask, dilute to volume with water, and mix.

Standard preparation
Proceed as directed under "test preparation", using "diluted stock standard solution" where "diluted stock test solution" is specified.

Polarographic test
Rinse a polarographic cell with a portion of the "standard preparation", then add a suitable volume to the cell, immerse it in a constant-temperature bath maintained at 25 ± 0.5^{o}, and de-aerate by bubbling oxygen-free nitrogen

through the solution for 10 min. Insert the dropping mercury electrode of a suitable polarograph, and record the polarogram from -0.6 to -1.2 volts at a sensitivity of 0.006 microampere per mm, using a saturated calomel electrode as the reference electrode. In the same manner, obtain a polarogram for a portion of the "test preparation". The diffusion current observed with the "test preparation" is not greater than 10% of the difference between the diffusion currents observed with the "standard preparation" and the "test preparation", respectively. (Note: An extra polarographic wave appearing ahead of the nitrilotriacetic acid-cadmium complex wave is probably due to uncomplexed cadmium. This wave should be ignored in measuring the diffusion current).

METHOD OF ASSAY

Transfer about 5 g, accurately weighed, of the sample, into a 250-ml volumetric flask, dissolve in water, dilute to volume and mix, to give the assay preparation. Place about 200 mg, accurately weighed, of reagent grade calcium carbonate of known purity in a 400-ml beaker, add 10 ml of water and swirl to form a slurry. Cover the beaker with a watch glass and introduce 2 ml of dilute hydrochloric acid TS from a pipette inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the outer surface of the pipette, the watch glass and the sides of the beaker, and dilute to about 100 ml with water. While stirring the solution, preferably with a magnetic stirrer, add about 30 ml of the assay preparation from a 50-ml burette. Add 15 ml of sodium hydroxide TS, 300 mg of hydroxynaphthol blue indicator and continue the titration with the assay preparation to a blue end point. Calculate the percentage of $C_{10}H_{14}N_2Na_2O_8$ " $2H_2O$ in the sample by the formula:

$$\%\, disodium\ EDTA = \frac{92{,}980 \times W_{CaCO_3}}{V_{ASSAY} \times W_{SAMPLE}}$$

where
W_{CaCO3} = the weight in grams of calcium carbonate;
V_{ASSAY} = the volume in ml of assay preparation; and
W_{SAMPLE} = the weight in gram of the sample taken.

ETHYL CELLULOSE

Prepared at the 26th JECFA (1982), published in FNP 25 (1982) and FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' was established at the 35th JECFA (1989).

SYNONYMS INS No. 462

DEFINITION Ethyl ether of cellulose, prepared from wood pulp or cotton by treatment with alkali and ethylation of the alkali cellulose with ethyl chloride. The article of commerce can be specified further by viscosity.

Chemical names Cellulose ethyl ether, ethyl ether of cellulose

C.A.S. number 9004-57-3

Assay Not less than 44% and not more than 50% of ethoxyl groups ($-OC_2H_5$) on the dried basis (equivalent to not more than 2.6 ethoxyl groups per anhydroglucose unit).

DESCRIPTION Free-flowing, white to light tan powder

FUNCTIONAL USES Tabletting aid, binder, filler, diluent of colour and other food additives

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water, in glycerol, and in propane-1,2-diol, but soluble in varying proportions in certain organic solvents, depending upon the ethoxyl content. Ethyl cellulose containing less than 46-48% of ethoxyl groups is freely soluble in tetrahydrofuran, in methyl acetate, in chloroform, and in aromatic hydrocarbon ethanol mixtures. Ethylcellulose containing 46-48% or more of ethoxyl groups is freely soluble in ethanol, in methanol, in toluene, in chloroform, and in ethyl acetate.

Film forming test Dissolve 5 g of the sample in 95 g of an 80:20 (w/w) mixture of toluene-ethanol. A clear, stable, slightly yellow solution is formed. Pour a few ml of the solution onto a glass plate, and allow the solvent to evaporate. A thick, tough continuous, clear film remains. The film is flammable.

pH (Vol. 4) Neutral to litmus (1 in 20 suspension)

PURITY

Loss on drying (Vol. 4) Not more than 3% (105°, 2 h)

Sulfated ash (Vol. 4) Not more than 0.4%
Test 1 g of the sample (Method I)

Lead (Vol. 4) Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Determine the ethoxyl content as directed under *Ethoxyl and Methoxyl Group Determination* (see Volume 4).

ETHYL HYDROXYETHYL CELLULOSE

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). A group ADI 'not specified' was established at the 35th JECFA (1989)

SYNONYM INS No. 467

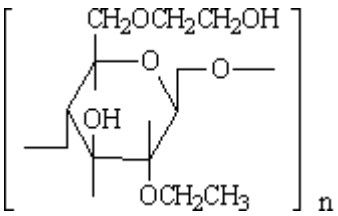
DEFINITION Ethyl Hydroxyethyl Cellulose is cellulose in which both ethyl and hydroxyethyl groups are attached to the anhydroglucose units by ether linkages. Ethyl hydroxyethyl cellulose is prepared from cellulose by treatment with alkali, ethylene oxide and ethyl chloride. The article of commerce may be specified further by the viscosity of its aqueous solutions.

Chemical names: Ethyl hydroxyethyl cellulose, 2-hydroxyethyl ether of ethyl cellulose

C.A.S. number: 9004-58-4

Chemical formula: $[C_6H_7O_2(OH)_x(OC_2H_5)_y[O(CH_2CH_2O)_mH]_z]_n$
where n is the degree of polymerisation
x + y + z = 3
y = 0.7 - 1.5 (degree of ethyl substitution, DS)
m + z = 0.5 - 2.5 (molar hydroxyethyl substitution, MS)

Structural formula



Possible structural formula for a repeating unit of an ethyl hydroxyethyl cellulose with a DS = 1.0 and a MS = 1.0

Formula weight:
Unsubstituted structural unit: 162.14
Structural unit with DS = MS = 1.0: 234
Structural unit with DS = 1.0; MS = 2.0: 278
Structural unit with DS = 1.5; MS = 0.5: 226
Macromolecules: from about 40,000 (n about 175)
up to about 350,000 (n about 1,300)

Assay: Not less than 7% and not more than 19% of ethoxyl groups ($-OC_2H_5$), and not less than 10% and not more than 38% of oxyethylene groups ($-OCH_2CH_2-$), on the dried and salt-free basis.

DESCRIPTION Hygroscopic white or slightly yellowish or greyish, odourless granules or fine powder.

FUNCTIONAL USES Emulsifier, stabilizer, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Swelling in water, producing a clear to opalescent, viscous, colloidal solution; insoluble in boiling water and ethanol.
Foam formation	Vigorously shake a 0.1% solution of the sample. A layer of foam appears. This test permits the distinction of cellulose ethers from sodium carboxymethyl cellulose, alginates, and natural gums.
Precipitate formation	To 5 ml of an 0.5% solution of the sample add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. This test permits the distinction of cellulose ethers from sodium carboxymethyl cellulose, gelatine, carob bean gum and tragacanth.
Precipitate formation by warming	A 1% water solution of a sample is slowly heated from room temperature, while stirring. At a distinct temperature above 35°, a cloudy precipitate occurs which disappears completely during cooling.
Substituent content	Determine the substituents by gas chromatography as given in Method of Assay

PURITY

Loss on drying (Vol. 4)	Not more than 10% (105° , to constant weight)
Chlorides	Not more than 2% See description under Tests
Ethylene oxides, 1,4-dioxane and ethylene chlorohydrin	Not more than 0.5 mg/kg, individually See description under TESTS
Mono and diethylene glycol	Not more than 1%, individually or in combination See description under TESTS
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Chlorides	Weigh 2 g of dried dried sample, and disperse in about 100 ml of boiling, distilled water by swirling the flask. When the solution is homogeneous, or after at least one hour, add a pinch of sodium bicarbonate and exactly 1.0 ml of the potassium chromate TS indicator. Titrate with 0.1 M silver nitrate to a weakly brown colour. Titrate to the same colour a blank, containing 100 ml distilled water, bicarbonate, and 1.0 ml of potassium chromate TS indicator.

Chloride content (%) = [35.5 x M x (A-B)]/10 x W
Sodium chloride content (%) = [58.5 x M x (A-B)]/10 x W

where:
W = weight of the sample (g)
M = molarity of silver nitrate (mol/l)
A = silver nitrate consumed by sample (ml)
B = silver nitrate consumed by blank (ml)

Ethylene oxide, dioxane, ethylene chlorohydrin

Principle
Ethylene oxide (EO), ethylene chlorohydrin (EKH) and dioxane are determined by head space gas chromatography with mass selective detection (GC-MSD).

Apparatus
Gas chromatograph (Hewlett Packard 5890 or equivalent) equipped with a mass selective detector (Hewlett Packard 5970 or equivalent).

Chromatographic conditions
EKH and dioxane: Column, 15 m, 0.25 mm i.d. DB 5 ms, 0.15 µm (or equivalent). Temperature programmed 40^{o} (2 min), increased to 150^{o} at 15^{o}C/min. Split injection, 150^{o}; Carrier, He, 20 ml/min; interface, 275^{o}; ms dwell time 100 msec.; monitor 43, 44, 57 and 88 amu.

Chromatographic conditions
EO: Column, 50 m, 0.32 mm i.d., CP Sil 5 CB, 5 µm (or equivalent). Temperature programmed 100^{o} (1 min) to 250^{o} at 15^{o}/min. Split injection, 150^{o}; Carrier, He, 20 ml/min.; interface, 275^{o}, ms dwell time 100 msec; monitor 29, 43, 44 and 72 amu.

Regents and Solutions Standards
Accurately weigh approximately 200 mg each of dioxane and EKH and 20 mg EO into a 100 ml volumetric flask containing 50 ml distilled water. Fill to 100 ml. Dilute this standard 1:10, 1:100, and 1:1000. An internal standard is made by accurately weighing approximately 40 mg of methyl ethyl ketone (MEK) and diluting to 100 ml with distilled water. A 1:1000 dilution of this solution gives a 0.4 µg/ml standard solution.

Equipment
Headspace vials, 20 ml, with teflon coated septa; gas tight syringe, 1,000 to 2,500 µl; block or oil bath heater, 80^{o}.

Procedure
Response factors: Accurately weigh 1.0 g of the sample into each of 4 vials, add 0.5 ml of internal standard to each and add 0, 0.2, 0.4, or 0.6 ml of the 1:1000 standards to one of each vial. Add sufficient buffer to bring the total volume in each vial to 1.5 ml. Cap and place vials into the heater at 80^{o} for 20 min. Inject 1.0 ml of the headspace into the GC-MSD. Monitor ions amu 44, EO; 43, MEK; 88, dioxane; 57, EKH. Plot the amount of each compound added to the vial against the ratio of the analyte peak area to the MEK peak area. The response factor, R_f, for each analyte is given by the inverse of the x coefficient of the linear regression of the data.

Analysis
Inject 1 ml headspace of unknowns containing MEK internal standard. Record peak areas of unknowns and the internal standard.

Calculation
The EO, EKH, and dioxane concentrations are calculated from the peak areas in the unknown by the following:

$$\text{Content (mg / kg)} = \frac{A_{UNK}}{A_{MEK}} \times C_{MEK} \times R_f$$

where
A_{UNK} = area of unknown peak
A_{MEK} = area of MEK peak
C_{MEK} = concentration of MEK in the sample
R_f = response factor of the unknown compared to MEK

Mono- and diethylene glycols

Principle
mono- and diethylene glycol are determined by gas chromatography after extraction with acetone.

Apparatus
Gas chromatograph (Hewlett Packard 5980 or equivalent) equipped with an FID detector. Chromatographic conditions: Column, 25 m, 0.52 mm id. CP wax 57 B, 0.5 µm (or equivalent). Temperature programmed 90° (2 min), increased to 250° at 20°/min., final hold, 10 min. Split injection, 250°. Carrier He, 27 ml/min. Injection volume, 1 µl.

Standard solution
Accurately weigh about 20 mg of each glycol into a 25 ml volumetric flask and fill to mark with acetone. This standard is diluted 1:5 and 1:20.

Procedure
Weigh 0.5 g EHEC sample into a 25 ml volumetric flask, add 20 ml acetone, stopper, and stir 2 h at room temperature. Fill flask to volume. Allow the EHEC to settle and inject 1 µl into the chromatograph. Similarly inject 1 µl of the standard solution.

Calculation
The amount of each ethylene glycol is calculated as follows:

$$\%\ \text{Glycol} = \frac{A_{SAM}}{A_{STD} \times W} \times C_{STD} \times V_{SAM} \times 100$$

where
A_{SAM} = peak area of the sample
A_{STD} = peak area of standard
C_{STD} = glycol concentration in the standard (mg/ml)
V_{SAM} = volume in which the sample was dissolved
W = weight of the sample in mg
The calculated amount of glycol is divided by 0.75 to account for the 75% recovery of the glycols.

METHOD OF ASSAY

Determination of the ethoxyl group

Principle

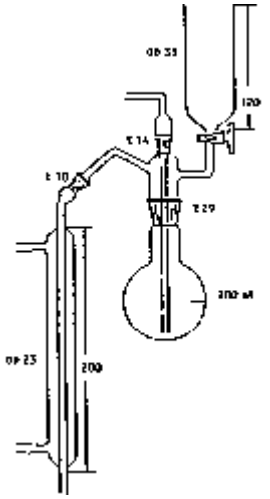
The sample is oxidized with chromium trioxide and the ethoxyl groups quantitatively transformed into acetic acid. Acetic acid is distilled and determined by titration.

Reagents

-0.020N sodium hydroxide (carbon dioxide-free)
-0.020N sodium thiosulfate: standardized against 0.020N iodine solution.
-Sodium hydrogen carbonate (analytical grade)
-Aqueous 30% solution of chromium trioxide (analytical grade)

Equipment

The apparatus shown in the following picture is used.



Procedure

Accurately weigh approximately 50 mg of the sample into a 100-ml round bottomed flask; add 10 ml of chromic acid solution and immerse the flask two thirds into an oil bath. The rest of the apparatus is fixed to the flask, and nitrogen is blown through at a rate of 1-2 bubbles per sec. The temperature of the bath is gradually raised over 30 min to 155^{o} and held. Distillation starts at 135-140^{o}. When 5 ml has been distilled, 5 ml of boiled distilled water is added from the graduated 50 ml dropping funnel. This procedure is continued until 50 ml of water has been added and consequently 55 ml of faintly yellow distillate has been collected. The distillate is quantitatively transferred to a flask and the distillate is titrated with 0.020N sodium hydroxide (carbon dioxide-free) to a phenolphthalein end-point. The solution is boiled 1 min and cooled to room temperature. The titration is continued until the pink colour remains stable for 10 sec. About 0.5 g of sodium hydrogen carbonate is added to the titrated solution followed by 10 ml of 10% sulfuric acid. When carbon dioxide evolution has ceased, 1 g of potassium iodide is added; the flask is shaken and kept 5 min in the dark. Liberated iodine is titrated with 0.020N sodium thiosulfate using 1% starch solution as the indicator.

Chromium trioxide solution (10 ml) is distilled and titrated as described above to provide a blank test. It is necessary to run a new blank when a new chromium trioxide solution has been prepared or if changes have been made in the apparatus.

Calculations

$$\% \text{ Ethoxyl} = \frac{45 \times 0.020 \times (A - (B \times E))}{10 \times I}$$

A = ml of sodium hydroxide used
B = ml of sodium thiosulfate used
C = ml of sodium hydroxide used in blank test
D = ml of sodium thiosulfate used in blank test
0.020 = normality of sodium hydroxide and sodium thiosulfate
I = grams of sample (calculated dry and free from ash)
E = C/D
The theoretical value for E is 0.667; in practice it is usually higher (0.7-0.8).

Determination of the total ethoxyl and oxyethylene groups

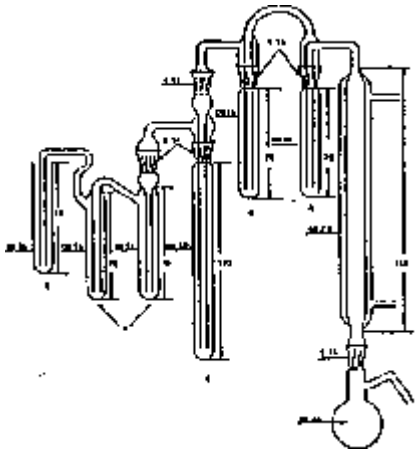
Principle
$ROC_2H_5 + HI = ROH + C_2H_5I$
$ROCH_2CH_2OH + HI = RI + ICH_2CH_2I + 2H_2O$
Ethylene iodide is partly converted to ethylene, partly to ethyl iodide. The former is absorbed in bromine solution, the latter in silver nitrate solution. Ethoxyl groups present in the sample will be transformed to ethyl iodide. Conversion of silver iodide to silver bromide with bromine is carried out, followed by thiosulfate titration of the iodine liberated.

Reagents
-Hydriodic acid: A constant boiling mixture with water (126-127^o) containing 57% HI is made in the following manner: Hypophosphorous acid (10-15 ml) is added to 250 ml of HI and the mixture boiled for 4-5 h under reflux in a carbon dioxide atmosphere. The acid is immediately put into 10-ml ampoules of brown glass, which are sealed and stored in a dark place.

-Silver nitrate solution: Silver nitrate (15 g) is dissolved in 50 ml of distilled water. The solution is poured into 400 ml of absolute ethanol, and a few drops of conc. nitric acid are added.
-Bromine solution: Bromine (1 ml) is added to 300 ml of glacial acetic acid which is saturated with dried potassium bromide (about 0.23 g per 100 ml of acetic acid).
-Potassium acetate: 100 g is dissolved in a mixture of 900 ml of acetic acid and 100 ml of acetic anhydride.
-Sodium thiosulfate solution 0.1N: Standardized against a 0.1N iodine solution which in turn has been standardized against 0.1N arsenic trioxide solution. Thiosulfate can also be standardized against potassium iodate purchased as a standard.

Equipment

The apparatus shown in the figure below is used. Tube A is filled halfway with 6% sodium hydrogen carbonate solution, and tube B is filled halfway with 10% cadmium sulfate solution. The silver nitrate solution (5 ml) is pipetted into tube C, and 10 ml of the bromine solution is transferred from the first to the second leg by careful tilting of the tube D. Tube E is filled halfway with 20% potassium iodide solution.



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Procedure

Weigh 50-60 mg of the sample into the reaction flask, and add 6 ml of hydriodic acid and a few boiling stones. Fix the flask to the apparatus and immerse it in an oil bath at 140-145^{o}. Circulate tap water through the condenser on top of the flask. Pass carbon dioxide through the apparatus at a rate of about 1 bubble per sec. Continue heating about 2 h (the end of the reaction manifests itself by agglomeration of the precipitate in tube C and clearing up of the solution). Five minutes before the end of the analysis, heat tube C to 50-60^{o} in a water bath. Loosen tubes D and E first, and then tube C. Add to tube D, 5 ml of 20% potassium iodide solution. Mix the bromine and the potassium iodide solutions by carefully purging the tube with carbon dioxide. During this whole procedure, tube E must be left connected to tube D. Then transfer the contents of tube D to a 500-ml Erlenmeyer flask containing 10 ml of 20% potassium iodide solution and 50 ml of distilled water. Rinse tube D well with distilled water, and flood down the contents of tube E in the flask; dilute to 150 ml, and add 5 ml of 10% sulfuric acid. Titrate liberated iodine with 0.05N sodium thiosulfate. Prepare a blank by adding 10 ml of the bromine solution to 10 ml of 20% potassium iodide solution, 150 ml of water, and 5 ml of 10% sulfuric acid and titrate it as described above.

In a 500-ml Erlenmeyer flask, mix 15 ml of the potassium acetate solution with 1 ml bromine. Quantitatively transfer the precipitate and solution in tube C into the Erlenmeyer flask. Allow the flask to stand for 10 min, then add 10 ml of the sodium acetate solution. Eliminate the excess of bromine by carefully adding formic acid dropwise. When the colour of the solution has disappeared, add three more drops. (The colour of the precipitate is always slightly yellow; it is necessary, therefore, to let it settle and observe the colour of the solution only). After 3 min, add 3 g of potassium iodide and 15 ml of 10% sulfuric acid, and titrate the liberated iodine with 0.1N sodium thiosulfate using 1% starch solution as the indicator. Prepare and titrate a blank containing reagents only.

Calculations

$$\text{Ethoxyl produced by } C_2H_4 \text{ (tubes D, E)} = \frac{22.5 \times N \times (B - A)}{10 \times I}$$

$$\text{Ethoxyl produced by } C_2H_5I \text{ (tube C)} = \frac{7.4 \times C \times N}{10 \times I}$$

where
A = ml of thiosulfate for titration of bromine solution
B = ml of thiosulfate for titration of the blank test on the bromine solution
C = ml of thiosulfate for titration of the silver nitrate solution plus the precipitate
N = normality of thiosulfate solution
I = grams of sample, calculated dry and free from ash
Both hydroxyethoxyl and ethoxyl are reported as ethoxyl.

FAST GREEN FCF

Prepared at the 30th JECFA (1986), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). Corrected at the 69th JECFA (2008). An ADI of 0-25 mg/kg bw was established at 30th JECFA (1986).

SYNONYMS CI Food Green 3, FD&C Green 3, CI (1975) No. 42053, INS No. 143

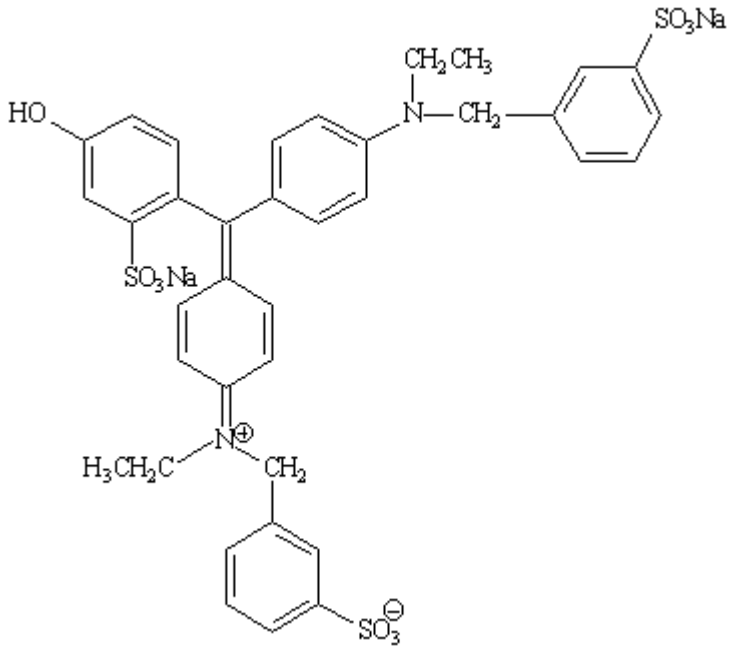
DEFINITION Consists essentially of disodium 3-[N-ethyl-N-[4-[[4-[N-ethyl-N-(3-sulfonatobenzyl)amino]phenyl](4-hydroxy-2-sulfonatophenyl)methylene]-2,5-cyclohexadien-1-ylidene]ammoniomethyl]benzenesulfonate and isomers and subsidiary colouring matters together with water, sodium chloride and/or sodium sulfate as the principal uncoloured components. May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names Disodium 3-[N-ethyl-N-[4-[[4-[N-ethyl-N-(3-sulfonatobenzyl) amino] phenyl](4-hydroxy-2-sulfonatophenyl)methylene]-2,5-cyclohexadien-1-ylidene] ammoniomethyl]-benzenesulfonate;
Inner disodium salt of N-ethyl-N-[4[[4-ethyl[(3-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl) methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzene-methanaminium hydroxide

C.A.S. number 2353-43-9

Chemical formula $C_{37}H_{34}N_2Na_2O_{10}S_3$

Structural formula



Formula weight 808.86

Assay	Not less than 85% total colouring matter

DESCRIPTION

Red to brown-violet powder or crystals

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Identification of colouring matters (Vol. 4)	Passes test

PURITY

Loss on drying at 135° (Vol. 4)	Not more than 15% together with chloride and sulfate calculated as sodium salts
Water insoluble matter (Vol. 4)	Not more than 0.2%
Chromium (Vol. 4)	Not more than 50 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Subsidiary colouring matters	Not more than 6% See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5%, sum of 2-, 3-, and 4-Formylbenzenesulfonic acids, sodium salts Not more than 0.3%, sum of 3- and 4-[N-Ethyl-N-(4-sulfophenyl)amino]methylbenzenesulfonic acid, disodium salts Not more than 0.5% of 2-Formyl-5-hydroxybenzenesulfonic acid, sodium salt Proceed as directed under *Column Chromatography*, using, for example, the following absorptivities: 3-formylbenzenesulfonic acid: 0.495 mg L^{-1} cm^{-1} at 246 nm in dilute HCl 3-[(ethyl)(4-sulfophenyl)amino]methylbenzenesulfonic acid: 0.078 mg L^{-1} cm^{-1} at 277 nm in dilute ammonia 2-formyl-5-hydroxybenzenesulfonic acid: 0.080 mg L^{-1} cm^{-1} at 335 nm in dilute ammonia

Leuco base (Vol. 4)	Not more than 5.0% Weigh accurately 130±5 mg sample and proceed as directed under *Leuco Base in Sulfonated Triarylmethane Colours* Absorptivity (a) = 0.156 mg L^{-1} cm^{-1} at approx. 625 nm Ratio = 0.971
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether extractable matter (Vol. 4)	Not more than 0.4%

TESTS

PURITY TESTS

Subsidiary colouring matters	Prepare a 1% solution of the colour. By means of a syringe spot as a band 1 mg of the colour on an 20 x 20 cm silica gel *thin layer chromatographic plate*. Allow the plate to dry about 20 min in the dark before placing it in a chromatographic tank containing the following developing solution: acetonitrile/isoamyl alcohol/methyl ethyl ketone/water/ammonia (50/50/15/10/5 by volume). Develop the plate until the solvent front is near the top of the plate. Remove the plate and allow it to dry. The subsidiary colours will appear in the following positions: the two top bands are the lower sulfonated subsidiary colours followed by the isometric and the main band or fast Green FCF near the bottom of the plate. Scrape off all the bands above the isometric colour. Extract the colour from the silica gel with ethyl alcohol. Filter through a sintered glass funnel and examine spectrophotometrically. The Standard absorptivity of the lower subsidiary colour is 0.126 $mgL^{-1}cm^{-1}$. NOTE: In the calculation of the subsidiary colour, it is assumed that its absorptivity at its peak is the same as that of the parent compound at its peak. Standard solutions must be prepared and their absorptivities measured within 1 h. Spectrophotometric measurements of the extracted subsidiary colours must be made as promptly as possible.

METHOD OF ASSAY

Proceed as directed under *Total Content by Titration with Titanous Chloride* (see Volume 4) using the following:
Weight of sample: 1.9 - 2.0 g
Buffer: 15 g sodium hydrogen tartrate
Weight (D) of colouring matters equivalent to
1.00 ml 0.1 N $TiCl_3$: 40.45 mg

FERRIC AMMONIUM CITRATE

Prepared at the 28th JECFA (1984), published in FNP 31/2 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A PMTDI of 0.8 mg/kg bw for iron was established at the 29th JECFA (1985).

SYNONYMS Iron ammonium citrate, ammonium ferric citrate, ammonium iron citrate, ammonium iron (III) citrate, INS No. 381

DEFINITION A complex salt of undetermined structure, composed of iron, ammonia and citric acid; there are two types of salts - brown and green - containing different amounts of iron

Chemical names: Ferric ammonium citrate, ammonium iron (III) citrate

Assay: Not less than 16.5% and not more than 22.5% of iron (Fe) for the brown salt, and not less than 14.5% and not more than 16.0% of iron (Fe) for the green salt.

DESCRIPTION Brown salt: thin, transparent brown, reddish brown, or garnet red scales or granules, or a brownish yellow powder; odourless or has a slight ammoniacal odour

Green salt: thin, transparent green scales, granules, powder, or transparent green crystals; odourless

FUNCTIONAL USES Nutrient, dietary supplement (brown salt)
Nutrient, dietary supplement, anticaking agent for sodium chloride(green salt)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Very soluble in water; insoluble in ethanol

Test for iron and ferric salts: Ignite 0.5 g of the sample gently, and dissolve the residue in 5 ml of dilute hydrochloric acid TS. The solution gives positive tests for *iron* and for *ferric salts*.

Test for citrate: To 5 ml of a 1-in-10 solution of the sample add 0.3 ml of potassium permanganate TS and 4 ml of mercuric sulfated TS and then heat the mixture to boiling. A white precipitated forms.

Test for ferric and ammonium salt: Dissolve 0.5 g of the sample in 5 ml of water, and add 5 ml of sodium hydroxide TS. A reddish brown precipitate forms and ammonia is evolved when the mixture is heated.

PURITY

Ferric citrate: Add potassium ferrocyanide TS to a 1 in 100 solution of the sample. No blue precipitation forms.

Oxalate

Transfer 1 g of the sample into a 125-ml-separator, dissolve in 10 ml of water, add 2 ml of hydrochloric acid, and extract successively with 50-ml portion and one 20-ml portion of ether. Transfer the combined ether extracts to a 150-ml beaker, add 10 ml of water, and remove the ether by evaporation on a steam bath. Add 1 drop of glacial acetic acid and 1 ml of calcium acetate solution (1 in 20) to the residual aqueous solution. No turbidity is produced within 5 min.

Sulfates

Not more than 0.3%
Dissolve a 100 mg sample in 1 ml of diluted hydrochloric acid TS, and dilute to 30 to 40 ml with water. Proceed as directed in the Limit Test for Sulfates, beginning with the addition of 3 ml of barium chloride TS. Any turbidity produced does not exceed that shown in a control containing 0.6 ml of 0.01 N sulfuric acid.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 1 g of the sample, accurately weighed, into a 250 ml glass-stoppered Erlenmeyer flask, and dissolve in 25 ml of water and 5 ml of hydrochloric acid. Add 4 g of potassium iodide, stopper, and allow to stand protected from light for 15 min. Add 100 ml of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Perform a blank determination and make any necessary correction. Each ml of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).

FERROCYANIDES of CALCIUM, POTASSIUM and SODIUM

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI of 0-0.025 mg/kg bw was established at the 18th JECFA (1974)

SYNONYMS Yellow prussiate of lime, potash or soda; hexacyanoferrate of calcium, potassium or sodium; INS No. Calcium salt 538, Potassium salt 536, Sodium salt 535

DEFINITION

Chemical names: Calcium (or Potassium or Sodium) ferrocyanide,
Calcium (or Potassium or Sodium) hexacyanoferrate (II)

C.A.S. number: 1327-39-5, Calcium salt
13943-58-3, Potassium salt
13601-19-9, Sodium salt

Chemical formula: $Ca_2Fe(CN)_6 \cdot 12H_2O$
$K_4Fe(CN)_6 \cdot 3H_2O$
$Na_4Fe(CN)_6 \cdot 10H_2O$

Formula weight: Calcium salt 508.3
Potassium salt 422.4
Sodium salt 484.1

Assay: Not less than 99.0% of the respective ferrocyanide

DESCRIPTION Yellow crystals or crystalline powder

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water; potassium and sodium salts are insoluble in ethanol

Test for ferrocyanide: To 10 ml of a 1% solution of the sample add 1 ml of ferric chloride TS. A dark blue precipitate is formed. (Retain the mixture for the Test for calcium).

Test for calcium (Vol. 4): Passes test
Test the mixture from the Test for ferrocyanide

Test for potassium (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

PURITY

Cyanide: Not detectable

Dissolve 10 mg of copper sulfate in a mixture of 8 ml of water and 2 ml of ammonia TS. Wet a strip of filter paper with this solution, and place the wet paper in a stream of hydrogen sulfide. When one drop of a 1% solution of the sample is placed on the brown reagent paper, a white circle should not be produced.

Ferricyanide

Not detectable
Dissolve about 10 mg of the sample in 10 ml of water and place one drop of this solution on a spot plate. Add one drop of a 1% solution of lead nitrate, followed by a few drops of a solution prepared by saturating cold 2 N acetic acid with benzidine. No blue precipitate or blue coloration should appear.

Arsenic (Vol. 4)

Not more than 3 mg/kg (Method II)

Lead (Vol. 4)

Not more than 5mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh 3 g of the sample to the nearest 0.1 mg and transfer into a 400-ml beaker. Dissolve in 225 ml of water, and add cautiously about 25 ml of sulfuric acid TS. Add, with stirring, 1 drop of orthophenanthroline TS, and titrate with 0.1 N ceric sulfate until the colour changes sharply from orange to pure yellow. Each ml of 0.1 N ceric sulfate is equivalent to 50.83 mg of $Ca_2Fe(CN)_6 \cdot 12H_2O$; 42.24 mg of $K_4Fe(CN)_6 \cdot 3H_2O$ or 48.41 mg of $Na_4Fe(CN)_6 \cdot 10H_2O$.

FERROUS GLUCONATE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). PMTDI 0.8 mg/kg bw for iron, established at the 27th JECFA in 1983.

SYNONYMS INS No. 579

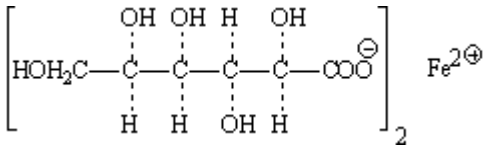
DEFINITION

Chemical names Iron (II) di-D-gluconate dihydrate, ferrous gluconate

C.A.S. number 299-29-6

Chemical formula $C_{12}H_{22}FeO_{14} \cdot 2H_2O$

Structural formula



Formula weight 482.17

Assay Not less than 95% on the dried basis

DESCRIPTION Fine yellowish-grey or pale greenish-yellow powder or granules having a slight odour resembling that of burnt sugar

FUNCTIONAL USES Colour, stabilizer, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble with slight heating in water; practically insoluble in ethanol

Test for ferrous salts (Vol. 4) Passes test

Test for gluconate (Vol.4) Passes test

PURITY

Loss on drying (Vol. 4) Not less than 6.5% and not more than 10.0% (105°, 16 h)

Reducing sugars Dissolve 0.5 g of the sample in 10 ml of water; warm, and make the solution alkaline with 1 ml of ammonia TS. Pass hydrogen sulfide gas into the solution to precipitate the iron, and allow the mixture to stand for 30 min to coagulate the precipitate. Filter, and wash the precipitate with two

successive 5 ml portions of water. Acidify the combined filtrate and washings with hydrochloric acid, and add 2 ml of dilute hydrochloric acid TS in excess. Boil the solution until the vapours no longer darken lead acetate paper, and continue to boil, if necessary, until concentrated to about 10 ml. Allow to cool, add 5 ml of sodium carbonate TS and 20 ml of water; filter, and adjust the volume of the filtrate to 100 ml. To 5 ml of the filtrate add 2 ml of alkaline cupric tartrate TS and boil for 1 min. No red precipitate should be formed within 1 min.

Iron (III)

Not more than 2%
Dissolve about 5 g of the sample, accurately weighed, in a mixture of 100 ml of water and 10 ml of hydrochloric acid in a 250-ml glass stoppered flask. Add 3 g of potassium iodide, shake well, and allow to stand in the dark for 5 min. Titrate any liberated iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (Fe III).

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 1.5 g of the dried sample, accurately weighed, in a mixture of 75 ml of water and 15 ml of dilute sulfuric acid TS in a 300-ml Erlenmeyer flask, and add 250 mg of zinc dust. Close the flask with a stopper containing a Bunsen valve, and allow to stand at room temperature for 20 min. Then filter through a Gooch crucible containing a glass fibre filter paper coated with a thin layer of zinc dust, and wash the crucible and contents with 10 ml of dilute sulfuric acid TS, followed by 10 ml of water. Add orthophenanthroline TS and titrate the filtrate in the suction flask immediately with 0.1 N ceric sulfate. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N ceric sulfate is equivalent to 44.61 mg of $C_{12}H_{22}FeO_{14}$.

FERROUS LACTATE

Prepared at the 35th JECFA (1989), published in FNP 49 (1990) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). A PMTDI of 0.8 mg/kg bw for iron was established at the 35th JECFA (1989)

SYNONYMS INS No. 585

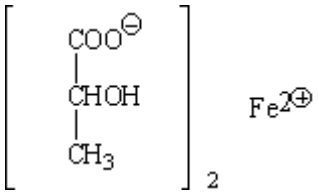
DEFINITION

Chemical names: Ferrous lactate, iron (II) lactate, iron (II) 2-hydroxypropanoate

C.A.S. number: 5905-52-2

Chemical formula: $C_6H_{10}FeO_6 \cdot xH_2O$, (x = 2 or 3)

Structural formula:



Formula weight: Dihydrate: 270.02
Trihydrate: 288.03

Assay: Not less than 96% on the dried basis

DESCRIPTION Greenish white crystals or light green powder having a weak, characteristic smell

FUNCTIONAL USES Colouring adjunct, nutrient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water; practically insoluble in ethanol

pH (Vol. 4): 5.0 - 6.0 (1 in 50 soln)

Test for lactate (Vol. 4): Passes test

Test for ferrous salts (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 18% (100° using vacuum, approx. 700 mm Hg)

Sulfates (Vol. 4): Not more than 0.1%

Test 0.5 g of the sample as directed in the Limited Test using 1 ml of 0.01 N sulphuric acid in the control

Chlorides (Vol. 4)

Not more than 0.1%
Test 0.5 g of the sample as directed in the Limit Test using 1.4 ml of 0.01 N hydrochloric acid in the control.

Iron (III)

Not more than 0.6%
Dissolve about 5 g of the sample, accurately weighed, in a mixture of 100 ml of water and 10 ml of hydrochloric acid in a 250-ml glass stoppered flask. Add 3 g of potassium iodide, shake well, and allow to stand in the dark for 5 min. Titrate liberated iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (III).

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 2 g of the dried sample, accurately weighed, to a 100-ml volumetric flask, dilute to the mark with water and mix. Pipet 20 ml of the sample solution into a 100-ml conical flask. Add 5 ml of formic acid (85% v/v). Titrate the solution with 0.1 N potassium permanganate until it turns pink. Each ml of 0.1 N potassium permanganate is equivalent to 23.40 mg of $C_6H_{10}FeO_6$.

FUMARIC ACID

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 35th JECFA (1989) and published in FNP 49 (1990). ADI "not specified" established at the 35th JECFA in 1989.

SYNONYMS INS No. 297

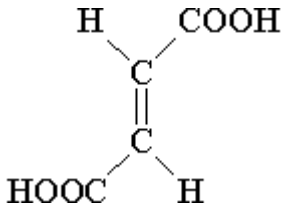
DEFINITION

Chemical names *trans*-Butenedioic acid, *trans*-1,2-Ethylene-dicarboxylic acid

C.A.S. number 110-17-8

Chemical formula $C_4H_4O_4$

Structural formula



Formula weight 116.07

Assay Not less than 99.0% calculated on the dried basis

DESCRIPTION Odourless, white crystalline powder or granules

FUNCTIONAL USES Acidity regulator, flavouring agent (see "Flavouring agents" monograph, JECFA no. 618)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in ethanol; slightly soluble in water and in diethyl ether

pH (Vol. 4) 2.0 - 2.5 (1 in 30 solution)

1,2-Dicarboxylic acid Place 50 mg of the sample in a test tube, add 2 to 3 mg of resorcinol and 1 ml of sulfuric acid, shake, heat at 130° for 5 min and cool. Dilute with water to 5 ml and add sodium hydroxide solution (2 in 5) dropwise to render the solution alkaline, cool and dilute with water to 10 ml. A greenish blue fluorescence is observed under an ultraviolet lamp.

Test for double bond Add 10 ml of water to 0.5 g of the sample and dissolve by boiling. Add 2 or 3 drops of bromine TS to the hot solution. The colour of bromine TS disappears.

PURITY

Loss on drying (Vol. 4)	Not more than 0.5% (120^{o}, 4 h)
Melting range (Vol. 4)	286 - 302^{o} (closed capillary, rapid heating)
Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Maleic acid (Vol. 4)	Not more than 0.1%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 1 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, add 50 ml of methanol, and dissolve the sample by warming gently on a steam bath. Cool, add phenolphthalein TS, and titrate with 0.5 N sodium hydroxide to the first appearance of a pink colour that persists for at least 30 sec. Perform a blank determination and make any necessary correction. Each ml of 0.5 N sodium hydroxide is equivalent to 29.02 mg of $C_4H_4O_4$.

GELLAN GUM

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996). An ADI 'not specified' was established at the 37th JECFA (1990)

SYNONYM INS No. 418

DEFINITION Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Pseudomonas elodea*, purified by recovery with isopropyl alcohol, dried, and milled. The high molecular weight polysaccharide is principally composed of a tetrasaccharide repeating unit of one rhamnose, one glucuronic acid, and two glucose units, and is substituted with acyl (glyceryl and acetyl) groups as the O-glycosidically-linked esters. The glucuronic acid is neutralized to a mixed potassium, sodium, calcium, and magnesium salt. It usually contains a small amount of nitrogen containing compounds resulting from the fermentation procedures.

C.A.S. number 71010-52-1

Formula weight Approximately 500,000

Assay Yields, on the dried basis, not less than 3.3% and not more than 6.8% of carbon dioxide (CO_2).

DESCRIPTION Off-white powder

FUNCTIONAL USES Thickening agent, gelling agent, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, forming a viscous solution; insoluble in ethanol

Gel test with calcium ion Add 1.0 g of the sample to 99 ml of water, and stir for about 2 h, using a motorized stirrer having a propeller-type stirring blade. Draw a small amount of this solution into a wide bore pipet and transfer into a 10% solution of calcium chloride. A tough worm-like gel will be formed immediately.

Gel test with sodium ion Add 1.0 g of the sample to 99 ml of water, and stir for about 2 h, using a motorized stirrer having a propeller-type stirring blade. Add 0.50 g of sodium chloride, heat to 80° with stirring, and hold at 80° for 1 min. Allow the solution to cool to room temperature. A firm gel is formed.

PURITY

Loss on drying (Vol. 4) Not more than 15% (105°, 2½ h)

Nitrogen (Vol. 4) Not more than 3%

Isopropyl alcohol — Not more than 750 mg/kg
See description under TESTS

Microbiological criteria — Total plate count: Not more than 10,000 colonies per gram
E. coli: Negative by test
Salmonella: Negative by test
Yeasts and moulds: Not more than 400 colonies per gram
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

PURITY TESTS

Isopropyl alcohol

Isopropyl alcohol (IPA) Standard Solution
Transfer 500.0 mg of chromatographic quality isopropyl alcohol into a 50-ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix.

Tertiary butyl alcohol (TBA) Standard Solution
Transfer 500.0 mg of chromatographic quality tert-butyl alcohol into a 50-ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix.

Mixed Standard Solution
Pipet 4 ml each of the IPA standard solution and of the TBA standard solution into a 125-ml graduated Erlenmeyer flask, dilute to about 100 ml with water, and mix. This solution contains approximately 40 µg each of isopropyl alcohol and of tert-butyl alcohol per ml.

Sample preparation
Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake the flask for 1 h, on a wrist-action mechanical shaker. Connect the flask to a fractionating column and distil about 100 ml; adjust the heat so that foam does not enter the column. Add 4.0 ml of TBA Standard Solution to the distillate to obtain the Sample Preparation.

Procedure
Inject about 5 µl of the Mixed Standard Solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m x 2.3-mm stainless steel column packed with 80/100-mesh Porapak QS or equivalent. The carrier is helium flowing at 80 ml per min. The injection port temperature is 200°, the column temperature 165°, and the detector temperature 200°. The retention time of isopropyl alcohol is about 2 min,

and that of tert-butyl alcohol about 3 min.

Determine the areas of the IPA and TBA peaks, and calculate the response factor, f, by the formula A_{IPA}/A_{TBA}, in which A_{IPA} is the area of the isopropyl alcohol peak, and A_{TBA} is the area of the tert-butyl alcohol peak.

Similarly, inject about 5 µl of the Sample Preparation, and determine the peak areas, recording the area of the isopropyl alcohol peak as a_{IPA}, and that of the tert-butyl alcohol peak as a_{TBA}.

Calculate the isopropyl alcohol content, in mg/kg, in the sample taken by the formula:
$(a_{IPA} \times 4000)(f \times a_{TBA} \times W)$
where W is the weight of the sample taken, in g.

Microbiological criteria

Total plate count

Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 12-15 ml of Plate Count Agar previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, allow the agar to solidify. Invert the plates and incubate for 48±2 h at 35±1°.

After incubation count the growing colonies visible on each plate and record the number of colonies. Take the average of both plates, and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

E. coli determination

Using aseptic technique, disperse 1 g of sample in 99 ml of Lactose broth using either a Stomacher, shaker or stirrer to fully dissolve the sample. Limit the dissolving time to about 15 min and then lightly seal the container and incubate the broth for 18-24 h at 35±1°. Using a sterile pipette, inoculate 1 ml of the incubate into a tube containing 10 ml GN broth. Incubate for 18-24 h and then streak any GN broths showing positive growth or gas production onto duplicate plates of Levine EMB agar. Incubate the plates for 24±2 h at 35±1° and then examine for colonies typical of *E. coli i.e.* showing strong purple growth with dark centre and a green metallic sheen sometimes spreading onto the agar. Record any typical *E. coli* colonies as presumptive positive, otherwise negative.
Streak any well isolated suspect colonies onto a plate of PCA and incubate for 18-24 h at 35±1°. Perform a Gram stain on any growth to confirm it is Gram negative. If so, disperse any colony growth into a small volume of 0.85% saline and perform chemical tests to confirm the identity of the bacterial growth. This can most conveniently be done by using API 20E or Micro ID strips or equivalent systems.

After completion of the tests, identify the organism from the Identification manual of the system used and record the final result.

Media

GN Broth (Gram Negative Broth)
Peptone 20.0 g
Dextrose 1.0 g
Mannitol 2.0 g
Sodium citrate 5.0 g
Sodium deoxycholate 0.5 g
Potassium phosphate (dibasic) 4.0 g
Potassium phosphate (monobasic) 1.5 g
Sodium chloride 5.0 g
Make up to 1 litre with distilled or de-ionised water, pH 7.0±0.2 at 25°.

Salmonella determination

Using aseptic technique, disperse 5 g of sample into 200 ml of sterile lactose broth using either a Stomacher, shaker or stirrer to maximise dissolution over a 15 min period. Loosely seal the container and incubate at 35±1° for 24±2 h.

Continue as per method on page 221 of *FNP 5/Rev. 2 (1991)*. Identification can be more conveniently done using API or Micro ID systems or equivalent.

Yeasts and moulds

Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 15-20 ml of Potato dextrose Agar (either acidified or containing antibiotic) previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, and allow the agar to solidify. Invert the plates and incubate for 5 days at 20-25°.
After incubation, count the growing colonies visible on each plate using a colony counter and record the number of colonies. Separate the yeasts from the moulds according to their morphology and count them separately. Take the average of both plates and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

METHOD OF ASSAY

Processed as directed in the test for Carbon Dioxide Determination by Decarboxylation in the *General Methods*, Volume 4*,* using about 1.2 g of the sample weighed accurately.

GLUCONO-δ-LACTONE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 30th JECFA (1986), published in FNP 37 (1986) and republished in FNP 52 (1992). Group ADI "not specified" for glucono-delta-lactone and gluconates, excluding ferrous gluconate, established at the 51st JECFA in 1998.

SYNONYMS Glucono-delta-lactone, gluconolactone, delta-gluconolactone, GDL; INS No. 575

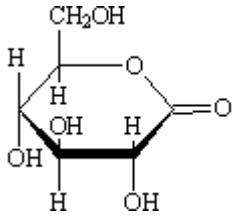
DEFINITION Glucono-delta-lactone is the cyclic 1,5-intramolecular ester of D-gluconic acid. In aqueous media it is hydrolyzed to an equilibrium mixture of D-gluconic acid (55-66%) and the delta- and gamma-lactones.

Chemical names D-Glucono-1,5-lactone, D-gluconic acid delta-lactone

C.A.S. number 90-80-2

Chemical formula $C_6H_{10}O_6$

Structural formula



Formula weight 178.14

Assay Not less than 99.0% on the dried basis

DESCRIPTION White, odourless or nearly odourless crystals or crystalline powder

FUNCTIONAL USES Acidifier, raising agent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water; sparingly soluble in ethanol

Colour reaction To 1 ml of a 1 in 50 solution, add 1 drop of ferric chloride TS. A deep yellow colour is produced

Test for gluconate (Vol. 4) Passes test

PURITY

Loss on drying (Vol. 4) Not more than 1% (105°, 2 h)

Sulfated ash (Vol. 4) Not more than 0.1%

Test 2 g of the sample (Method I)

Reducing substances — Not more than 0.5% (as D-glucose)
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
.

TESTS

PURITY TESTS

Reducing substances — Weigh accurately 10 g into a 400-ml beaker, dissolve the sample in 40 ml of water, add phenolphthalein TS, and neutralize with sodium hydroxide solution (1 in 2). Dilute to 50 ml with water, and add 50 ml of alkaline cupric tartrate TS. Heat the mixture on an asbestos gauze over a Bunsen burner, regulating the flame so that boiling begins in 4 min, and continue the boiling for exactly 2 min. Filter through a Gooch crucible, wash the filter with 3 ml or more small portions of water, and place the crucible in an upright position in the original beaker. Add 5 ml of water and 3 ml of nitric acid to the crucible, mix with a stirring rod to ensure complete solution of the cuprous oxide, and wash the solution into a beaker with several ml of water. To the beaker add sufficient bromine TS (5 to 10 ml) until the colour becomes yellow, and dilute with water to about 75 ml. Add a few glass beads, boil over a Bunsen burner until the bromine is completely removed, and cool. Slowly add ammonium hydroxide until a deep blue colour appears, then adjust the pH to approximately 4 with glacial acetic acid, and dilute to about 100 ml with water. Add 4 g of potassium iodide, and titrate with 0.1 N sodium thiosulfate, adding starch TS just before the endpoint is reached. Not more than 16.1 ml is required.

METHOD OF ASSAY

Dissolve about 0.6 g of the dried sample, accurately weighed, in 50 ml of 0.1 N sodium hydroxide, and allow to stand for 20 min. Add 3 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.1 N sulfuric acid. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N sodium hydroxide is equivalent to 17.81 mg of $C_6H_{10}O_6$.

GLUCOSE OXIDASE and CATALASE from *ASPERGILLUS NIGER* var.

Prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). An ADI not specified' was established at 18th JECFA (1974)

SYNONYMS

1. Glucose oxyhydrase, glucose aerodehydrogenase, notatin, aero-glucose dehydrogenase; INS No. 1102
2. None

SOURCES

Commercial enzyme preparations are produced by the controlled fermentation of *Aspergillus niger* var.

Active principles

1. Glucose oxidase
2. Catalase

Systematic names and numbers

1. ß-D-glucose: oxygen 1-oxidoreductase (EC 1.1.3.4)
2. Hydrogen-peroxide: hydrogen-peroxide oxidoreductase (EC 1.11.1.6)

Reactions catalyzed

1. ß-D-glucose + O_2 --> D-glucono-delta-lactone + H_2O_2
2. H_2O_2 + H_2O_2 --> $2H_2O$ + O_2

Secondary enzyme activities

Invertase (EC 3.2.1.26)

DESCRIPTION

Off-white to brown liquids; soluble in water and practically insoluble in ethanol, chloroform and ether

FUNCTIONAL USES

Enzyme preparation
Used in the preparation of and/or use in milk, cheese, eggs, beverages and salads

GENERAL SPECIFICATIONS

Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Glucose oxidase activity (Vol. 4)

The sample shows glucose oxidase activity

Catalase activity (Vol. 4)

The sample shows catalase activity

L-GLUTAMIC ACID

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for glutamic acid and its Ammonium, Ca, K, Mg & Na salts, was established at the 31st JECFA (1987)

SYNONYMS Glutamic acid, INS No. 620

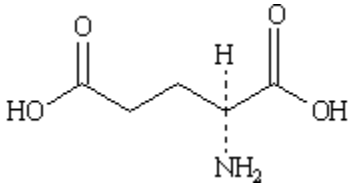
DEFINITION

Chemical names: L-Glutamic acid, L-(+)-glutamic acid, L-2-amino-pentanedioic acid, L-alpha-aminoglutaric acid

C.A.S. number: 56-86-0

Chemical formula: $C_5H_9NO_4$

Structural formula:



Formula weight: 147.13

Assay: Not less than 99.0% on the dried basis

DESCRIPTION Colourless or white crystals or crystalline powder

FUNCTIONAL USES Flavour enhancer, salt substitute

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Sparingly soluble in water; practically insoluble in ethanol or ether

Test for glutamate (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 0.2% (80°, 3 h)

pH (Vol. 4): 3.0 - 3.5 (saturated solution)

Specific rotation (Vol. 4): [alpha] 20, D: Between +31.5 and + 32.2° (10%(w/v) soln in 2N hydrochloric acid)

Sulfated ash (Vol. 4): Not more than 0.2%

	Test 1 g of the sample (Method I)
Chlorides (Vol. 4)	Not more than 0.2% Test 0.07 g of the sample as directed in the Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control
Pyrrolidone carboxylic acid (Vol. 4)	Passes test
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank. Each ml of 0.1 N perchloric acid is equivalent to 14.713 mg of $C_5H_9NO_4$.

GLYCEROL

Prepared at the 20th JECFA (1976), published in FNS 1B (1977) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not specified' was established at the 20th JECFA (1976)

SYNONYMS Glycerin; INS No. 422

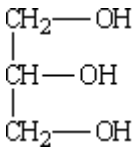
DEFINITION

Chemical names 1,2,3-Propanetriol, glycerol, trihydroxypropane

C.A.S. number 56-81-5

Chemical formula $C_3H_8O_3$

Structural formula



Formula weight 92.10

Assay Not less than 99% of on the anhydrous basis

DESCRIPTION Clear, colourless, hygroscopic, syrupy liquid, having a not more than a slight characteristic odour, which is neither harsh nor disagreeable

FUNCTIONAL USES Humectant, solvent, bodying agent, plasticizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Miscible with water and with ethanol; immiscible with ether

Test for glycerol (Vol. 4) Passes test

PURITY

Water (Vol. 4) Not more than 5% (Karl Fischer Method)

Colour The colour of the sample, when viewed downward against a white surface in a 50-ml Nessler tube, is not darker than the colour of a standard made by diluting 0.4 ml of ferric chloride TSC with water to 50 ml and similarly viewed in a Nessler tube of approximately the same diameter and colour as that containing the sample.

Sulfated ash Not more than 0.01%
Heat 50 g in a tared, open dish, and ignite the vapours, allowing them to burn until the sample has been completely consumed. After cooling, moisten the residue with 0.5 ml of concentrated sulfuric acid, and complete

the ignition by heating for 15 min periods at 800±25° to constant weight.

Chlorides (Vol. 4)

Not more than 10 mg/kg
Test 10 g of the sample as directed in the Limit Test using 0.1 mg of chloride ion in the control

Chlorinated compounds

Not more than 30 mg/kg (as chloride ion)
Transfer 5 g of the sample into a dry 100 ml round bottom ground joint flask and add 15 ml of morpholine. Connect the flask with a ground joint reflux condenser, and reflux the mixture gently for 3 h. Rinse the condenser with 10 ml of water, receiving the washing into the flask, and cautiously acidify with nitric acid. Transfer the solution to a suitable comparison tube, add 0.5 ml of silver nitrate TS, dilute with water to 50 ml, and mix thoroughly. Any turbidity does not exceed that produced by 150 µg of chloride ion (Cl) in an equal volume of solution containing the quantities of reagents used in the test, omitting the refluxing.

Fatty acids and esters

Not more than 30 mg/kg
To a 40 ml (50 g) of the sample add 50 ml of recently boiled water and 5 ml of 0.5 N sodium hydroxide, then mix. Boil the mixture for 5 min, cool, add phenolphthalein TS, and titrate the excess alkali with 0.5 N hydrochloric acid. Not more than 1 ml of 0.5 N sodium hydroxide is consumed.

Readily carbonizable substances

Rinse a glass-stoppered, 25-ml cylinder with sulfuric acid TS, and allow to drain for 10 min. Add 5 ml of the sample and 5 ml of sulfuric acid TS, shake vigorously for 1 min, and allow to stand for 1 h. The mixture is no darker than *Matching Fluid H.*

Butanetriols

Not more than 0.2%
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Butanetrioles

Reagents
- Chromatographic Siliceous Earth: Use 80- to 100-mesh Chromosorb W or other comparable grade of purified chromatographic siliceous earth.
- 1,4-Butanediol: Purify the commercial product by vacuum distillation, collecting the portion distilling between 120° and 121° at 8 mm of mercury.
- 1,2,4-Butanetriol: Purify the commercial product by vacuum distillation, collecting the portion distilling between 151° and 153° at 2 mm of mercury.

Preparation of Column Material
Place about 500 g of chromatographic siliceous earth in a large beaker, add sufficient 6 N hydrochloric acid to cover the material, and allow to

stand overnight. Decant the acid, wash the siliceous earth on a Buchner funnel with water until the wash water is neutral to pH indicator paper then wash with acetone until free from water, and spread out to dry in the air. Transfer the washed and dried material to a sintered glass funnel, cover with chloroform, stir, and remove the chloroform, by aspiration. Repeat the washing with chloroform, and again dry in the air at room temperature. Weigh 87.5 g of the dried siliceous earth into a dish, and add sufficient acetone to form a slurry. Transfer 12.5 g of polyoxyethylene-(8)-ethylenediamine into a beaker, and dissolve in acetone. Place the dish on a steam bath, and heat gently, with stirring, while adding the solution of polyoxyethylene-(8)-ethylenediamine. Continue heating until enough acetone has evaporated to cause the mixture to become free-flowing, and spread out to dry at room temperature.

(Note: The column prepared with polyoxyethylene-(8)-ethylene-diamine does not have long-term stability, particularly when used with a flame-ionization detector; it is more stable, however, when used with a thermal-ionization detector. To prolong stability in either case, the column should be kept sealed against exposure to air when not in use.)

Weigh accurately about 10 g of the sample, add 1 drop of 1,4-butanediol, accurately weighed, as internal standard, dilute with 5 ml of methanol, and mix. Inject a 10- portion of this solution into a gas chromatographic apparatus equipped with a linear temperature programming device. The operating conditions of the apparatus may vary, depending upon the particular instrument used, but a suitable chromatogram is obtained with a copper column, 1.5 m in length and 6.3 mm in outside diameter, packed with the column material previously described. In addition, the carrier is helium, flowing at the rate of 100 ml per min; the injector block temperature is 320^{o}, the detector block temperature is 250^{o}, and the column temperature is programmed to rise from 150^{o} to 180^{o} at a rate of 5.60 per min. The detector bridge current should be maintained at 250 mA when the operating conditions described are employed.

The resolution factor, R. should be not less than 1.9 between the threo- and the erythro-butanetriols peaks, not less than 2.5 between the erythro-1,2,3-butanetriol and the glycerol peaks, and not less than 4.5 between the glycerol and the 1,2,4-butanetriol peaks. (These values for R are obtained when mixtures of equal quantities of glycerol and the butanetriols are determined in an apparatus programmed as described above).

Prepare a 1 in 1,000 solution in glycerol of 1,2,4-butane-triol, accurately weighed, and calculate the percent (P) of 1,2,4-butanetriol in the standard mixture. Weigh accurately about 10 g of the standard mixture, add 1 drop of 1,4-butane-diol, accurately weighed, as the internal standard, and dilute with 5 ml of methanol. Inject about 10 µl of this solution, and obtain a standard chromatogram under the same operating conditions as employed for the sample, applying attenuation of the detector signal as necessary. Under the conditions described, the 1,4-butanediol is eluted in about 8 min, and an area of about 10 cm^2 is generated as compared to an area of 1.0-1.5 cm^2 for the butanetriols when present in a concentration of about 0.1%. In addition, the following retention times have been obtained: 1.00 for 1,4-butanediol, 2.14 for threo-1,2,3-butanetriol, 2.52 for erythro-1,2,3-

butanetriol, and 5.26 for 1,2,4-butanetriol. Retention times will vary if programming different from that described is used.

Calculation

Measure the areas of the peaks produced by the 1,4-butanediol (a) and by the 1,2,4-butanetriol (A), and calculate the response factor (f) by the formula:

$$\frac{W \times P \times a}{100 \times w \times A}$$

where
W = the exact weight of the standard mixture used for dilution with the methanol
w = the exact weight of the drop of 1,4-butanediol internal standard added to the standard mixture.
Calculate the percent of each butanetriol in the sample by the formula:

$$\frac{100 \times f \times W' \times Ax}{A' \times W}$$

where
f = the response factor previously determined
W' = the exact weight of 1,4-butanediol internal standard added to the sample solution
Ax = the area of the peak produced by each butanetriol
A' = the area of the 1,4-butanediol peak
W = the weight of the sample
The sum of the percents found does not exceed 0.2.

METHOD OF ASSAY

Weigh accurately about 1 g of the sample and dissolve in water to make 100 ml. Add 100 ml of 0.3% potassium periodate solution to a 5 ml portion of the solution, shake thoroughly, and allow to stand for 1 h. Add 1 ml of propylene glycol, allow to stand for 10 min, and titrate with 0.05 N sodium hydroxide, using 3 drops of phenol red TS as the indicator, until a pink colour persists. Perform a blank test in the same manner as the sample. Each ml of 0.05 N sodium hydroxide is equivalent to 4.605 mg of $C_3H_8O_3$.

GLYCEROL ESTER OF WOOD ROSIN
(TENTATIVE)

Tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). A temporary group ADI of 0-12.5 mg/kg bw for glycerol ester of gum rosin and glycerol ester of wood rosin was established at the 74th JECFA (2011).

Updated information required:

- *Composition of the refined wood rosin currently used as the source rosin with regard to the levels of resin acids and "neutrals" (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the glycerol ester of wood rosin with regard to the levels of:*
  - *a) glycerol esters;*
  - *b) free resin acids; and*
  - *c) neutrals (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the total glycerol ester of resin acids with regard to the levels of:*
  - *a) monoglycerol esters;*
  - *b) (1,2) diglycerol esters;*
  - *c) (1,3) diglycerol esters;*
  - *d) triglycerol esters; and*
  - *e) dimeric esters*
- *Composition of the neutrals, including identification of the major classes of compounds and the concentration of the major constituents within the classes*

NOTE:
Validated methods should be used for the analysis of substances considered in the specification. Detailed information on the methods used, including sample preparation and validation parameters should be provided. It is recommended that representative samples of commercially available glycerol ester of gum rosin be analysed by independent laboratories.

SYNONYMS INS No. 445(iii)

DEFINITION Glycerol ester of wood rosin is a complex mixture of tri- and diglycerol esters of resin acids from wood rosin, with a residual fraction of monoglycerol esters. Besides these esters, up to x % resin acids (data on percentage required) and up to x % non-acidic saponifiable and unsaponifiable substances (data on percentage required) are present. It is obtained by the solvent extraction of aged pine stumps (*Pinus palustris* (longleaf) and *Pinus elliottii* (slash) species) followed by a liquid-liquid solvent refining process.

The refined wood rosin composed of x% resin acids (data on percentage required) and x% neutrals (non-acidic saponifiable and unsaponifiable substances) (data on percentage required). The resin-acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main component is abietic acid. The substance is purified by steam stripping or by countercurrent steam distillation.

These specifications do not cover substances derived from gum rosin, an exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing.

C.A.S. number 8050-30-4

Assay Sum of tri- and diglycerol esters: information required

DESCRIPTION

Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES

Emulsifier, density adjustment agent (flavouring oils in beverages), stabilizer, chewing gum base component

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, soluble in acetone

Infrared absorption (Vol. 4) The infrared spectrum of a thin film of the sample (potassium bromide disc) corresponds with the typical infrared spectrum below

Sulfur test Negative
Weigh 40-50 mg of sample into a test tube and add 1- 2 drops of a 20% (w/v) solution of sodium formate. Place a strip of lead acetate test paper over the mouth of the test tube. Heat the tube until fumes are formed that contact the test paper. Continue heating for 2-5 min. The formation of a black spot of lead sulfide indicates the presence of sulfur-containing compounds. (Detection Limit: 50 mg/kg sulfur)

PURITY

Monoglycerol esters Information required

Neutrals (non-acidic saponifiable and unsaponifiable substances) Information required

Specific gravity (Vol. 4) d^{20}_{25}: Not less than 0.935 (50% solution in d-limonene)

Ring and ball softening point (Vol. 4) Not less than 82° (see “Specific Methods, Glycerol Esters of Rosins”)

Acid value (Vol. 4) Between 3 and 9 (see “Specific Methods, Fats, Oils, and Hydrocarbons”)

Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

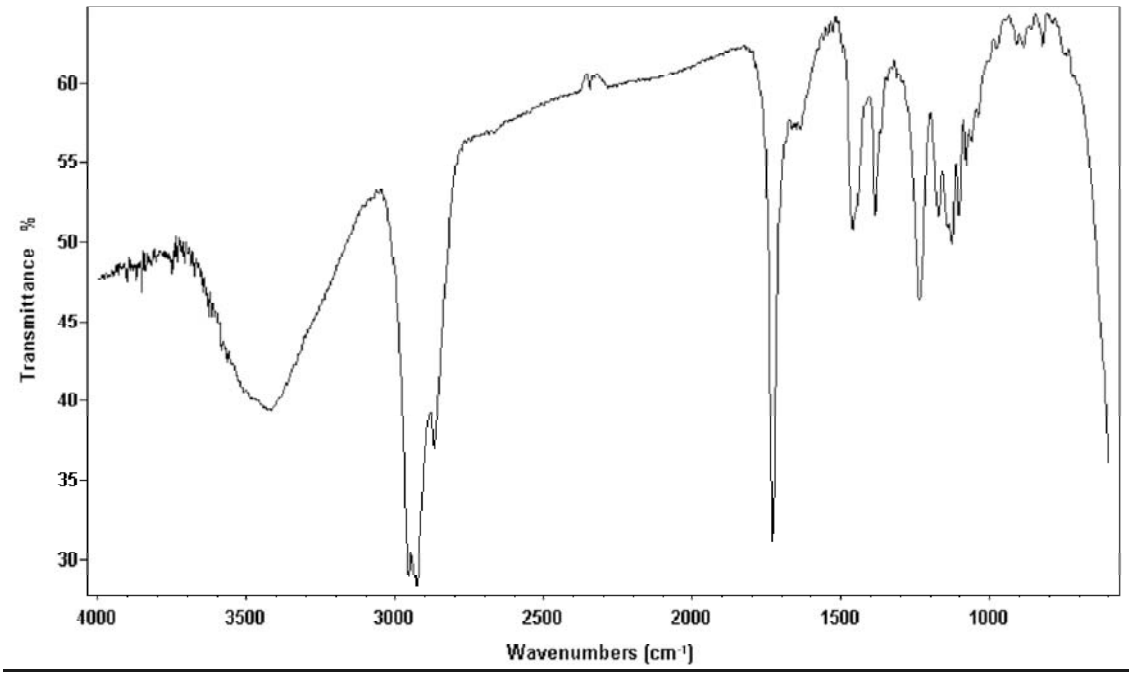
PURITY TESTS

Monoglycerol esters:	Information required
Neutrals (non-acidic saponifiable and unsaponifiable substances)	Information required

METHOD OF ASSAY

Tri- and diglycerol esters	Information required

Infrared spectrum



NOTE: The IR spectrum for glycerol ester of wood rosin is referenced from the Food Chemicals Codex, 7th Edition, 2010, p. 449. Reprinted with permission from the US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

GRAPE SKIN EXTRACT

Prepared at the 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-2.5 mg/kg bw was established at the 26th JECFA (1982).

SYNONYMS Enociania, Eno; INS No. 163(ii)

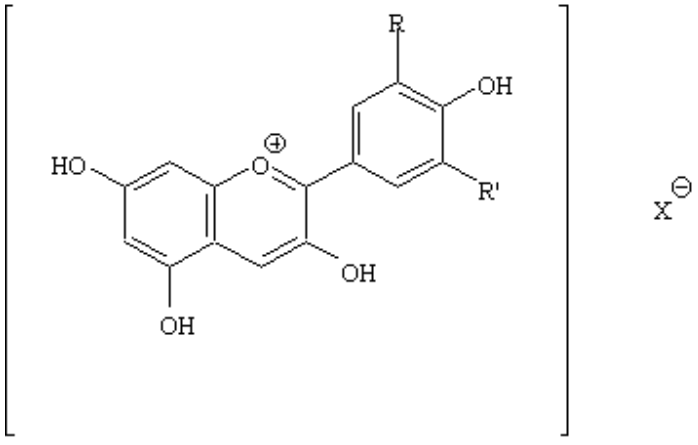
DEFINITION Obtained by aqueous extraction of grape skin or marc after the juice has been expressed from it; contains the common components of grape juice, namely: anthocyanine, tartaric acid, tannins, sugars, minerals, etc., but not in the same proportions as found in grape juice. During the extraction process, sulphur dioxide is added and most of the extracted sugars are fermented to alcohol; the extract is concentrated by vacuum evaporation during which practically all the alcohol is removed; a small amount of sulphur dioxide may be present.

Chemical names The principal colouring matters are anthocyanins, glucosides of anthocyanidins (2-phenylbenzopyrylium salts) such as peonidin, malvidin, delphinidin, and petunidin.

Chemical formula

Peonidin: $C_{16}H_{13}O_6X$
Malvidin: $C_{17}H_{15}O_7X$
Delphinidin: $C_{15}H_{11}O_7X$
Petunidin: $C_{16}H_{13}O_7X$
X: acid moiety

Structural formula



Peonidin: R = OCH_3; R' = H
Malvidin: R, R' = OCH_3
Delphinidin: R, R' = OH
Petunidin: R = OCH_3; R' = OH
X^-: acid moiety

Assay The colour intensity is not less than declared

DESCRIPTION Purplish-red liquid, lump, powder or paste, having a slight characteristic odour

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Soluble in water

Spectrophotometry (Vol. 4) — At pH 3 the absorbance maximum is about 525 nm

Colour reaction — Add 0.1 g of the sample to 50 ml of water and shake thoroughly. Filter if necessary. The solution shows red to purplish-red colour and it turns to blue or dark green on the addition of sodium hydroxide TS.

PURITY

Sulfur dioxide — Not more than 0.005% per 1 colour value
Distil 1 g of the sample with 100 ml of water and 25 ml of phosphoric acid solution (2 in 7) in a distilling flask with the Wagner tube (Figure 1). In an absorption flask, place 25 ml of lead acetate solution (1 in 50) previously prepared. Insert the lower end of condenser into lead acetate solution in the absorption flask. Distil until the liquid in the absorption flask reaches about 100 ml and rinse the end of the condenser with a little amount of water. To the distilled solution add 5 ml of hydrochloric acid and 1 ml of starch TS, and titrate with 0.01 N iodine. Each ml of 0.01 N iodine is equivalent to 0.3203 mg of SO_2.

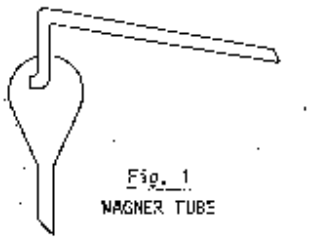


Fig. 1
WAGNER TUBE

Basic colouring matters — Add 1 g of the sample to 100 ml sodium hydroxide solution (1 in 100) and shake well. Take 30 ml of this solution and extract with 15 ml of ether. Extract this ether extract twice with each 5 ml of dilute acetic acid TS. The acetic acid extract is colourless.

Other acidic colouring matters — Add 1 ml of ammonia TS and 10 ml of water to 1 g of the sample and following the directions *Chromatography* place 0.002 ml of the solution on the chromatographic sheet and dry it. Use a mixture of pyridine and ammonia TS (2:1 by volume) as developing solvent and stop the development when the solvent front reaches about 15 cm height from the

point where the sample solution was placed. No spot is observed at the solvent front after drying under daylight. If any spot is observed, it should be decolourized when sprayed with a solution of stannous chloride in hydrochloric acid (2 in 5).

Arsenic (Vol. 4) — Not more than 3 mg/kg

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

In the absence of an assay method, a measurement of colour intensity by the following method may be used.

Prepare approximately 200 ml of pH 3.0 citric acid - dibasic sodium phosphate buffer solution: Mix 159 volumes of 2.1% citric acid solution and 41 volumes of 0.16% dibasic sodium phosphate solution, and adjust the pH to 3.0, using the citric acid solution or dibasic sodium phosphate solution. Weigh accurately an adequate amount of the sample so that the measured absorbance is between 0.2 and 0.7, and add pH 3.0 citric acid - dibasic sodium phosphate buffer solution to make up a 100-ml solution. Measure the absorbance A of this solution in a 1 cm cell at the wavelength of maximum absorption around 525 nm, using pH 3.0 citric acid - dibasic sodium phosphate buffer solution as the blank.

$$\text{Colour value} = \frac{A \times 10}{\text{weight of sample (g)}}$$

GUAIAC RESIN

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 17th JECFA (1973) and published in FNP 4 (1978) and in FNP 52 (1992). An ADI of 0-2.5 mg/kg bw was established at the 17th JECFA (1973).

SYNONYMS Guaiac gum, Gum guaiac, Gum guaiacum, Guaiacum; INS No. 314

DEFINITION The resin from the wood of Guajacum officinale L., or of *Guajacum sanctum* L., (Fam. *Zygophyllaceae*), consisting of approximately 70% alpha- and beta-guaiaconic acids, 10% guaiaretic acid, and 15% guaiac beta-resin and small quantities of guaiac yellow, vanillin, etc.

DESCRIPTION Irregular lumps enclosing fragments of vegetable tissues; or large, nearly homogeneous masses and occasionally more or less rounded or ovoid tears; externally, it is brownish black to dusky brown, acquiring a greenish colour on long exposure, the fractured surface having a glassy lustre, the thin pieces being transparent and varying in colour from brown to yellowish orange; the powder is moderate yellow brown, becoming olive brown on exposure to air. It has a mild balsamic odour.

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in fats; dissolves readily but incompletely in ethanol, ether, and solutions of alkalis

Melting range (Vol. 4) 85 - 90°

Colour reactions Add 1 drop of ferric chloride TS to 5 ml of an ethanolic solution of the sample (1 in 100). A blue colour is produced which gradually changes to green, finally becoming greenish yellow.
A mixture of 5 ml of an ethanolic solution of the sample (1 in 100) and 5 ml of water becomes blue upon shaking with 20 mg of lead peroxide. Filter the solution, and boil a portion of the filtrate. The colour disappears but may be restored by the addition of lead peroxide and shaking. Add a few drops of diluted hydrochloric acid TS to a second portion of the filtrate. The colour is immediately discharged.

PURITY

Total ash (Vol. 4)) Not more than 5%

Acid-insoluble ash (Vol. 4) Not more than 2%

Ethanol-insoluble residue Not more than 15%
Place 2 g of the sample, finely powdered and accurately weighed, in a dry, tared extraction thimble, and extract it with ethanol in a suitable continuous extraction apparatus for 3 h or until completely extracted. Dry the insoluble

residue in a thimble for 4 h at 105° and weigh. The weight of the residue shall not exceed 300 mg.

Rosin

A 10% solution of the sample in petroleum ether is colourless and when shaken with an equal quantity of a fresh solution of cupric acetate 0.5% is not more green than a similar solution of cupric acetate in petroleum ether.

Lead (Vol. 4)

Not more than 2 mg/kg.
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

5'-GUANYLIC ACID

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for 5'guanylic acid and its Ca & Na salts was established at the 18th JECFA (1974)

SYNONYMS Guanylic acid, GMP, INS No. 626

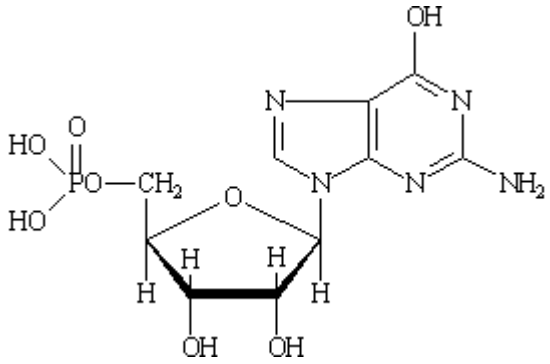
DEFINITION

Chemical names: Guanosine-5'-monophosphoric acid

C.A.S. number: 85-32-5

Chemical formula: $C_{10}H_{14}N_5O_8P$

Structural formula



Formula weight: 363.22

Assay: Not less than 97.0% and not more than 102.0% of on the dried basis

DESCRIPTION Odourless, colourless or white crystals, or a white crystalline powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water; practically insoluble in ethanol

Spectrophotometry (Vol. 4): A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 256±2nm. The ratio A250/A260 is between 0.95 and 1.03, and the ratio A280/260 is between 0.63 and 0.71.

Test for ribose (Vol. 4): Passes test

Test for organic phosphate (Vol. 4): Passes test
Test 5 ml of a 1 in 400 soln

PURITY

Loss on drying (Vol. 4)	Not more than 1.5% (120°, 4 h)
pH (Vol. 4)	1.5 - 2.5 (1 in 400 soln)
Related foreign substances (Vol. 4)	Chromatographically not detectable Test 2 µl of a 1 in 400 soln
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 260 nm using 0.01 N hydrochloric acid as the reference.

Calculate the content of $C_{10}H_{14}N_5O_8P$, in % in the sample by the formula:

$$\frac{A}{325} \times \frac{250{,}000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{loss on drying (\%)}} \times 100$$

GUAR GUM

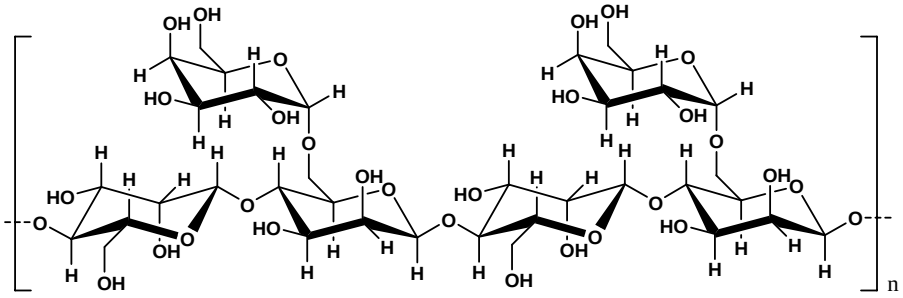
Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding tentative specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not specified" was established at the 19th JECFA (1975).

SYNONYMS Gum cyamopsis, guar flour; INS No. 412

DEFINITION Primarily the ground endosperm of the seeds from *Cyamopsis tetragonolobus* (L.) Taub. (Fam. *Leguminosae*) mainly consisting of high molecular weight (50,000-8,000,000) polysaccharides composed of galactomannans; the mannose:galactose ratio is about 2:1. The seeds are crushed to eliminate the germ, the endosperm is dehusked, milled and screened to obtain the ground endosperm (native guar gum). The gum may be washed with ethanol or isopropanol to control the microbiological load (washed guar gum).

C.A.S. number 9000-30-0

Structural formula



DESCRIPTION White to yellowish-white, nearly odourless, free-flowing powder

FUNCTIONAL USES Thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol

Gel formation Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.

Viscosity Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add 200 ml of water with vigorous stirring until the gum is completely and uniformly dispersed. An opalescent, viscous solution is formed. Transfer

	100 ml of this solution into another 400-ml beaker, heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is no substantial increase in viscosity (differentiating guar gums from carob bean gums).
Gum constituents (Vol. 4)	Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 to 10 µl of the hydrolysate instead of 1 to 5 µl. Use galactose and mannose as reference standards. These constituents should be present.
Microscopic examination	Place some ground sample in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under a microscope. Guar gum shows close groups of round to pear formed cells, their contents being yellow to brown.
PURITY	
Loss on drying (Vol. 4)	Not more than 15.0% (105°, 5 h)
Borate	Absent by the following test Disperse 1 g of the sample in 100 ml of water. The dispersion should remain fluid and not form a gel on standing. Mix 10 ml of dilute hydrochloric acid with the dispersion, and apply one drop of the resulting mixture to turmeric paper. No brownish red colour is formed.
Total ash (Vol. 4)	Not more than 1.5% (800°, 3-4 h)
Acid-insoluble matter (Vol. 4)	Not more than 7.0%
Protein (Vol. 4)	Not more than 10.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method) in Volume 4 (under “General Methods, Inorganic components”). The percentage of nitrogen determined multiplied by 6.25 gives the percentage of protein in the sample.
Residual solvents	Not more than 1% of ethanol or isopropanol, singly or in combination See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenizing the mixture in a high-speed blender.

Total (aerobic) plate count : Not more than 5,000 CFU/g
E. coli: Negative in 1g
Salmonella: Negative in 25g
Yeasts and moulds: Not more than 500 CFU/g

TESTS

PURITY TESTS

Residual solvents

Determine by gas chromatography in Volume 4 (under "Analytical Techniques, Chromatography").

Chromatography conditions
Column: 25% Diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm i.d., 0.25 μm film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injector: 280°
- column: Hold for 6 min at 40°, then 40-110° at 4°/min, 110-250° at 25°/min, hold for 10 min at 250°
- detector: 250°

Standard solutions
Solvent standard solution: Transfer 100 mg each of chromatography grade ethanol and isopropanol into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
TBA standard solution: Transfer 100 mg of chromatography grade tertiary-butyl alcohol (TBA) into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
Mixed standard solutions: Transfer 1, 2, 3, 4 and 5 ml of Solvent standard solution into each of five 100-ml volumetric flasks. Add 4 ml of TBA standard solution to each flask and dilute to volume with water.

Sample preparation
Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 4 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distil about 95 ml, adjusting the heat so that foam does not enter the column. Add 4 ml of TBA standard solution to the distillate and make up to 100 ml with water to obtain the Sample solution.

Standard curves
Inject 1 μl of each Mixed standard solution into the chromatograph. Measure the peak areas for each solvent and TBA. Construct the standard curves by plotting the ratios of the peak areas of each of the solvents/TBA against the concentrations of each solvent (mg/ml) in the Mixed standard solutions.

Procedure

Inject 1 µl of the Sample solution into the chromatograph. Measure the peak areas for each solvent and TBA. Calculate the ratios of the peak areas of each solvent/TBA, and obtain the concentration of each solvent from the standard curves.

Calculate the percentage of each solvent from:

$$\% \text{ Solvent} = (C \times 100/W \times 1000) \times 100$$

where C is the concentration of solvent (mg/ml)
W is weight of sample (g)

GUAR GUM (CLARIFIED)

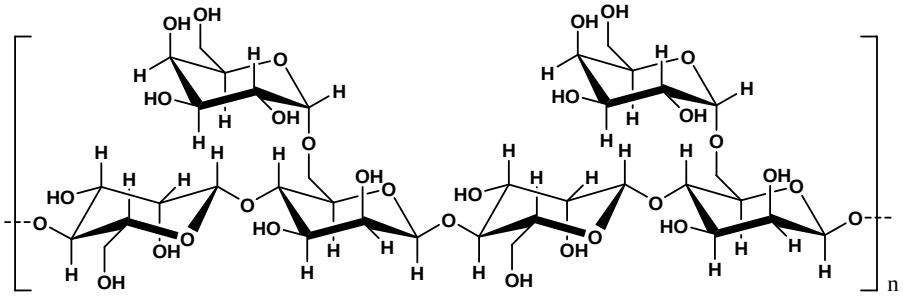
Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding tentative specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not specified" was established at the 19th JECFA (1975) for guar gum.

SYNONYMS INS No. 412

DEFINITION Primarily the ground endosperm of the seeds from *Cyamopsis tetragonolobus* (L.) Taub. (Fam. *Leguminosae*) mainly consisting of high molecular weight (50,000-8,000,000) polysaccharides composed of galactomannans; the mannose:galactose ratio is about 2:1. The seeds are crushed to eliminate the germ, the endosperm is dehusked, milled and screened to obtain the ground endosperm (native guar gum). The gum is clarified by dissolution in water, filtration and precipitation with ethanol or isopropanol. Clarified guar gum does not contain cell wall materials. Clarified guar gum in the market is normally standardized with sugars.

C.A.S. number 9000-30-0

Structural formula



DESCRIPTION White to yellowish white, nearly odourless, free-flowing powder

FUNCTIONAL USES Thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol

Gel formation Add small amounts of sodium borate TS to an aqueous solution of the sample; a gel is formed.

Viscosity Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add 200 ml of water with vigorous stirring until the gum is completely and uniformly

	dispersed. An opalescent, viscous solution is formed. Transfer 100 ml of this solution into another 400-ml beaker, heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is no substantial increase in viscosity (differentiating guar gums from carob bean gums).
Gum constituents (Vol. 4)	Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 to 10 µl of the hydrolysate instead of 1 to 5 µl. Use galactose and mannose as reference standards. These constituents should be present.
PURITY	
Loss on drying (Vol. 4)	Not more than 15.0% (105°, 5 h)
Borate	Absent by the following test Disperse 1 g of the sample in 100 ml of water. The dispersion should remain fluid and not form a gel on standing. Mix 10 ml of dilute hydrochloric acid with the dispersion, and apply one drop of the resulting mixture to turmeric paper. No brownish red colour is formed.
Total ash (Vol. 4)	Not more than 1.0% (800°, 3-4 h)
Acid-insoluble matter (Vol. 4)	Not more than 1.2%
Protein (Vol. 4)	Not more than 1.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method) in Volume 4 (under “General Methods, Inorganic components”). The percentage of nitrogen determined multiplied by 6.25 gives the percentage of protein in the sample.
Residual solvents	Not more than 1% of ethanol or isopropanol, singly or in combination See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenizing the mixture in a high-speed blender. Total (aerobic) plate count: Not more than 5,000 CFU/g *E. coli:* Negative in 1g *Salmonella*: Negative in 25g Yeasts and moulds: Not more than 500 CFU/g

TESTS

PURITY TESTS

Residual solvents

Determine by gas chromatography in Volume 4 (under “Analytical Techniques, Chromatography”).

Chromatography conditions
Column: 25% Diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm i.d., 0.25 μm film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injector: 280°
- column: Hold for 6 min at 40°, then 40-110° at 4°/min, 110-250° at 25°/min, hold for 10 min at 250°
- detector: 250°

Standard solutions
Solvent standard solution: Transfer 100 mg each of chromatography grade ethanol and isopropanol into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
TBA standard solution: Transfer 100 mg of chromatography grade tertiary-butyl alcohol (TBA) into a 100-ml volumetric flask containing about 90 ml water and dilute to 100 ml with water.
Mixed standard solutions: Transfer 1, 2, 3, 4 and 5 ml of Solvent standard solution into each of five 100-ml volumetric flasks. Add 4 ml of TBA standard solution to each flask and dilute to volume with water.

Sample preparation
Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 4 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distil about 95 ml, adjusting the heat so that foam does not enter the column. Add 4 ml of TBA standard solution to the distillate and make up to 100 ml with water to obtain the Sample solution.

Standard curves
Inject 1 μl of each Mixed standard solution into the chromatograph. Measure the peak areas for each solvent and TBA. Construct the standard curves by plotting the ratios of the peak areas of each of the solvents/TBA against the concentrations of each solvent (mg/ml) in the Mixed standard solutions.

Procedure
Inject 1 μl of the Sample solution into the chromatograph. Measure the peak areas for each solvent and TBA. Calculate the

ratios of the peak areas of each solvent/TBA, and obtain the concentration of each solvent from the standard curves.

Calculate the percentage of each solvent from:

$$\% \text{ Solvent} = (C \times 100/W \times 1000) \times 100$$

where C is the concentration of solvent (mg/ml)
W is weight of sample (g)

GUM ARABIC

Prepared at the 51st JECFA (1998) and published in FNP 52 Add 6 (1998); republished in FNP 52 Add 7 (1999) to include editorial changes. Supersedes specifications prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997). ADI "not specified", established at the 35th JECFA in 1989.

SYNONYMS Gum arabic (*Acacia senegal*), gum arabic (*Acacia seyal*), Acacia gum, arabic gum, INS No. 414

DEFINITION Gum arabic is a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (fam. *Leguminosae*)
Gum arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food.

C.A.S. number 9000-01-5

DESCRIPTION Gum arabic (*A. senegal*) is a pale white to orange-brown solid, which breaks with a glassy fracture. The best grades are in the form of whole, spheroidal tears of varying size with a matt surface texture. When ground, the pieces are paler and have a glassy appearance.
Gum arabic (*A. seyal*) is more brittle than the hard tears of gum arabic (*A. senegal*).

Gum arabic is also available commercially in the form of white to yellowish-white flakes, granules, powder, roller dried, or spray-dried material.

An aqueous solution of 1 g in 2 ml flows readily and is acid to litmus.

FUNCTIONAL USES Emulsifier, stabilizer, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) One gram dissolves in 2 ml of water; insoluble in ethanol

Gum constituents (Vol. 4) Proceed as directed under Gum Constituents Identification (FNP 5) using the following as reference standards: arabinose, galactose, mannose, rhamnose, galacturonic acid, glucuronic acid and xylose. Arabinose, galactose, rhamnose and glucuronic acid should be present. Additional spots corresponding to mannose, xylose and galacturonic acid should be absent.

Optical rotation Gum from *A. senegal* : aqueous solutions are levorotatory
Gum from *A. seyal* : aqueous solutions are dextrorotatory
Test a solution of 10 g of sample (dry basis) in 100 ml of water (if necessary, previously filtered through a No. 42 paper or a 0.8 µm millipore

filter), using a 200-mm tube.

PURITY

Loss on drying (Vol. 4)	Not more than 15% (105º, 5 h) for granular and not more than 10% (105º, 4 h) for spray dried material Unground samples should be powdered to pass through a No. 40 sieve and mixed well before weighing
Total ash (Vol. 4)	Not more than 4%
Acid-insoluble ash (Vol. 4)	Not more than 0.5%
Acid-insoluble matter (Vol. 4)	Not more than 1%
Starch or dextrin	Boil a 1 in 50 solution of the sample, cool and add a few drops of Iodine T.S. No bluish or reddish colour should be produced.
Tannin-bearing gums	To 10 ml of a 1 in 50 solution of the sample, add about 0.1 ml of ferric chloride TS. No blackish colouration or blackish precipitate should be formed.
Microbiological criteria (Vol. 4)	*Salmonella* spp.: Negative per test *E. coli:* Negative in 1 g
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

HEXAMETHYLENETETRAMINE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-0.15 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Hexamine, methenamine, INS No. 239

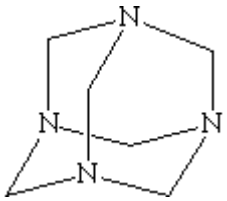
DEFINITION

Chemical names 1,3,5,7-Tetraazatricyclo[3.3.1.$1^{3,7}$]-decane, hexamethylenetetramine

C.A.S. number 100-97-0

Chemical formula $C_6H_{12}N_4$

Structural formula



Formula weight 140.19

Assay Not less than 99.0% on the dried basis

DESCRIPTION Nearly odourless, colourless lustrous crystals, or white crystalline powder

FUNCTIONAL USES Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water, soluble in ethanol

Test for formaldehyde Heat a 1 in 10 solution of the sample with dilute sulfuric acid TS. Formaldehyde is liberated, recognizable by its odour and by its darkening of paper moistened with silver ammonium nitrate TS.

Test for ammonia (Vol. 4) Passes test

PURITY

Loss on drying (Vol. 4) Not more than 2.0% (over P_2O_5, 4 h)

Sulfated ash (Vol. 4) Not more than 0.05%
Test 2 g of the sample (Method I)

Ammonium salts Add 1 ml of Nessler's reagent TS to 10 ml of a 5% solution of the sample. The mixture should not be darker than a mixture of 1 ml of the reagent in 10

ml of water.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh, to the nearest 0.1 mg, 1 g of the sample, previously dried for 4 h over phosphorus pentoxide. Transfer into a beaker and add 40.00 ml of N sulfuric acid. Boil gently adding water from time to time, if necessary, until the odour of formaldehyde is no longer perceptible. Cool, add 20 ml of water, and methyl red TS and titrate the excess acid with N sodium hydroxide. Each ml of N sulfuric acid is equivalent to 35.05 mg of $C_6H_{12}N_4$.

HYDROCHLORIC ACID

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 20th JECFA (1976), published in FNS 1B (1977) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 9th JECFA (1965)

SYNONYMS Muriatic acid, INS No. 507

DEFINITION

Chemical names	Hydrochloric acid
C.A.S. number	7647-01-0
Chemical formula	HCl
Formula weight	36.46
Assay	Not less than 97.0% and not more than 103.0% of the labelled amount

DESCRIPTION Clear colourless or slightly yellowish liquid with a pungent odour. Various concentrations are supplied as products of commerce.

FUNCTIONAL USES Acid

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water and in ethanol
Test for acid	A 1 in 100 solution of the sample is acid to litmus paper
Test for chloride (Vol. 4)	Passes test

PURITY

Non-volatile residue	Not more than 0.5% Transfer 1 g into a tared glass dish, evaporate to dryness on a steam bath, dry at 110º for 1 h, cool in a desiccator and weigh. The weight of the residue does not exceed 5 mg.
Reducing substances	Not more than 70 mg/kg as sulfur dioxide ; Transfer 1 ml of reagent grade hydrochloric acid into a 30-ml test tube, dilute to 20 ml with freshly boiled and cooled water, and add 1 ml of potassium iodide TS, 1 ml of starch TS and 2 ml of 0.001N iodine. Stopper the test tube and mix thoroughly. The blue colour produced is not discharged by 1 ml of the sample.
Oxidizing substances	Not more than 30 mg/kg as chlorine Transfer 1 ml of the sample into a 30-ml test tube, dilute to 20 ml with freshly boiled and cooled water, and add 1 ml of potassium iodide TS and 1 ml of starch TS. Stopper the test tube and mix thoroughly. The intensity of any blue colour developed does not exceed that produced in a control

prepared similarly but containing 1 ml of 0.001N iodine (instead of potassium iodide TS) and 1 ml of reagent grade concentrated hydrochloric acid (instead of sample).

Sulfate — Not more than 0.5%
Dilute 1 g of the sample to 100 ml with water, transfer 5 ml of this dilution to a 50-ml tall-form Nessler tube and dilute to 20 ml with water. Add a drop of phenolphthalein TS, neutralize the solution with ammonia TS, and then add 1 ml of hydrochloric acid TS prepared from reagent grade hydrochloric acid. To the clear solution (filtered if necessary) add 3 ml of barium chloride TS, dilute to 50 ml with water and mix. Prepare a control consisting of 1 ml of reagent grade concentrated hydrochloric acid and 250 µg of sulfate (SO_4) and the same quantities of the reagents as used for the sample. Any turbidity shown in the sample does not exceed that in the control.

Total organic compounds — Total organic compounds (non-fluorine): Not more than 5 mg/kg
Benzene: Not more than 0.05 mg/kg
Fluorinated organic compounds (total): Not more than 25 mg/kg
See description under TESTS

Iron — Not more than 5 mg/kg
Dilute 5 g (4.3 ml) of the sample to 40 ml and add about 40 mg of ammonium persulfate and 10 ml of ammonium thiocyanate TS. Any red colour developed does not exceed that in a control prepared by mixing 2.5 ml of Iron standard solution in an equal volume of a solution containing the same quantities of reagent grade hydrochloric acid and the reagents as used in the test.

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Organic compounds — Carry out analyses by gas chromatography employing Vapour Partitioning or Solvent Extraction, depending upon the characteristics of the compound being determined. It is necessary, however, to use the Vapour Partitioning method for the determination of benzene.

Vapour Partitioning Method
This method is suitable for the determination of extractable organic compounds at 0.05 to 100 mg/kg but is most appropriate for organic compounds with a vapour pressure greater than 10 mm Hg at 25°. Use a gas chromatograph equipped with a flame ionization detector and a 4-m x 2-mm (id) stainless-steel column packed with 15%, by weight, methyl trifluoropropyl silicone (DCFS 1265, or QF-1, or OV-210, or SP-2401) stationary phase on 80/100 mesh Gas Chrom R or the equivalent. A newly packed column should be conditioned at 120° and 30 ml/min helium flow for at least 2 h (preferably overnight) before it is attached to the detector. For

analysis, the column is maintained isothermally at 105°, the injection port and detector are maintained at 250°, the carrier gas flow rate is set at 11 ml/min, fuel gas flows should be optimized for the gas chromatograph and detector in use. The experimental conditions may be changed as necessary for optimal resolution and sensitivity. The signal-to-noise ratio should be at least 10:1.

Preparation of Standard Solutions

Prepare a standard solution of the organic compounds to be quantitated in Hydrochloric Acid (known to be free of interfering impurities) at approximate concentrations of 5 mg/kg, or within ±50% of the concentrations in the samples to be analyzed.

Place a stirring bar in a one-litre volumetric flask equipped with a ground-glass stopper, and tare the combination. Fill the flask with reagent-grade hydrochloric acid so that no air space is present when the flask is stoppered, and determine the weight of the Hydrochloric Acid. Calculate the volume (V) in 1l of each organic component to be added from the formula

$$V = (C \times W)/(D \times 1000)$$

where C is the desired concentration, in mg/kg; W is weight, in g, of the Hydrochloric Acid; D is the density, in mg/1l, of the organic compound; and 1000 is a conversion factor with the units g/kg. Add the calculated amount of each component to the Hydrochloric Acid with a syringe (ensure that the syringe tip is under the solution surface), stopper the flask, and stir the solution for at least 2 h using a magnetic stirrer.

Calibration

Treat the standard in the same way as described for the sample under Procedure (below). Determine a blank for each lot of reagent-grade Hydrochloric Acid, and calculate a response factor (R) by dividing the concentration (C) in mg/kg for each component by the peak area (A) for that component (subtract any area obtained from the blank sample):
R - C/(A - area of blank)

Gaseous compounds present special problems in the preparation of standards. Therefore, to determine response factors for gaseous compounds use the following method, which will be referred to as the Method of Multiple Extractions. Dilute a sample of Hydrochloric Acid known to contain the gaseous compound of interest with an equal volume of water. Draw 20 ml of this solution into a 50-ml glass syringe; then draw 20 ml of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Withdraw 1 ml of the vapour through the septum, and inject it into the chromatograph. Expel the vapour phase from the 50-ml syringe, draw in another 20 ml of air, repeat the extraction, and inject another 1-ml vapour sample into the gas chromatograph. Repeat the extraction, and GC analysis on the same sample of acid a total of six times. For each impurity, plot the area (A_N) determined for extraction N against the difference between A_N and the area determined for extraction (N + 1); that is, plot A_N against $[A_N - A_{N+1}]$. The slope of this line is the extraction efficiency (E) for that impurity into the air.

Inject into the chromatograph 1 ml of 0.1% (by volume) standard gas sample of each impurity in air and determine the absolute factor (F_A) in g, per peak area (A) by the following formula:

$F_A = (M \times 4.0816 \times 10^{-8})/A$,

where M is the molecular weight of the compound.
The concentration (C), in mg/kg, of the component in the original sample is calculated by the formula

$C = (A \times F_A \times 1.6949 \times 10^{6})/E$.

where A is the peak area corresponding to the compound (as above), F_A is the absolute factor, and E is extraction efficiency. The response factor is then calculated as R = C/A

Procedure
Dilute a 10-ml sample of Hydrochloric Acid to be analyzed with an equal volume of water. Draw this solution into a 50-ml glass syringe. Then draw 20 ml of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Draw 1 ml of the vapour through the septum, and inject it into the gas chromatograph. Approximate elution times in min for some specific organic compounds are as follows:

Methane and acetylene: 1.70
Methyl chloride: 2.21
Vinyl chloride: 2.29
1,1,1-Trichlorofluoromethane: 2.62
Ethyl chloride: 2.90
Vinylidene chloride: 3.20
Methylene chloride: 3.64
Chloroform: 4.49
1,1-Dichloroethane: 4.53
Carbon tetrachloride: 4.86
1,1,1-Trichloroethane: 5.50
Benzene: 6.00
Trichloroethylene: 6.22
Ethylene dichloride: 6.61
Propylenedichloride: 8.41
Perchloroethylene: 9.73

Alternate columns may be required to resolve some combinations of components. Methyl chloride and vinyl chloride are resolved by a 3.7-m x 3-mm (id) squalene column at 45^{o} and a helium flow of 10 ml/min. Chloroform and 1,1-dichloroethane are resolved by a 4-m x 3-mm (id) DC 550R column at 110^{o} and a helium flow of 12 ml/min.
Calculation
Calculate the concentration (C) in mg/kg of each compound by multiplying its corresponding peak area (A) by the appropriate response factor (R) determined in the Calibration protocol:
C = R x A

Precision

The relative standard deviation at 5 mg/kg should not exceed 15% for five analyses.

Solvent Extraction Method

The solvent extraction technique is suitable for the determination of extractable organic compounds at 0.3 to 100 mg/kg, but is most appropriate for organic compounds with vapour pressures less than 10 mm Hg at 25º. The conditions for the gas chromatograph are the same as for the Vapour Partitioning method, except that the column temperature is 120º, and the carrier-gas flow is 21 ml/min.

Preparation of Standards

Prepare the Standard Solution as described under Vapour Partitioning.

Calibration

Extract a sample of the Standard Solution as directed under Procedure (below) and inject it into the gas chromatograph. Determine a blank for each lot of reagent-grade Hydrochloric Acid and perchloroethylene by extracting the Hydrochloric Acid in the same way as the standard. Calculate a response factor (R) by dividing the concentration (C) in mg/kg for each component by the peak area (A) for that component (subtract any area obtained from the blank sample):
R = C/(A - area of blank)

Procedure

Accurately transfer 90 ml of the Hydrochloric Acid sample and 10 ml of perchloroethylene (free of interfering impurities) into a narrow-mouth, 125-ml bottle. Place the bottle in a mechanical shaker for 30 min. Separate the two phases (perchloroethylene on the bottom) and inject 3 µl of the perchloroethylene extract into the gas chromatograph. Approximate elution times in min for some chlorinated organic compounds are as follows:

Vinylidene chloride: 2.94
Methylene chloride: 3.27
Chloroform: 3.83
Carbon tetrachloride: 4.07
1,1,1-Trichloroethane: 4.50
Trichloroethylene: 4.97
Ethylene dichloride: 5.26
Propylene dichloride: 6.36
Perchloroethylene: 6.95
1,1,1,2-Tetrachloroethane: 10.12
1,1,2,2-Tetrachloroethane: 13.70
Pentachloroethane: 16.19

To determine perchloroethylene and higher-boiling impurities, substitute methylene chloride (free of interfering impurities) for perchloroethylene in the extraction step. For higher-boiling impurities such as monochlorobenzene and the three dichloro- benzenes, use a 2.74-m x 2.1-mm (id) stainless steel column packed with 10% Carbowax 20M/20% KOH on 80/100 mesh Chromasorb W (acid washed) at 150º and a nitrogen flow of 35 ml/min.

Calculation

Calculate the concentration (C), in mg/kg, of each compound by multiplying the corresponding peak area (A) (subtracted any area obtained from the blank sample) by the appropriate response factor (R) determined in the Calibration protocol:

C = R x (A - area of blank)

Precision

The relative standard deviation at 5 mg/kg should not exceed 15% for five analyses.

METHOD OF ASSAY

Tare accurately a 125-ml glass-stoppered conical flask containing 50 ml of 1N sodium hydroxide. Partially fill, without the use of vacuum, a 10-ml serological pipet from near the bottom of a representative sample, remove any acid adhering to the outside and discard the first ml flowing from the pipet. Hold the tip of the pipet just above the surface of the sodium hydroxide solution, and transfer between 2.5 and 3 ml of the sample into the flask, mix the contents, and weigh accurately to obtain the weight of the sample. Add methyl orange TS and titrate the excess of sodium hydroxide with 1N hydrochloric acid. Each ml of 1N sodium hydroxide is equivalent to 36.46 mg of HCl.

ETHYL p-HYDROXYBENZOATE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). Group ADI 0-10 mg/kg bw for ethyl, methyl and propyl p-hydroxybenzoate, established at the 17th JECFA in 1973.

SYNONYMS Ethylparaben, ethyl p-oxybenzoate, INS No. 214

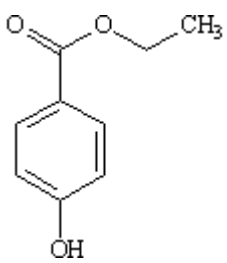
DEFINITION

Chemical names: Ethyl p-hydroxybenzoate, ethyl ester of p-hydroxybenzoic acid

C.A.S. number: 120-47-8

Chemical formula: $C_9H_{10}O_3$

Structural formula:



Formula weight: 166.18

Assay: Not less than 99.0% on the dried basis

DESCRIPTION Almost odourless, small, colourless crystals or a white, crystalline powder

FUNCTIONAL USES Preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in ethanol, ether and propylene glycol.

Melting range (Vol. 4): 115 - 118º

Test for p-hydroxybenzoate: Melting range of p-hydroxybenzoic acid derived from the sample is 212-217º
To 0.5 g of the sample add 10 ml of sodium hydroxide TS. Boil for 30 min and concentrate to about 5 ml. Cool, acidify with dilute sulfuric acid TS, collect the precipitate on a filter, and wash thoroughly with water. Dry in a desiccator over sulfuric acid. Determine the melting range of p-hydroxybenzoic acid so obtained.

PURITY

Loss on drying (Vol. 4): Not more than 0.5% (80º, 2 h)

Sulfated ash (Vol. 4)	Not more than 0.05% Test 2 g of the sample (Method I)
Acidity	Heat 750 mg of the sample with 15 ml of water at 80^{o} for 1 min, cool, and filter. The filtrate should be acid or neutral to litmus. To 10 ml of the filtrate add 0.2 ml of 0.1 N sodium hydroxide and 2 drops of methyl red TS. The solution should be yellow without even a light cast of pink.
p-Hydroxybenzoic acid and salicylic acid	Dissolve 0.5 g of the sample, accurately weighed, in 30 ml of ether, add 20 ml of a 1 in 100 sodium hydrogen carbonate solution, shake, and separate the water layer. Wash the water layer with two 20 ml portions of ether, add 5 ml of dilute sulfuric acid and 30 ml of ether, and shake. Separate the ether layer, and shake with about 10 ml of water. Filter the ether layer, and wash the vessel and the filter with a small amount of ether. Combine the washings and the filtrate, evaporate ether on a water bath, and dry the residue over sulfuric acid to constant weight. The weight of the residue should not exceed 5 mg. Dissolve any residue in 25 ml of water, heat to about 70^{o}, filter, and add a few drops of dilute ferric chloride TS. No violet to reddish violet colour should be produced.
Lead (Vol. 4)	Not more than 2 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh, to the nearest mg, 2 g of the dried sample and transfer into a flask. Add 40 ml of N sodium hydroxide and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h and cool. Add 5 drops of bromothymol blue TS and titrate the excess sodium hydroxide with N sulfuric acid, comparing the colour with a buffer solution (pH 6.5) containing the same proportion of indicator. Perform a blank determination with the reagents and make any necessary correction. Each ml of N sodium hydroxide is equivalent to 166.18 mg of $C_9H_{10}O_3$.

METHYL p-HYDROXYBENZOATE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). Group ADI 0-10 mg/kg bw for ethyl, methyl and propyl p-hydroxybenzoate, established at the 17th JECFA in 1973.

SYNONYMS Methylparaben, methyl p-oxybenzoate; INS No. 218

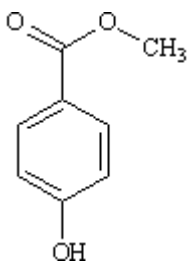
DEFINITION

Chemical names: Methyl p-hydroxybenzoate, methyl ester of p-hydroxybenzoic acid

C.A.S. number: 99-76-3

Chemical formula: $C_8H_8O_3$

Structural formula:



Formula weight: 152.15

Assay: Not less than 99.0% on the dried basis

DESCRIPTION Almost odourless, small colourless crystals or white crystalline powder

FUNCTIONAL USES Preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water; freely soluble in ethanol and propylene glycol; soluble in ether

Melting range (Vol. 4): 125 - 128°

Test for p-hydroxybenzoate: Melting range of p-hydroxybenzoic acid derived from the sample is 212-217°

To 0.5 g of the sample add 10 ml of sodium hydroxide TS. Boil for 30 min and concentrate to about 5 ml. Cool, acidify with dilute sulfuric acid TS, collect the precipitate on a filter, and wash thoroughly with water. Dry in a desiccator over sulfuric acid. Determine the melting range of p-hydroxybenzoic acid so obtained.

PURITY

Loss on drying (Vol. 4)	Not more than 0.5% (over silica gel, 5 h)
Sulfated ash (Vol. 4)	Not more than 0.05%. Test 2 g of the sample (Method I)
Acidity	Heat 750 mg of the sample with 15 ml of water at 80° for 1 min, cool, and filter. The filtrate should be acid or neutral to litmus. To 10 ml of the filtrate add 0.2 ml of 0.1 N sodium hydroxide and 2 drops of methyl red TS. The solution should be yellow without even a light cast of pink.
p-Hydroxybenzoic acid and salicylic acid	Dissolve 0.5 g of the sample, accurately weighed, in 30 ml of ether, add 20 ml of a 1 in 100 sodium hydrogen carbonate solution, shake, and separate the water layer. Wash the water layer with two 20 ml portions of ether, add 5 ml of dilute sulfuric acid and 30 ml of ether, and shake. Separate the ether layer, and shake with about 10 ml of water. Filter the ether layer, and wash the vessel and the filter with a small amount of ether. Combine the washings and the filtrate, evaporate ether on a water bath, and dry the residue over sulfuric acid to constant weight. The weight of the residue should not exceed 5 mg. Dissolve any residue in 25 ml of water, heat to about 70°, filter, and add a few drops of dilute ferric chloride TS. No violet to reddish violet colour should be produced.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh, to the nearest mg, 2 g of the dried sample and transfer into a flask. Add 40 ml of N sodium hydroxide and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h and cool. Add 5 drops of bromothymol blue TS and titrate the excess sodium hydroxide with N sulfuric acid, comparing the colour with a buffer solution TS (pH 6.5) containing the same proportion of indicator. Perform a blank determination with the reagents and make any necessary correction. Each ml of N sodium hydroxide is equivalent to 152.2 mg of $C_8H_8O_3$

HYDROXYPROPYL CELLULOSE

Revised specification prepared at the 63rd JECFA (2004) and published in FNP52 Add 12 (2004) superseding specifications prepared at the 29th JECFA (1985) and published in FNP 52. An ADI 'not specified' was established for modified celluloses at the 35th JECFA (1989).

SYNONYMS Cellulose hydroxypropyl ether; modified cellulose; INS No. 463

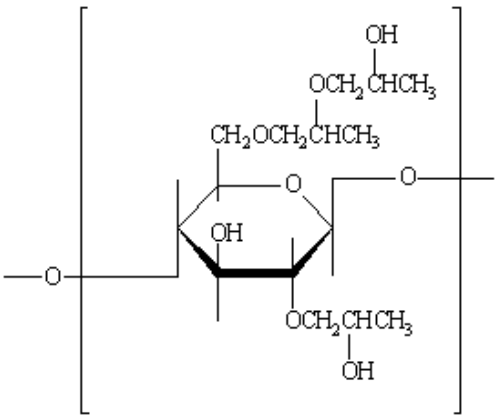
DEFINITION An ether of cellulose containing hydroxypropyl substitution prepared from cellulose by treatment with alkali and propylene oxide. The article of commerce can be specified further by viscosity.

Chemical names: Hydroxypropyl ether of cellulose, cellulose hydroxypropyl ether

C.A.S. number: 9004-64-2

Chemical formula: $[C_6H_7O_2(OH)_X(OCH_2CHOHCH_3)_y(OCH_2CH[R_w]CH_3)_z]_n$
where
x + y + z = 3
y + z (1+w) = not greater than 4.6
R = A substituent comprising "w" hydroxypropoxy groups

Structural formula



One of many possible structural formulae for the repeating unit of a hydroxypropyl cellulose with molar substitution of 3.0 and a degree of polymerization of n, showing a monomeric hydroxypropyl substitution at C_2 and a dimeric hydroxypropyl substitution at C_6.

Formula weight: Unsubstituted structural unit: 162.14
Trisubstituted structural unit: 336.37
Macromolecules: from about 30 000 (n about 100) up to about 1 million (n about 2500)

Assay: Not more than 80.5% of hydroxypropoxy groups equivalent to not more than 4.6 hydroxypropyl groups per anhydroglucose unit on the dried basis

DESCRIPTION Slightly hygroscopic, white or off-white, almost odourless, granular or fibrous powder

FUNCTIONAL USES Emulsifier, thickener, stabiliser, binder, suspension agent, film coating

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Swells in water, producing a clear to opalescent, viscous colloidal solution; insoluble in ethanol; insoluble in ether
Foam formation	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.
Precipitate formation	To 5 ml of a 0.5% solution of the sample, add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.
Substituents	See description under METHOD OF ASSAY

PURITY

Loss on drying (Vol. 4)	Not more than 10.0% (105° to constant weight)
pH (Vol. 4)	Not less than 5.0 and not more than 8.0 (1 in 100 soln)
Sulfated ash (Vol. 4)	Not more than 0.5%. Test 1 g of the sample
Lead (Vol. 4)	Not more than 2 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, "Instrumental Methods"
Propylene chlorohydrins	Not more than 0.1 mg/kg See description under TESTS

TESTS

PURITY TESTS

Propylene chlorohydrins — Determine by gas liquid chromatography (see Volume 4) using the following procedure:

Preparation of Standards

Stock Standard Solution: Weigh 0.1 g propylene chlorohydrin (C.A.S. No. 127-00-4, mixture of 1-Chloro-2-propanol-70% and 2-Chloro-1-propanol-30%) to the nearest 0.0001g and bring to a final volume of 100 ml with diethyl ether.

Working Standard Solution: Perform serial dilutions (in diethyl ether) of stock standard to achieve a working calibration range of 6-25 ng/ml.

Note**:** All standard solutions should be prepared with diethyl ether of the highest purity

Gas Chromatography

Gas Chromatograph with a Halogen Specific Detector, on-column injector, and linear column temperature programming.

Column: 30 m x 0.53 mm x 1 μm DB-WAX or equivalent.

Temperature programming:

Initial Temperature	35^{o}
Initial Hold Time	7.0 min
Ramp Rate	8.0^{o}/min
Final Temperature	200^{o}
Final Hold Time	5.0 min
Inlet	200^{o}
Detector (XSD)	1000^{o}

Flow rates:

Helium (carrier gas) 5 psi (column head pressure at 35^{o})
Detector Make-up Gas (air) 40 psi
Retention times (min):

1-Chloro-2-propanol	~11.7
2-Chloro-1-propanol	~12.5

Procedure:
Weigh ~1 g of sample into a centrifuge tube and record weight to the nearest 0.01 g. Quantitatively add 5.0 ml diethyl ether to the sample and sonicate for 10 minutes. Centrifuge the sample to separate the mixture. Remove a portion of the diethyl ether extract for GC analysis.

Calculations:
Prepare a calibration curve by plotting the concentration (ng/ml) versus detector response (in a linear range of 6-25 ng/ml). From the linear regression of this curve, calculate ng/g using the following equation:

$$ng/g = (V \times (R-b)/m)/W$$

where:

- R= detector response for the sample
- b = y-intercept of the linear regression curve
- m = slope of the linear regression curve
- V= final volume (5.0 ml)
- W= weight of the sample in grams

METHOD OF ASSAY

Determination of the hydroxypropoxy group content

Apparatus
The apparatus for hydroxypropoxy group determination is shown in the accompanying diagram. The boiling flask, D, is fitted with an aluminium foil-covered Vigreux column, E, on the sidearm and with a bleeder tube through the neck and to the bottom of the flask for the introduction of steam and nitrogen. A steam generator, B, is attached to the bleeder tube through Tube C, and a condenser, F, is attached to the Vigreux column. The boiling flask and steam generator are immersed in an oil bath, A, equipped with a thermo-regulator such that a temperature of 155^{o} and the desired heating rate may be maintained. The distillate is collected in a 150 ml beaker, G, or other suitable container.

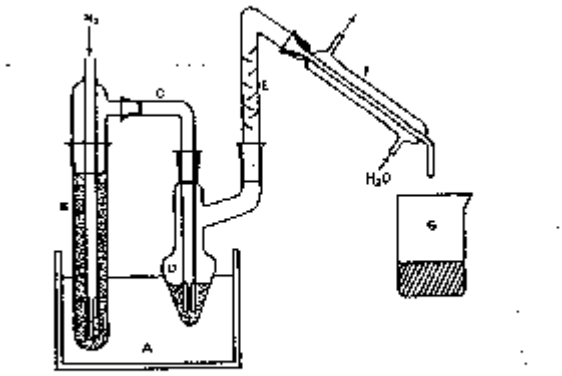


Figure Apparatus for Hydroxypropyl Determination

Procedure

Transfer about 100 mg of the sample, previously dried at 105° for 2 h and accurately weighed, into the boiling flash, and add 10 ml of chromium trioxide solution (60 g in 140 ml of water). Immerse the steam generator and the boiling flask in the oil bath (at room temperature) to the level of the top of the chromium trioxide solution. Start cooling water through the condenser and pass nitrogen gas through the boiling flask at the rate of one bubble per sec. Starting at room temperature, raise the temperature of the oil bath to 155° over a period of not less than 30 min, and maintain this temperature until the end of the determination. Distil until 50 ml of the distillate is collected. Detach the condenser from the Vigreux column, and wash it with water, collecting the washings in the distillate container. Titrate the combined washings and distillate with 0.02 N sodium hydroxide to a pH of 7.0, using a pH meter set at the expanded scale.
NOTE: Phenolphthalein TS may be used for this titration, if it is also used for all standards and blanks.

Record the volume, V_a of the 0.02 N sodium hydroxide used. Add 500 mg of sodium bicarbonate and 10 ml of dilute sulfuric acid TS, and then after evolution of carbon dioxide has ceased, add 1 g of potassium iodide. Stopper the flask, shake the mixture, and allow it to stand in the dark for 5 min. Titrate the liberated iodine with 0.02 N sodium thiosulfate to the sharp disappearance of the yellow colour, confirming the end-point by the addition of a few drops of starch TS. Record the volume of 0.02 N sodium thiosulfate required as Y_a.
Make several reagent blank determinations, using only the chromium trioxide solution in the above procedure. The ratio of the sodium hydroxide titration (V_b) to the sodium thiosulfate titration (Y_b), corrected for variation in normalities, will give the acidity-to-oxidizing ratio, $V_b/Y_b = K$, for the chromium trioxide carried over in the distillation. The factor K should be constant for all determinations.
Make a series of blank determinations using 100 mg of methyl cellulose (containing no foreign material) in place of the sample, recording the average volume of 0.02 N sodium hydroxide required as V_m and the average volume of 0.02 N sodium thiosulfate required as Y_m.

Calculate the hydroxypropoxy group content of the sample, in mg, by the formula:

$75.0 \times [N_1 (V_a - V_m) - k N_2 (Y_a - Y_m)]$
where
N_1 = the exact normality of the 0.02 N sodium hydroxide solution
N_2 = the exact normality of the 0.02 N sodium thiosulfate solution
$k = V_b N_1 / Y_b N_2$

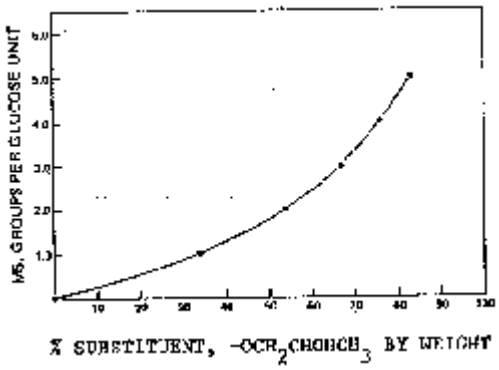


Chart for converting percentage of substitution, by weight, of hydroxypropoxy groups to molecular substitution per glucose unit.

Determination of the methoxy group
See Apparatus and Procedure in *Ethoxy and Methoxy Group Determination* and determine the content of methoxy group ($-OCH_3$).

Calculation
Calculate as percentage. Correct the % of methoxy groups thus determined by the formula:

$$A - (B \times 0.93 \times 31 / 75)$$

where

A = the total % of $-OCH_3$ groups determined
B = the % of $-OCH_2CHOHCH_3$ determined in the Method of Assay for Hydroxypropoxy group content
0.93 = an average obtained by determining, on a large number of samples, the propylene produced from the reaction of hydriodic acid with hydroxypropoxy groups during the Method of Assay for methoxy groups ($-OCH_3$).

HYDROXYPROPYLMETHYL CELLULOSE

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding the specifications prepared at the 63rd JECFA (2004), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI "not specified" for modified celluloses (ethyl cellulose, ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, methyl cellulose, methyl ethyl cellulose, and sodium carboxymethyl cellulose) was established at the 35th JECFA (1989).

SYNONYMS INS No. 464

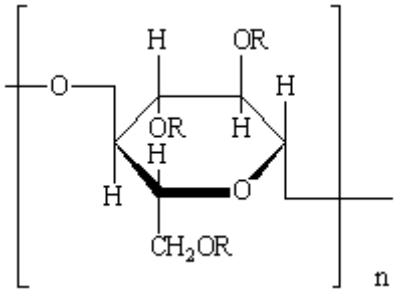
DEFINITION Hydroxypropylmethyl cellulose is a methyl cellulose modified by treatment with alkali and propylene oxide by which a small number of 2-hydroxypropyl groups are attached through ether links to the anhydroglucose units of the cellulose. The article in commerce may be further specified by viscosity.

Chemical names: Hydroxypropylmethyl cellulose, 2-hydroxypropyl ether of methyl cellulose

C.A.S. number: 9004-65-3

Chemical formula: $[C_6H_7O_2(OH)_x(OCH_3)_y(OCH_2CHOHCH_3)_z]_n$
where
z = 0.07 - 0.34
y = 1.12 - 2.03
x = 3-(z + y): (z + y = degree of substitution)

Structural formula



where R = H or CH_3 or $CH_2CHOHCH_3$

Formula weight: Unsubstituted structural unit: 162.14
Structural unit with 1.19 degree of substitution: approx. 180
Structural unit with 2.37 degree of substitution: approx. 210
Macromolecules: from about 13,000 (n about 70) up to about 200,000 (n about 1000)

Assay: Not less than 19% and not more than 30% of methoxy groups (-OCH_3) and not less than 3% and not more than 12% hydroxypropoxy groups (-$OCH_2CHOHCH_3$), on the dried basis

DESCRIPTION Hygroscopic white or off-white powder, or granules or fine fibres

FUNCTIONAL USES Emulsifier, thickening agent, stabiliser

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Swells in water, producing a clear to opalescent, viscous colloidal solution; insoluble in ethanol
Foam formation	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.
Precipitate formation	To 5 ml of a 0.5% solution of the sample, add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.
Substituents	See description under METHOD OF ASSAY

PURITY

Loss on drying (Vol. 4)	Not more than 10.0% (105° to constant weight)
pH (Vol. 4)	Not less than 5.0 and not more than 8.0 (1 in 100 solution)
Sulfated ash (Vol. 4)	Not more than 1.5% for products with viscosities of 50 centipoise or above, and not more than 3% for products with viscosities below 50 centipoise Test 1 g of the sample
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Propylene chlorohydrins	Not more than 1 mg/kg See description under TESTS

TESTS

PURITY TESTS

Propylene chlorohydrins	Determine by Gas Chromatography–Mass Spectrometry (GC-MS) (Vol. 4) using the following procedure. **Note:** Propylene chlorohydrins (PCH) are present as 2 isomers namely: 1-chloro-2-propanol (1C2P) and 2-chloro-1-propanol (2C1P). Internal standard solutions *Internal Standard Stock Solution #1 (1 mg/ml):* Weigh 0.1 g to nearest 0.1 mg (approximately 100 µl) of o-xylene-d_{10} (CAS 56004-61-6) into a 100 ml volumetric flask and make up to volume with methanol.

Internal Standard Stock Solution #2 (100 µg/ml): Pipette 5ml of Internal Standard Stock Solution #1 into a 50 ml volumetric flask and make up to volume with methanol.

Internal Standard Stock Solution #3 (4 µg/ml): Pipette 1 ml of Internal Standard Stock Solution #2 into a 25 ml volumetric flask and make up to volume with methanol:

Internal Standard Solution #4 (16 ng/ml): Add 1 ml of Internal Standard Stock Solution #3 into a 250 ml volumetric flask and dilute to volume with diethyl ether.

Internal Standard Solution #5 (8 ng/ml): Pipette 25 ml of Internal Standard Stock Solution #4 into a 50 ml volumetric flask and dilute to volume with diethyl ether.

Standards

Stock Standard Solution #1 (1mg/ml): Weigh 0.1 g to the nearest 0.1 mg of propylene chlorohydrin, mixture of 1-Chloro-2-propanol-75% and 2-Chloro-1-propanol-25%, Eastman Kodak, Cat. # P1325 or equivalent) into a 100 ml volumetric flask and make up to volume with diethyl ether.

Stock Standard Solution #2 (100 µg/ml): Pipette 5 ml of Standard Stock Solution #1 into a 50 ml volumetric flask and make up to volume with diethyl ether.

Stock Standard Solution #3 (10 µg/ml): Pipette 5 ml of Standard Stock Solution #2 into a 50 ml volumetric flask and make up to volume with diethyl ether.

Stock Standard Solution #4 (500 ng/ml): Pipette 5 ml of Standard Stock Solution #3 into a 100 ml volumetric flask and make up to volume with diethyl ether.

Note: All standard solutions should be prepared with diethyl ether of the highest purity available.

Prepare working standard solutions by pipetting the volumes shown in the table below in to a 10 ml volumetric flask and make up to volume with diethyl ether.

Vol. of Stock Standard Solution #4, ml	Vol. of Internal standard solution #4, ml	Vol. made up, ml	Conc. of Standard (ng/ml)	Conc. of Internal Standard (ng/ml)
0.50	5.0	10.0	25	8
1.0	5.0	10.0	50	8
2.0	5.0	10.0	100	8
4.0	5.0	10.0	200	8
5.0	5.0	10.0	300	8

Instrument:

A gas chromatograph with a mass selective detector (GCMS) in Selective Ion Monitoring (SIM) mode, Electron impact ionisation (EI)

source, pulsed-splitless injector and a data station.

GCMS Conditions:

	Inlet temperature	225°
	Pulse pressure	50 psi until 2 min
	Inlet purge flow	40 ml/min at 2 min
	Injection volume	5 µl
Guard Column	Deactivated fused silica, 10 m x 0.25 mm i.d. x 0.35 mm o.d.	
Column	30 m x 0.25 mm i.d. x 1.4 µm film DB-624 or equivalent	
Temperature programming:	Initial temperature	40°
	Initial hold Time	5.0 min
	Ramp rate	10°/min
	Temperature 2	80°
	Hold time	3.0 min
	Ramp rate	25°/min
	Final temperature	230°
	Final hold time	5.0 min
Carrier	Gas	Helium
	Flow rate	1.4 ml/min
	Column he d pressure	11.5 psi
Detector	Ion source temperature.	230°
	Transfer line temperature	260°
SIM ions:	o-Xylene-d_{10}	Target ion m/z = 116
		Qualifier ion m/z = 98
	1-Chloro-2-Propanol	Target ion m/z = 79
		Qualifier ion m/z = 81
	2-Chloro-1-Propanol	Target ion m/z= 58
		Qualifier ion m/z= 31
Retention times	o-Xylene-d_{10}	13.7 min
	1-Chloro-2-Propanol	9.5 min
	2-Chloro-1-Propanol	10.4 min

Procedure:

Weigh about 1.00 g, to nearest 0.1 mg, of sample into a glass vial. Pipette 5.0 ml of Internal Standard Solution #5 into the vial, securely close the vial and sonicate for 10 minutes. Centrifuge the vial to separate the mixture. Remove a portion of the diethyl ether layer for GCMS analysis.

Calculations:

Calculate the ratios of detector responses for 1C2P and 2C1P versus detector response for o-xylene-d_{10} at each working standard concentration using the following equation:

$$AR_{(std)} = R_{(std)}/R_{(IS)}$$

where

$AR_{(std)}$ is the ratio of detector response for 1C2P or 2C1P versus the detector response for o-xylene-d_{10} in the standard;

$R_{(std)}$ is the detector response of the target ion for 1C2P or 2C1P in the standard; and

$R_{(IS)}$ is the detector response of the target ion for o-xylene-d_{10} in the standard.

Prepare standard curves for 1C2P and 2C1P by plotting the concentration of 1C2P or 2C1P (ng/ml) versus the ratios of detector response ($AR_{(std)}$) for each isomer in the working standards

Calculate the ratio of detector response for 1C2P and 2C1P versus the detector response for o-xylene-d_{10} in the sample using the following equation:

$$AR_{(sample)} = R_{(Sample)}/R_{(IS)}$$

where

$AR_{(sample)}$ is the ratio of detector response for 1C2P or 2C1P versus the detector response for o-xylene-d_{10} in the sample;

$R_{(sample)}$ is the detector response of the target ion for 1C2P or 2C1P in the sample; and

$R_{(IS)}$ is the detector response of the target ion for o-xylene-d_{10} in the sample.

From the linear regression of the standard curves for each isomer, calculate ng/g using the following equation:

$$ng/g = (V \times (AR_{(sample)} - b)/m)/W$$

where

$AR_{(sample)}$ is the Ratio of detector response for 1C2P or 2C1P versus the detector response for o-xylene-d_{10} in the sample;

b is the y-intercept of the linear regression curve;

m is the slope of the linear regression curve;

V is the final volume (5.0 ml); and

W is the weight of the sample in grams.

Report the PCH content in mg/kg as the sum of the 2 isomers (1C2P and 2C1P).

METHOD OF ASSAY **Determination of the content of hydroxypropoxy groups**

Apparatus

The apparatus for hydroxypropoxy group determination is shown in the accompanying diagram. The boiling flask, D, is fitted with an aluminium foil-covered Vigreux column, E, on the sidearm and with a bleeder tube through the neck and to the bottom of the flask for the introduction of steam and nitrogen. A steam generator, B, is attached to the bleeder tube through Tube C, and a condenser, F, is attached to the Vigreux column. The boiling flask and steam generator are immersed in an oil bath, A, equipped with a thermo-regulator such that a temperature of 155° and the desired heating rate may be maintained. The distillate is collected in a 150 ml beaker, G, or other suitable container.

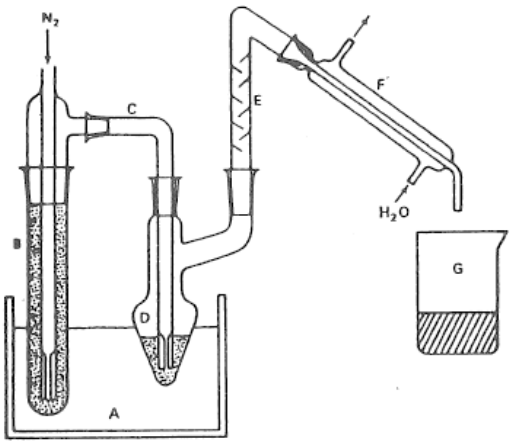


Figure. Apparatus for hydroxypropyl determination

Procedure

Accurately weigh about 100 mg of the sample, previously dried at 105° for 2 h, transfer into the boiling flask and add 10 ml of chromium trioxide solution (60 g in 140 ml of water). Immerse the steam generator and the boiling flask in the oil bath (at room temperature) to the level of the top of the chromium trioxide solution. Start cooling water through the condenser and pass nitrogen gas through the boiling flask at the rate of one bubble per sec. Starting at room temperature, raise the temperature of the oil bath to 155° over a period of not less than 30 min, and maintain this temperature until the end of the determination. Distil until 50 ml of the distillate is collected. Detach the condenser from the Vigreux column, and wash it with water, collecting the washings in the distillate container. Titrate the combined washings and distillate with 0.02 N sodium hydroxide to a pH of 7.0, using a pH meter set at the expanded scale.

Note: Phenolphthalein TS may be used for this titration instead of pH meter, if it is also used for all standards and blanks.

Record the volume, V_a of the 0.02 N sodium hydroxide used. Add

500 mg of sodium bicarbonate and 10 ml of dilute sulfuric acid TS, and then after evolution of carbon dioxide has ceased, add 1 g of potassium iodide. Stopper the flask, shake the mixture, and allow it to stand in the dark for 5 min. Titrate the liberated iodine with 0.02 N sodium thiosulfate to the sharp disappearance of the yellow colour, confirming the end-point by the addition of a few drops of starch TS. Record the volume of 0.02 N sodium thiosulfate required as Y_a.

Make several reagent blank determinations, using only the chromium trioxide solution in the above procedure. The ratio of the sodium hydroxide titration (V_b) to the sodium thiosulfate titration (Y_b), corrected for variation in normalities, will give the acidity-to-oxidizing ratio, $V_b/Y_b = K$, for the chromium trioxide carried over in the distillation. The factor K should be constant for all determinations.

Make a series of blank determinations using 100 mg of methyl cellulose (containing no foreign material) in place of the sample, recording the average volume of 0.02 N sodium hydroxide required as V_m and the average volume of 0.02 N sodium thiosulfate required as Y_m.

Calculate the content of hydroxypropoxy groups (in mg) in the sample using the formula:

$$75.0 \times [N_1 (V_a - V_m) - k\, N_2 (Y_a - Y_m)]$$

where

N_1 is the exact normality of the 0.02 N sodium hydroxide solution;
N_2 is the exact normality of the 0.02 N sodium thiosulfate solution; and
k is $V_b N_1 / Y_b N_2$

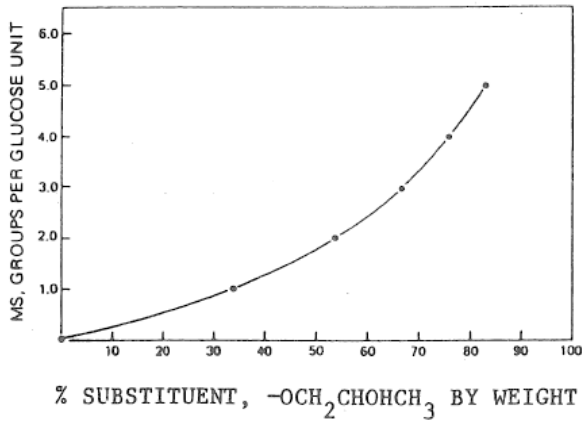


Chart for converting percentage of substitution, by weight, of hydroxypropoxy groups to molecular substitution per glucose unit.

Determination of the content of methoxy groups

Volume 4, under ASSAY METHODS, Cellulose Derivatives Assay, *Ethoxyl and Methoxyl Group Determination*.

See Apparatus and Procedure in *Ethoxy and Methoxy Group Determination* and determine the content of methoxy groups ($-OCH_3$).

Calculation

Calculate as percentage. Correct the % of methoxy groups thus determined by the formula:

$$A - (B \times 0.93 \times 31 / 75)$$

where

A is the total % of $-OCH_3$ groups determined;

B is the % of $-OCH_2CHOHCH_3$ determined in the Method of Assay for hydroxypropoxy group content; and

0.93 is an average obtained by determining, on a large number of samples, the propylene produced from the reaction of hydriodic acid with hydroxypropoxy groups during the Method of Assay for methoxy groups ($-OCH_3$).

INDIGOTINE

Prepared at the 73rd JECFA (2010) and published in FAO Monographs 10 (2010), superseding specifications prepared at the 28th JECFA (1984) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0 - 5 mg/kg bw was established at the 18th JECFA (1974).

SYNONYMS CI Food Blue 1, FD&C Blue No. 2, Indigo Carmine, CI (1975) No. 73015, INS No. 132

DEFINITION Consists essentially of a mixture of disodium 3,3' -dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate (principal component) and disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate (isomer) and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

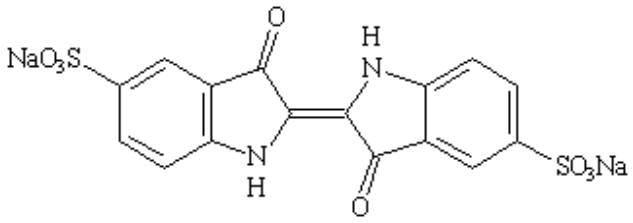
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names: Disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate (principal component)

C.A.S. number: 860-22-0 (principal component)

Chemical formula: $C_{16}H_8N_2Na_2O_8S_2$ (principal component)

Structural formula



Principal component

Formula weight: 466.36 (principal component)

Assay: Not less than 85% total colouring matters.
Not more than 18% of disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate.

DESCRIPTION Blue powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Identification of colouring matters (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 15% at 135° together with chloride and sulfate calculated as sodium salts. (See Volume 4 under "SPECIFIC METHODS, Food Colours.")
Water insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Not more than 1% See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5% of sum of isatin-5-sulfonic acid, 5-sulfoanthranilic acid and anthranilic acid. (See Volume 4 under "SPECIFIC METHODS, Food Colours.") Proceed as directed under *Determination by High Performance Liquid Chromatography* using an elution gradient of 2 to 100% at 4% per min (linear) followed by elution at 100%.
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline (See Volume 4 under "SPECIFIC METHODS, Food Colours.")
Ether extractable matter (Vol. 4)	Not more than 0.2% (See Volume 4 under "SPECIFIC METHODS, Food Colours, Method II.") Use 2 g of sample for the test.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Inorganic Components, Metallic Impurities").

TESTS

PURITY TESTS

Subsidiary colouring matters	Subsidiary colouring matters are determined by high performance liquid chromatography using the following conditions: Chromatographic system - HPLC system with a UV/VIS detector or a diode array detector,

auto sampler or injector
- Detector wavelength: 610 nm
- Column: C18 on silica gel (250 x 4.6 mm, 5 µm) ACE 5 C18 or equivalent
- Mobile phase: solvent A: 0.02 mol/l ammonium acetate and solvent B: acetonitrile: water (7:3 v/v)
- Gradient elution: A:B 92:8 v/v to A:B 70:30 v/v (0-15 min); to A:B 40:60 v/v (15-25 min); to A:B 92:8 v/v (25-30 min); A:B 92:8 v/v (30-35 min).
- Column temperature: 40°
- Flow rate: 1.0 ml/min
- Injection volume: 20 µl

The subsidiary colours are separated from the principal component and its isomer. The subsidiary colouring matter monosodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5 sulfonate elutes at approximately 21 min.

METHOD OF ASSAY

Total colouring matters content

Proceed as directed under *Total Content by Titration with Titanous Chloride* in Volume 4, using the following (See "SPECIFIC METHODS, Food Colours"):
Weight of sample: 1.0-1.1 g
Buffer: 15 g sodium hydrogen tartrate
Weight (D) of colouring matters equivalent to
1.00 ml of 0.1 N $TiCl_3$: 23.32 mg.

Disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate,disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]- 5,7'-disulfonate (5,7' isomer) and subsidiary colouring matters by HPLC

The isomers get separated under the HPLC conditions detailed under the separation of subsidiary colouring matters, and the amounts present can be quantified using an external standard calibration.

Reagents
- Acetonitrile, HPLC grade
- Ammonium acetate, HPLC grade
- Reference standards of disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate and disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]- 5,7'-disulfonate

Standard stock solutions (1000 µg/ml): Weigh accurately 0.10 g (±0.1 mg) of each reference standard and transfer to a 100 ml volumetric flask and bring to volume with water.

Standard solutions: Prepare five solutions from the standard stock solutions in the concentration range of 1 to 20 µg/ml.

Sample solution: Weigh accurately 0.10 g (±0.1 mg) (w_2) of the sample and transfer to a 100 ml volumetric flask (v) and bring to

volume with water (sample solution S_A).
Transfer 1.00 ml (v_{s1}) and 5.00 ml (v_{s5}) of the solution S_A to two 50 ml volumetric flasks (v_S), respectively, and bring to volume with water (sample solutions S_1 and S_5).

Procedure

Inject the five standard solutions for each isomer using the conditions detailed under TESTS (Subsidiary colouring matters by HPLC) and integrate peak areas at 6.5 min for the 5,5' isomer (disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate) and 10.8 min for the 5,7' isomer (disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]- 5,7'-disulfonate). Construct standard curve for each compound (Area vs. standard concentration, µg/ml).
Inject sample solutions S_A, S_1 and S_5. The peak area of the 5,7' isomer for the sample solution should be in the linear range of the calibration graph, otherwise increase the volume v_{s5}. Sample solutions S_1, S_5 and S_A are to quantify disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate, disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate (5,7' isomer) and subsidiary colouring matters, respectively.

Calculations
Calculate the concentrations (C, % (w/w)) of the two isomers and subsidiary colouring matters in the sample using the standard curves and the following formulas:

$$C_{5.5'\,\text{isomer}\,\%\,(w/w)} = \left(\frac{A_{5,5} - b_{5,5'}}{m_{5,5'}} \right) \times 10^{-6} \times \frac{v_S}{v_{s1}} \times v \times \frac{100}{w_S}$$

$$C_{5.7'\,\text{isomer}\,\%\,(w/w)} = \left(\frac{A_{5,7} - b_{5,7'}}{m_{5,7'}} \right) \times 10^{-6} \times \frac{v_S}{v_{s5}} \times v \times \frac{100}{w_S}$$

$$C_{\text{Subsidiary colouring matters}\,\%\,(w/w)} = \left(\frac{A_{sum} - b_{5,5'}}{m_{5,5'}} \right) \times 10^{-6} \times v \times \frac{100}{w_S}$$

where

- $A_{5,5'}$ is the area of the peak of the 5,5' isomer in the sample chromatogram (area units);
- $A_{5,7'}$ is the area of the peak of the 5,7' isomer in the sample chromatogram (area units);
- A_{sum} is the sum of the areas of the peaks in the chromatogram (610 nm), except for the two isomers;
- $b_{5,5'}$ and $b_{5,7'}$ are the linear coefficients of the calibration graphs for the 5,5' isomer and 5,7' isomer, respectively;
- $m_{5,5'}$ and $m_{5,7'}$ are is the slope of the calibration graph (area units ml/µg) for the 5,5' isomer and 5,7' isomer, respectively;
- w_s is the sample weight (g);

v is the volume of the sample solution s_A (ml);
v_{s1} is the volume of the sample solution s_1 (ml); and
v_{s5} is the volume of the sample solution s_5 (ml).

5'-INOSINIC ACID

Prepared at the 29th JECFA (1974), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for inosinic acid and its Ca, K and Na salts, was established at the 29th JECFA (1985)

SYNONYMS Inosinic acid, IMP, INS No. 630

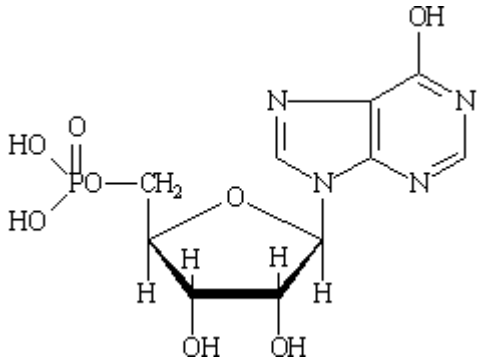
DEFINITION

Chemical names: Inosine-5'-monophosphoric acid

C.A.S. number: 131-99-7

Chemical formula: $C_{10}H_{13}N_4O_8P$

Structural formula



Formula weight: 348.21

Assay: Not less than 97.0% and not more than 102.0% on the dried basis

DESCRIPTION Odourless, colourless or white crystals, or a white crystalline powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; slightly soluble in ethanol

Spectrophotometry (Vol. 4): A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 250±2nm. The ratio A250/A260 is between 1.55 and 1.65, and the ratio A280/260 is between 0.20 and 0.30

Test for ribose (Vol. 4): Passes test

Test for organic phosphate (Vol. 4): Passes test
Test 5 ml of a 1 in 20 solution

PURITY

Loss on drying (Vol. 4)	Not more than 3% (120°, 4 h)
pH (Vol. 4)	1.0 - 2.0 (1 in 20 soln)
Related foreign substances (Vol. 4)	Chromatographically not detectable Test 1 µl of a 1 in 200 soln
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 250 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of $C_{10}H_{13}N_4O_8P$, in % in the sample by the formula:

$$\frac{A}{349} \times \frac{250{,}000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{loss on drying (\%)}} \times 100$$

IRON OXIDES

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding the specifications prepared at the 63rd JECFA (2004), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-0.5 mg/kg bw was established at the 53rd JECFA (1999).

SYNONYMS

Iron Oxide yellow: CI Pigment Yellow 42 and 43; CI(1975) No. 77492; INS No. 172(iii)
Iron Oxide Red: CI Pigment Red 101 and 102; CI (1975) No. 77491; INS No. 172(ii)
Iron Oxide Black: CI Pigment Black 11; CI (1975) No. 77499; INS No. 172(i)

DEFINITION

Iron oxides are produced from ferrous sulfate by heat soaking, removal of water, decomposition, washing, filtration, drying and grinding. They are produced in either anhydrous or hydrated forms. Their range of hues includes yellows, reds, browns and blacks. The food-quality iron oxides are primarily distinguished from technical grades by their comparatively low levels of contamination by other metals; this is achieved by the selection and control of the source of the iron or by the extent of chemical purification during the manufacturing process.

Chemical names

Iron Oxide Yellow:	Hydrated ferric oxide, hydrated iron (III) oxide
Iron Oxide Red:	Iron sesquioxide, anhydrous ferric oxide, anhydrous iron (III) oxide
Iron Oxide Black:	Ferroso ferric oxide, iron (II,III) oxide

C.A.S. number

Iron Oxide Yellow:	51274-00-1
Iron Oxide Red:	1309-37-1
Iron Oxide Black:	1317-61-9

Chemical formula

Iron Oxide Yellow:	$FeO(OH) \cdot xH_2O$
Iron Oxide Red:	Fe_2O_3
Iron Oxide Black:	$FeO \cdot Fe_2O_3$

Formula weight

88.85	FeO(OH)
159.70	Fe_2O_3
231.55	$FeO \cdot Fe_2O_3$

Assay

Not less than 60% of iron

DESCRIPTION

Yellow, red, brown or black powder

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water and organic solvents; soluble in concentrated mineral acids

PURITY

Loss on drying (Vol. 4)

Iron Oxide Red : Not more than 1.0% (105°, 4 h)

Water-soluble matter — Not more than 1.0%
See description under TESTS

Arsenic (Vol. 4) — Not more than 3 mg/kg
Determine by the atomic absorption hydride technique. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

Cadmium (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption/ICP technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

Lead (Vol. 4) — Not more than 10 mg/kg
Determine using an atomic absorption/ICP technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

Mercury (Vol. 4) — Not more than 1 mg/kg
Determine by the cold vapour atomic absorption technique.

TESTS

PURITY TESTS

Water-soluble matter — Weigh accurately 5.0 g of iron oxide, transfer to a 250 ml beaker, add 200 ml of water and boil for 5 minutes; stir to avoid bumping. Cool the mixture, transfer the contents to a 250 ml volumetric flask, rinse the beaker with 25 ml of water, adding the rinsings to the flask; bring to volume with water and mix. Allow the mixture to stand for 10 minutes and filter the solution. Transfer 100 ml of filtrate into a clean dry tared beaker and carefully evaporate the solution to dryness on a boiling water bath. Dry the residue at 105 -110^{o} for 2 hours, cool the beaker with residue in a desiccator, weigh the beaker, and calculate the amount of residue.

$$\text{Water-soluble matter (\%)} = 250 \times W_R/W_S$$

where W_R is the weight of residue (g) and W_S is the weight of sample taken (g).

METHOD OF ASSAY

Weigh accurately about 0.2 g of the sample, add 10 ml of 5 N hydrochloric acid, and heat cautiously to boiling in a 200-ml conical flask until the sample has dissolved. Allow to cool, add 6 to 7 drops of 30% hydrogen peroxide solution and again heat cautiously to boiling until all the excess hydrogen peroxide has decomposed (about 2-3 min). Allow to cool, add 30 ml of water and about 2 g of potassium iodide and allow to stand for 5 min. Add 30 ml of water and titrate with 0.1 N sodium thiosulfate adding starch TS as the indicator towards the end of the titration. Each ml of 0.1N sodium thiosulfate is equivalent to 5.585 mg of Fe (III).

ISOMALT

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 46th JECFA (1996), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established at the 29th JECFA (1985).

SYNONYMS Hydrogenated isomaltulose; INS No. 953

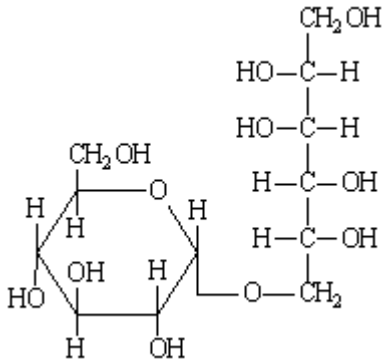
DEFINITION A mixture of hydrogenated mono- and disaccharides whose principal components are the disaccharides:

Chemical names
6-O-alpha-D-Glucopyranosyl-D-sorbitol (1,6-GPS) and
1-O-alpha-D-Glucopyranosyl-D-mannitol dihydrate (1,1-GPM)

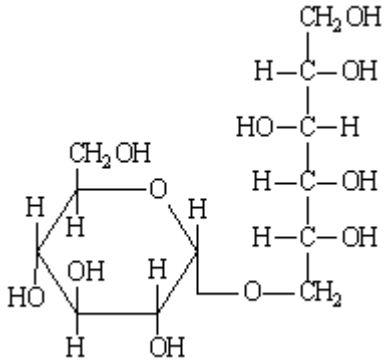
C.A.S. number
64519-82-0

Chemical formula
6-O-alpha-D-Glucopyranosyl-D-sorbitol: $C_{12}H_{24}O_{11}$
1-O-alpha-D-Glucopyranosyl-D-mannitol dihydrate: $C_{12}H_{24}O_{11} \cdot 2H_2O$

Structural formula



6-O-alpha-D-Glucopyranosyl-D-sorbitol



1-O-alpha-D-Glucopyranosyl-D-mannitol (without molecules of crystal water)

Formula weight
6-O-alpha-D-Glucopyranosyl-D-sorbitol: 344.32
1-O-alpha-D-Glucopyranosyl-D-mannitol dihydrate: 380.32

Assay	Not less than 98% of hydrogenated mono- and disaccharides and not less than 86% of the mixture of 6-O-alpha-D-glucopyranosyl-D-sorbitol and 1-O-alpha-D-glucopyranosyl-D-mannitol on the anhydrous basis

DESCRIPTION

Odourless, white, crystalline slightly hygroscopic substance

FUNCTIONAL USES

Sweetener, bulking agent, anticaking agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water, very slightly soluble in ethanol
Thin layer chromatography (Vol. 4)	Passes test See description under TESTS

PURITY

Water (Vol. 4)	Not more than 7.0% (Karl Fischer Titrimetric Method, “General Methods, Inorganic Components”)
Sulfated ash (Vol. 4)	Not more than 0.05% Test 5 g of the sample (Method I)
D-Mannitol	Not more than 3% See Method of Assay
D-Sorbitol	Not more than 6% See Method of Assay
Reducing sugars (Vol. 4)	Not more than 0.3% Proceed as directed under *Reducing Substances (as glucose),* Method II (under “General Methods, Organic Components”). The weight of cuprous oxide shall not exceed 50 mg.
Nickel (Vol. 4)	Not more than 2 mg/kg Proceed as directed under *Nickel in Polyols* (under “General Methods, Inorganic Components”).
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

IDENTIFICATION TESTS

Thin layer chromatography

TLC plates

TLC aluminium foils or plates of approx. 12 cm length and coated with a layer of approx. 0.2 mm, Kieselgel 60 F_{254}, Art. 5554, Merck, or equivalent

Reference solution

Dissolve 500 mg of each of the following sugar alcohols in 100 ml of water: Sorbitol, mannitol, lactitol, maltitol, 1-O-alpha-D-gluco-pyranosyl-D-mannitol (1,1-GPM), and 6-O-alpha-D-glucopyranosyl-D-sorbitol (1,6-GPS)

Test solution

Dissolve 500 mg of sample in 100 ml of water

Solvent A

Isopropanol:n-butanol:aqueous boric acid solution (25 mg/ml):acetic acid:propionic acid (50:30:20:2:16;v/v)

Solvent B

Ethylacetate:pyridine:water:acetic acid:propionic acid (50:50:10:5:5;v/v)

Detecting solutions

I 0.1% Na-metaperiodate in water (w/w)
II ethanol:sulfuric acid:anisaldehyde:acetic acid (90:5:1:1;v/v)

Procedure

Apply approximately 0.3 µl each of the reference and test solution to the bottom of the TLC plate. Dry the spots in warm air. Develop the plate to a height of 10 cm in a developing chamber containing either solvent A or solvent B. Allow the plate to dry in warm air and dip the plate for up to 3 sec into Detecting solution I.

Dry the plate in hot air. Note: The plate should be completely dry on both sides. Dip the plate in Detecting solution II up to 3 sec and dry in hot air until coloured spots become visible. Optionally, the background colour may be brightened in warm steam.

The approximate R_f values and colours of the spots on the TLC-plate of the substances specified above are described as "Compound / Colour / Solvent A(R_f) / Solvent B(R_f)". See below.

mannitol / reddish (light) / 0.36 / 0.40
sorbitol / brown / 0.36 / 0.36
GPM / blue-grey / 0.28 / 0.16
GPS / blue-grey / 0.25 / 0.13
maltitol / green / 0.26 / 0.22
lactitol / olive-green / 0.23 / 0.14

The R_f values may vary slightly depending on the commercial source of the silica gel plates.

The principal spots in the chromatogram obtained from a test solution of isomalt are similar in R_f value and colour to GPM and GPS.

PURITY TESTS

METHOD OF ASSAY

Internal standard solution

Dissolve suitable quantities of phenyl-ß-D-glucopyranoside and maltitol in water to obtain a solution of about 1 mg phenyl-ß-D-glucopyranoside and 50 mg maltitol per g water.

Standard solutions

Dissolve accurately weighed quantities of 1-O-alpha-D-glucopyranosyl-D-mannitol (1,1-GPM) and 6-O-alpha-D-glucopyranosyl-D-sorbitol (1,6-GPS), calculated as dry substance, in water to obtain two separate solutions having a concentration of about 50 mg per g each. Also prepare an aqueous standard solution containing approx. 1 mg mannitol and 1 mg sorbitol per g.

Sample solution

Dissolve an accurately weighed quantity of the sample (approx. 1 g) in water to obtain a concentration of about 10 g per 100 g.

Procedure

Pipet 100.0 mg of standard solution or sample solution into a glass tube fitted with a screw cap and add 100.0 mg of internal standard solution. Remove the water by lyophilization and dissolve the residue in 1.0 ml of pyridine. Add 4 mg O-benzyl-hydroxylamine hydrochloride, and cap the tube and set it aside for 12 h at room temperature. Then, add 1 ml of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) and heat to 80º for 12 h shaking occasionally and allow to cool. Inject 1 µl portions of these solutions directly into a gas chromatograph under the following operating conditions:

- Column: Fused silica HT-8 (25 m x 0.22 mm x 0.25 µm), or equivalent
- Injector: Programmed temperature vaporizer: 30º; 270º/min to 300º (49 min)
- Detector: Flame ionization detector; 360º
- Temperature program: 80º (3 min); 10º/min to 210º; 5º/min to 350º (6 min)
- Carrier gas: Helium
- Flow rate: initial flow rate: approx. 1 ml/min at 80º and 1 atm; split flow: 25 ml/min

Approximate retention times
Hydrogenated monosaccharides:
Mannitol 19.5 min
Sorbitol 19.6 min
Internal standards:
Phenyl-ß-D-glucopyranoside 26.8 min
Maltitol 33.5 min
Hydrogenated disaccharides (32 - 36 min)
1,1-GPS 33.9 min

1,1-GPM 34.5 min
1,6-GPS 34.6 min

Calculate the percentages of the individual components, w_I, in the sample according to the following formula:

$$W_I\ (\%) = \frac{a_I \times m_s}{F_I \times a_s \times m_{ISOMALT}} \times 100$$

where
a_I = peak area of component I (µV·s)
a_S = peak area of internal standard (µV·s)
m_S = mass of internal standard used for derivatization (mg d.s.)
$m_{ISOMALT}$ = mass of sample used for derivatization (mg d.s.)
F_I = relative response factor f_I/f_S
f_I = response factor of component I: $f_I = (a_I/m_I) \times (100/\%\ \text{purity})$
f_S = response factor of internal standard: $f_S = (a_S/m_S) \times (100/\%\ \text{purity})$
m_I, m_S = mass of component I or internal standard used for derivatization of standard sample (mg d.s.)

(NOTE: Use maltitol as internal standard for the calculation of hydrogenated disaccharides (e.g. 1,1-GPM, 1,6-GPS) and phenyl-ß-D-glucoside for the calculation of hydrogenated monosaccharides (mannitol, sorbitol). For the total of other saccharides (hydrogenated or not), subtract the sum of 1,1-GPM, 1,6-GPS, sorbitol and mannitol from 100%.)

ISOPROPYL CITRATE MIXTURE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-14 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS

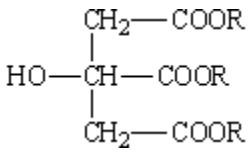
INS No. 384

DEFINITION

Chemical names

Citric acid mixed ester of 2-propanol. The article of commerce, monoisopropyl citrate mixture, is composed of approximately 38 parts by weight of isopropyl citrate in 62 pars by weight of mono- and diglycerides

Structural formula



where R is either hydrogen or a isopropyl group. The major component of the 38 parts of isopropyl citrate mixture is monoisopropyl citrate (approximately 25 parts), the remainder being diisopropyl citrate (approximately 9 parts) and triisopropyl citrate (approximately 4 parts)

Approximate composition:
Monoisopropyl citrate - 27 parts by weight
Diisopropyl citrate - 9 parts by weight
Triisopropyl citrate - 2 parts by weight

DESCRIPTION

Oil miscible semi-solid material. The commercial product, monoisopropyl citrate mixture, is a viscous, colourless syrup exhibiting some crystallization upon standing, and may be further specified as to saponification value, acid value, citric acid and isopropyl content.

FUNCTIONAL USES

Antioxidant, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Soluble in water and ethanol

Test for citrate

Reflux 3 g of sample with 50 ml of sodium hydroxide TS for 1 h, and let stand to cool. This solution is used for the following tests:
(1) Neutralize the solution with a (1 in 20) sulfuric acid solution, add an excess of mercuric sulfate TS, heat to boil, and add potassium permanganate TS. The permanganate colour of the solution disappears, and a white precipitate forms.
(2) Neutralize the solution with hydrochloric acid, add an excess of calcium chloride TS, and boil. A white crystalline precipitate is formed which is insoluble in sodium hydroxide TS, but soluble in dilute hydrochloric acid

TS.

Test for isopropanol	Reflux 2 g of sample with 50 ml of sodium hydroxide TS for 1 h. Distil off 20 ml. Place 8 g of chromic oxide in a flask, add 15 ml water and 2 ml concentrated sulfuric acid. Provide the flask with a reflux condenser and add 5 ml distillate slowly through the condenser. Reflux for 30 min, then cool and distil off 2 ml. Add 3 ml water and 10 ml mercuric sulfate TS to the distillate. Heat in a boiling water bath for 3 min. A white or yellow precipitate within 3 min indicates the presence of isopropanol.

PURITY

Acids other than citric acid	Should be absent
Alcohols other than isopropanol	Should be absent
Sulfated ash (Vol. 4)	Not more than 0.3%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

KARAYA GUM

Prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 33rd JECFA (1988).

SYNONYMS Karaya, gum karaya, *Sterculia*, gum *sterculia*, Kadaya, Katilo, Kullo, Kuterra; INS No. 416

DEFINITION A dried exudation from the stems and branches of *Sterculia urens* Roxburgh and other species of *Sterculia* (Fam. *Sterculiaceae*) or from *Cochlospermum gossypium* A.P. De Candolle or other species of *Cochlospermum* (Fam. *Bixaceae*); consists mainly of high molecular-weight acetylated polysaccharides, which on hydrolysis yield galactose, rhamnose, and galacturonic acid, together with minor amounts of glucuronic acid.

C.A.S. number 9000-36-6

DESCRIPTION Unground product: occurs in tears of variable size and in broken irregular pieces having a characteristic semi-crystalline appearance; pale yellow to pinkish brown; translucent and horny
Powdered product: pale grey to pinkish brown; a distinctive odour of acetic acid. Items of commerce may contain extraneous materials such as pieces of bark which must be removed before use in food.
Unground samples should be powdered to pass a standard ISO sieve of 355 µm (USA No. 45) and mixed well before performing any of the following tests.

FUNCTIONAL USES Emulsifier, stabilizer, thickening agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) 2 g added to 50 ml of water swells to form a granular, stiff, slightly opalescent gel which is acid to litmus; insoluble in ethanol

Swelling by ethanol solution Karaya gum swells in 60% ethanol distinguishing it from other Gums

Colour reaction Boil 1 g of the sample with 20 ml of water until a mucilage is formed. Add 5 ml of hydrochloric acid and boil the mixture for 5 min. A permanent red or pink colour develops.

Warm 0.5 g of the sample with 2 ml of 5 M sodium hydroxide; a brown colour is produced.

Precipitate formation Shake 1 g of the sample with 80 ml of water for 24 h. Boil 4 ml of the resulting mucilage with 0.5 ml of concentrated hydrochloric acid, add 1 ml of 5 M sodium hydroxide and filter. To the filtrate add 3 ml of potassium

cupric tartrate solution and heat. A red precipitate is formed.

Gum constituents — Proceed as directed under *Gum Constituents Identification* using the following as reference standards: galactose, rhamnose, galacturonic acid, glucuronic acid, mannose, arabinose and xylose. Galactose, rhamnose galacturonic acid, and glucuronic acid should be present and mannose, arabinose and xylose should be absent.

PURITY

Loss on drying (Vol. 4) — Not more than 20% (105°, 5 h)

Total ash (Vol. 4) — Not more than 8%

Acid insoluble ash — Not more than 1%
Weigh 3 g of the sample to the nearest 0.1 mg in a tared crucible. Ignite at a low temperature (about 550°), not to exceed a very dull redness, until free from carbon, cool in a desiccator, and weigh. If a carbon-free ash is not obtained, wet the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and ignite, in the crucible, the residue and filter paper until the ash is white or nearly so. Add the filtrate, evaporate it to dryness, and heat the whole to a dull redness. If a carbon-free ash is still not obtained, cool the crucible, add 15 ml of ethanol, break up the ash with a glass rod, burn off the ethanol, again heat the whole to a dull redness and cool. Boil this ash with 25 ml of dilute hydrochloric acid TS for 5 min. Collect the insoluble matter on a tared Gooch crucible or ashless filter, wash with hot water, ignite, cool and weigh. Calculate the percentage of acid-insoluble ash from the weight of the sample.

Acid insoluble matter — Not more than 3%
Weigh about 5 g of the sample, to the nearest 0.1 mg and transfer into a 250 ml beaker or Erlenmeyer containing 100 ml of 5% weight/volume hydrochloric acid. Cover with a watch glass or attach the flask to a condenser having cold water running through it. Boil gently until the gum is completely dissolved (about 3 h). Filter the solution through a tared porcelain or glass fritted crucible 10 to 20 µm porosity. Wash the residue several times with hot water until the washings are free from acid (pH paper). Dry the crucible to constant weight at 105°, cool to room temperature in a desiccator and weigh. Calculate as percentage.

Volatile acid — Not less than 10%, calculated as acetic acid.
To 1 g contained in a 700 ml long necked flash add 100 ml of water and 5 ml of syrupy orthophosphoric acid, allow to stand for several h., or until the gum is completely swollen, and boil gently for two h. under a reflux condenser; steam-distil until 800 ml of distillate is obtained and the acid residue measures about 20 ml, and titrate the distillate with 0.1 M sodium hydroxide using phenolphthalein TS as indicator. Repeat the procedure without gum. The difference between the titrations represents the amount of alkali required to neutralise the volatile acid. Each ml of 0.1 M sodium hydroxide is equivalent to 0.00600 g of volatile acid, calculated as acetic acid.

Starch — Not detectable
To a 1 in 10 solution of the sample add a few drops of iodine TS. No blue

	colour should be produced
Microbiological criteria (Vol. 4)	*Salmonella* spp.: Negative in 1 g *E. coli*: Negative in 1 g
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

KONJAC FLOUR

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 39th JECFA (1993), published in FNP 52 Add 3 (1993). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 46th JECFA (1996)

SYNONYMS Konjac mannan, Konjac, konnyaku; INS No. 425

DEFINITION The hydrocolloidal polysaccharide obtained from the tubers of various species of *Amorphophallus*; principal component is a high molecular weight, slightly branched, non-ionic glucomannan consisting of mannose and glucose, connected by ß-1,4 linkages, at a respective molar ratio of approximately 1.6-4:1; acetyl groups along the glucomannan back-bone contribute to solubility properties and are located, on average, every 9 to 19 sugar units

C.A.S. number 37220-17-0

Formula weight The main component, glucomannan, has an average molecular weight of 200,000 to 2,000,000

Assay Not less than 75% carbohydrate

DESCRIPTION White or cream to light tan powder

FUNCTIONAL USES Gelling agent, thickener, emulsifier, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Dispersible in hot or cold water forming a highly viscous solution with a pH between 4.0 and 7.0

Solubility is increased by heat and mechanical agitation. Addition of mild alkali to the solution results in the formation of a heat-stable gel that resists melting, even under extended heating conditions

Gel formation Add 5 ml of a 4% sodium borate solution to a 1% solution of the sample in a test tube, and shake vigorously. A gel forms.

Formation of heat-stable gel Prepare a 2% solution of the sample by heating it in a boiling water bath for 30 min, with continuous agitation and then cooling the solution to room temperature. For each g of the sample used to prepare the 2% solution, add 1 ml of 10% potassium carbonate solution to the fully hydrated sample at ambient temperature. Heat the mixture in a water bath to 85º, and maintain for 2 h without agitation. Under these conditions a thermally stable

gel is formed. Related hydrocolloids such as guar gum and locust bean gum do not form thermally stable gels and are negative by this test.

PURITY

Loss on drying (Vol. 4)	Not more than 15% (105º, 5 h)
Total ash (Vol. 4)	Not more than 5% (800º, 3-4 h)
Protein	Not more than 8% Proceed as directed under *Nitrogen Determination (Kjeldahl method)*. The percentage of nitrogen in the sample multiplied by 5.7 gives the percent of protein in the sample
Lead (Vol. 4)	Not more than 2 mfg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

The remainder, after subtracting from 100% the sum of the percentages of total ash, loss on drying and protein, represents the percentage of carbohydrate (glucomannans) in the sample.

LACTIC ACID

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 21st JECFA (1977), published in NMRS 57 (1977) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not limited' for lactic acid and its salts was established at the 23rd JECFA (1979)

SYNONYMS INS No. 270

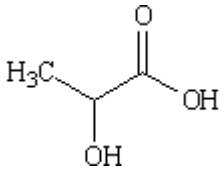
DEFINITION Obtained by the lactic fermentation of sugars or is prepared synthetically; may contain condensation products such as lactic acid, lactate and dilactide. Common products of commerce are 50-90% solutions. Solid products containing about 100-125% of titratable lactic acid also exist. (Note: Lactic acid is hygroscopic and when concentrated by boiling or by distillation it forms condensation products which hydrolyze to lactic acid on dilution and heating in water).

Chemical names: Lactic acid, 2-hydroxypropanoic acid, 2-hydroxypropionic acid

C.A.S. number: 50-21-5 (L-: 79-33-4; D-: 10326-41-7; DL-: 598-82-3)

Chemical formula: $C_3H_6O_3$

Structural formula



Formula weight: 90.08

Assay: Not less than 95.0% and not more than 105.0% of the labelled concentration. For the purity tests, prepare an aqueous solution containing 40% of lactic acid, using the labelled concentration. To dissolve the sample, use warming if necessary. When the labelled concentration is less than 40%, use the product for the test without dilution. The amount of sample to be tested in the tests is the amount of lactic acid calculated from the labelled concentration of the products, except in the tests for "sugars" and for "readily carbonizable substances". In the latter two tests, the term "sample" refers to the 40% solution of lactic acid. The limit of the tests is based on the amount of lactic acid, calculated from the labelled concentration.

DESCRIPTION Colourless, syrupy liquid or white to light yellow solid or powder

FUNCTIONAL USES Acid, acidifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Liquid: Soluble in water and in ethanol

	Solid: Sparingly soluble in water, soluble in acetone
Test for acid	A 1 in 10 solution or dispersion of the sample is acid to litmus paper
Test for lactate (Vol. 4)	Passes test
PURITY	
Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I). Retain the ash for use in the test for iron.
Chloride	Not more than 0.2% Weigh accurately a portion of the sample solution equivalent to about 5 g of lactic acid, dissolve in 50 ml of water, and neutralize to litmus with sodium hydroxide solution (1 in 4). Add 2 ml of potassium chromate TS and titrate with 0.1N silver nitrate to the first appearance of a red tinge. Each ml of 0.1N silver nitrate is equivalent to 3.545 mg of Cl.
Sulfate	Not more than 0.25% Weigh accurately a portion of the sample solution equivalent to about 50 g of lactic acid, transfer into a 600-ml beaker, dissolve in 200 ml of water, and neutralize to between pH 4.5 and 6.5 with sodium hydroxide solution (1 in 2), making the final adjustment with a more dilute alkali solution. Filter, if necessary, and heat the filtrate or clear solution to just below the boiling point. Add 10 ml of barium chloride TS, stirring vigorously, boil the mixture gently for 5 min, and allow to stand for at least 2 h, or preferably overnight. Collect the precipitate of barium sulfate in a tared Gooch crucible, wash until free from chloride, dry, and ignite at 600° to constant weight. The weight of barium sulfate so obtained, multiplied by 0.412, represents the weight of SO_4 in the sample taken.
Iron	Not more than 10 mg/kg To the ash obtained in the test for Sulfated ash add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, dilute to 40 ml with water, and add 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink colour does not exceed that produced by 2.0 ml of Iron Standard Solution (20 µg Fe) in an equal volume of solution containing the quantities of reagents used in the test.
Cyanide	To 0.1 g of the sample add 3 ml of a 20% of sodium hydroxide solution and heat on a water bath for 10 min. After cooling, add 1 drop of phenolphthalein TS and add dropwise dilute acetic acid TS until the pink colour has disappeared. Add 3 drops of dilute acetic acid TS and water to make 40 ml. Add 0.6 ml of chloramine-T solution (dissolve 1 g of chloramine-T ($C_7H_7NNaO_2SCl \cdot 3H_2O$) in water to make 100 ml; prepare freshly before use) and allow to stand for 3 min. Add 10 ml of pyridine-pyrazolone (dissolve 0.5 g of 1-phenyl-3-methyl-5-pyrazolone in 100 ml of hot water at 75° and cool to room temperature; mix with 20 ml of pyridine containing 0.025 g of bis-(1-phenyl-3-methyl-5-pyrazolone); prepare freshly before use) and allow to stand for 25 min. No blue colour is produced (limit approx. 1 mg/kg).

Citric, oxalic, phosphoric or tartaric acid	Dilute 1 g of the sample to 10 ml with water, add 40 ml of calcium hydroxide TS, and boil for 2 min. No turbidity is produced
Sugars	Add 5 drops of the sample solution to 10 ml of hot alkaline cupric tartrate TS. No red precipitate is formed.
Readily carbonizable substances	Superimpose carefully 5 ml of the sample solution kept at 15^{o} on 5 ml of sulfuric acid TS kept at 15^{o}. No deep grey colour is produced within 15 min at the contact zone of the two liquids.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh accurately a portion of the sample equivalent to about 3 g of lactic acid, transfer to a 250-ml flask, add 50 ml of 1N sodium hydroxide, mix, and boil for 20 min. Add phenolphthalein TS, titrate the excess alkali in the hot solution with 1N sulfuric acid, and perform a blank determination. Each ml of 1N sodium hydroxide is equivalent to 90.08 mg of $C_3H_6O_3$.

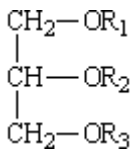
LACTIC and FATTY ACID ESTERS of GLYCEROL

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI not limited' was established at the 17th JECFA (1973)

SYNONYMS Lactic acid esters of mono- and diglycerides; lactoglycerides; INS No. 472b

DEFINITION Mixed glycerol esters of lactic acid and fatty acids of food fats
The article of commerce may be further specified as to monoglyceride content, total lactic acid, acid value, saponification value, free fatty acid content, solidification point of the free fatty acids, iodine value, free glycerol content and water content.

Structural formula



where R_1, R_2 and R_3 each may be a fatty acid moiety, a lactic acid moiety, or hydrogen (approximate composition)

DESCRIPTION Waxy solids of variable consistency and conforms to the following specifications

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water but dispersible in hot water

Tests for fatty acids (Vol. 4) Passes tests

Test for lactic acid (Vol. 4) Passes tests

Test for glycerol (Vol. 4) Passes tests

PURITY

Acids Acids other than lactic and fatty acids shall not be detectable

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

LACTITOL

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 33rd JECFA (1988), published in FNP 38 (1988). Metals and arsenic specifications revised at the 57th JECFA (2001), An ADI 'not specified' was established at the 27th JECFA (1983)

SYNONYMS Lactit, lactositol, lactobiosit, INS No. 966

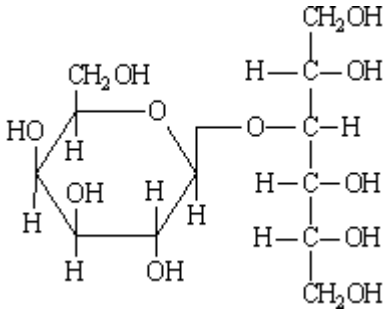
DEFINITION

Chemical names 4-O-ß-D-Galactopyranosyl-D-glucitol

C.A.S. number 585-86-4

Chemical formula $C_{12}H_{24}O_{11}$

Structural formula



Formula weight 344.32

Assay Not less than 95.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION Sweet tasting crystalline powders or colourless solutions; crystalline products occur in both monohydrate and dihydrate forms

FUNCTIONAL USES Sweetener, texturiser

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water

Specific rotation (Vol. 4) [alpha] 25, D: Between +13 and +15º (10% w/v aqueous solution)

Main peak in HPLC The main HPLC peak exhibited by the sample in the assay has the same elution time as that of the lactitol standard
See Method of Assay

PURITY

Water (Vol. 4) Crystalline products: Not more than 10.5% (Karl Fischer Method)
Solutions: Not more than 31% (Karl Fischer Method)

Sulfated ash (Vol. 4)	Not more than 0.1% on the anhydrous basis Test 2 g (anhydrous basis) (Method I)
Chlorides (Vol. 4)	Not more than 100 mg/kg on the anhydrous basis Test an amount of sample equivalent to 10 g of the anhydrous substance by the Limit Test using 3.0 ml of 0.01N hydrochloric acid in the standard
Sulfates (Vol. 4)	Not more than 200 mg/kg on the anhydrous basis Test an amount of sample equivalent to 10 g of the anhydrous substance by the Limit Test using 4.0 ml of 0.01N sulfuric acid in the standard
Other polyols	Not more than 2.5% on the anhydrous basis See Method of Assay
Reducing sugars	Not more than 0.1% Proceed as directed under *Reducing Substances (as glucose),* Method II. The weight of cuprous oxide shall not exceed 20 mg.
Nickel	Not more than 2 mg/kg See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Nickel

Test solution

Dissolve 20.0 g of the substance to be examined in a mixture of equal volumes of dilute acetic acid TS* and water and dilute to 100 ml with the same mixture of solvents. Add 2.0 ml of a 1% w/v solution of ammonium pyrrolidinedithiocarbamate and 10 ml of methyl isobutyl ketone. Mix and allow the layers to separate and use the methyl isobutyl ketone layer.

Standard solution

Prepare three standard solutions in the same manner as the test solution but adding 0.5 ml, 1.0 ml, and 1.5 ml, respectively, of a standard nickel solution containing 10 mg/kg Ni, in addition to the 20.0 g of the sample.

Procedure

Set the instrument to zero using methyl isobutyl ketone as described for the preparation of the test solution but omitting the substance to be examined. Measure the absorbance at 232.0 nm using a nickel hollow-cathode lamp as source of radiation and an air-acetylene flame.

METHOD OF

Principle

ASSAY

Determine lactitol as well as other polyols resulting as by-products during the manufacture of lactitol by *liquid chromatography*. Principal by-product polyols are the hexitols: sorbitol, mannitol, galactitol (dulcitol), and lower polyols such as glycitols.

Apparatus
Liquid chromatograph with elevated temperature capability, differential refractometric detector and 0.45 µm membrane filter before column.

Column
Aminex HPX 87 (calcium form) with dimensions 300 x 7.8 mm, or equivalent column designed for carbohydrate analyses

Standards
Lactitol, sorbitol, mannitol

Eluent
Water (degassed)

Procedure
Equilibrate chromatography column to 85º. Adjust eluent flow rate through column to 0.6 ml/min. Accurately prepare an aqueous solution of sample about 40% by weight. Inject 10 µl of the 40% sample solution onto the column. Record the chromatogram for peaks occurring at the retention time of lactitol and thereafter.

Approximate retention times for lactitol and other polyols using the recommended column are:

Lactitol 12 min
Ribitol 15 min
Erythritol 16 min
Mannitol 18 min
Galactitol 20 min
Sorbitol 21 min

For Assay, compare the sample response relative to the response of a standard sample of lactitol of known purity.

For other polyols, measure the area of all peaks occurring between Lactitol and Sorbitol. The sum of the areas of these peaks is not greater than 2.5 % of the dry weight of the sample.

ETHYL LAUROYL ARGINATE

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0-4 mg/kg bw per day for ethyl-N$^{\alpha}$-lauroyl-L-arginate was established at the 69th JECFA (2008).

SYNONYMS Lauric arginate ethyl ester; lauramide arginine ethyl ester; ethyl-N$^{\alpha}$-lauroyl-L-arginate·HCl; LAE; INS No. 243

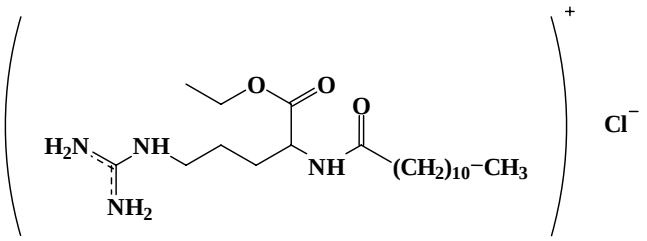
DEFINITION Ethyl lauroyl arginate is synthesized by esterifying arginine with ethanol, followed by reacting the ester with lauroyl chloride. The resultant ethyl lauroyl arginate is recovered as the hydrochloride salt, which is filtered and dried.

Chemical name Ethyl-N$^{\alpha}$-dodecanoyl-L-arginate·HCl

C.A.S. number 60372-77-2

Chemical formula $C_{20}H_{41}N_4O_3Cl$

Structural formula



Formula weight 421.02

Assay Not less than 85% and not more than 95%

DESCRIPTION White powder

FUNCTIONAL USES Preservative

CHARACTERISTICS

IDENTIFICATION

pH (Vol.4) 3.0-5.0 (1% solution)

Solubility (Vol. 4) Freely soluble in water, ethanol, propylene glycol and glycerol

Chromatography The retention time for the major peak in a HPLC chromatogram of the sample is approx. 4.3 min using the conditions described under the Method of Assay.

PURITY

Total ash (Vol. 4)	Not more than 2% (700°)
Water (Vol. 4)	Not more than 5%. Determine by the methods described in Volume 4 under “General Methods, Water Determination (Karl Fischer Method)”.
N^{α}-Lauroyl-L-arginine	Not more than 3% See description under TESTS
Lauric acid	Not more than 5% See description under TESTS
Ethyl laurate	Not more than 3% See description under TESTS
L-Arginine·HCl	Not more than 1% See description under TESTS
Ethyl arginate·2HCl	Not more than 1% See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

N^{α}-Lauroyl-L-arginine	Determine by HPLC in Volume 4 (under “Analytical Techniques, Chromatography”) using the conditions described in the Method of Assay. NOTE: The retention time of N^{α}-lauroyl-L-arginine is approx. 2.2 min. Calculate the percentage of N^{α}-lauroyl-L-arginine in the test sample as follows: $$\% N^{\alpha}\text{-Lauroyl-L-arginine} = \frac{C \times 50}{W \times 1000} \times 100$$ where C is the N^{α}-lauroyl-L-arginate HCl concentration determined (μg/ml); W is the weight of sample (mg); and 50 is the volume of sample solution (ml).
Lauric acid and ethyl laurate	Determine by HPLC in Volume 4 (under “Analytical Techniques, Chromatography”) using the following conditions.

Chromatography
Liquid chromatograph equipped with a spectrophotometric detector.
Column: Symmetry C18, 150 x 3.9 mm, 5μm (Waters) or equivalent
Column temperature: room temperature
Mobile phase: acetonitrile/water (85:15) containing 0.1% trifluoroacetic acid
Flow rate: 1 ml/min
Wavelength:212 nm
Injection volume: 10 µl

Standard solution
Weigh accurately about 125 mg of lauric acid standard and 75 mg ethyl laurate standard into a 50-ml volumetric flask. Dissolve and dilute with the mobile phase to obtain a solution of about 2500 μg/ml of lauric acid and 1500μg/ml of ethyl laurate. Take 5, 10 and 15 ml of the solution and dilute to 50 ml with mobile phase for the standard curves.

Sample solution
Weigh accurately about 500 mg of test sample into a 50-ml volumetric flask. Dissolve and dilute to 50 ml with mobile phase.

Procedure
Inject the standard and sample solutions into the chromatograph and measure their concentration (C μg/ml) from their peak area and their standard curves.
NOTE: The retention time of lauric acid is approx. 3.65 min and that of ethyl laurate is approx. 11.2 min.

Calculate their percentage in the test sample as follows:

$$\% \text{ Lauric acid or ethyl laurate} = \frac{C \times 50}{W \times 1000} \times 100$$

where
C is the lauric acid or ethyl laurate concentration determined (μg/ml);
W is the weight of sample (mg); and
50 is the volume of sample solution (ml).

L-Arginine·HCl and ethyl arginate·2HCl

Determine by HPLC in Volume 4 (under "Analytical Techniques, Chromatography") with post-column derivatization using the following conditions:
NOTE: Use deionized water

Chromatography
Liquid chromatograph equipped with a post-column derivatization and a spectrophotometric detector.
Column and packing: μ Bondapack C18, 300 x 3.9 mm, 10μm (Waters) or equivalent
Mobile phase: A-B-C-D (1:1:1:1.5)
A: 15 mmole/l sodium heptanesulphonate, B: 27 mmole/l phosphoric acid solution, C: 3 mmole/l sodium di-hydrogen phosphate solution, D: methanol

Flow rate: 0.8 ml/min
Flow rate of reagent solution: 0.8 ml/min
Column temperature: 65°
Wavelength: 340 nm
Injection volume: 10 µl

Standard solution

L-Arginine·HCl: Weigh accurately about 40 mg of L-arginine·HCl standard into a 100-ml volumetric flask. Dissolve and dilute to 100 ml with water to obtain a solution of about 400 µg/ml of L-arginine·HCl.

Ethyl arginate·2HCl: Weigh accurately about 200 mg of ethyl arginate·2HCl standard into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with water to obtain a solution of about 8000 µg/ml of ethyl arginate·2HCl.

Take 1, 2 and 3 ml of each solution and dilute to 20 ml with mobile phase separately for the standard curves.

Sample solution

To analyze L-arginine·HCl, weigh accurately about 100 mg of test sample into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with water.

To analyze ethyl arginate·2HCl, weigh accurately about 2 g of test sample into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with water.

Derivatizing solution

Mix 1 liter of 0.2M borate buffer solution (pH 9.4) with 0.8 g of *o*-phtaldialdehyde dissolved in 5 ml of methanol and 2 ml of 2-mercaptoethanol. The solution is stable 48 h at room temperature and without additional preventive measure but It is advisable to keep the solution under nitrogen and to prepare it freshly every 24-48 h.

Procedure

Inject the standard and sample solutions into the chromatograph. After column, derivatization reaction is produced employing the derivatizing solution at 65° in a teflon tubular reactor (650-800 x 0.3 mm). Then, measure the area of the peak at 340 nm.

NOTE: The retention time of L-arginine·HCl is approx. 5.03 min and ethyl arginate·2HCl is approx. 6.70 min.

Calculate the percentage of L-arginine·HCl and ethyl arginate·2HCl in the test sample as follows:

$$\% \text{ L-arginine·HCl or ethyl arginate·2HCl} = \frac{C \times 25}{W \times 1000} \times 100$$

where

C is the L-arginine·HCl or ethyl arginate·2HCl concentration determined (µg/ml);

W is the weight of sample (mg); and

25 is the volume of sample solution (ml).

METHOD OF ASSAY Determine by HPLC in Volume 4 (under "Analytical Techniques, Chromatography") using the following conditions:
NOTE: Use deionized water

Standards
Ethyl-N^{α}-lauroyl-L-arginate·HCl standard
N^{α}-lauroyl-L-arginine standard
(available from Laboratorios Miret, S.A, Géminis 4, Políg. Ind. Can Parellada, 08228 Terrassa, Spain)

Chromatography
Liquid chromatograph equipped with a spectrophotometric detector.
Column and packing: Symmetry C18, 150 x 3.9 mm, 5μm (Waters) or equivalent
Column temperature: room temperature
Mobile phase: acetonitrile/water (50:50) containing 0.1% trifluoroacetic acid
Flow rate: 1 ml/min
Wavelength: 215 nm
Injection volume: 10 µl

Standard solution
Weigh accurately about 25 mg of N^{α}-lauroyl-L-arginine standard into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with mobile phase (solution A). Weigh accurately about 150 mg of ethyl-N^{α}-lauroyl-L-arginate·HCl standard into a 50-ml volumetric flask and dissolve with some milliliters of the mobile phase. Then, add 5 ml of solution A and dilute to 50 ml with mobile phase to obtain a solution of about 3000 μg/ml of ethyl-N^{α}-lauroyl-L-arginate·HCl and 100 μg/ml of N^{α}-lauroyl-L-arginine (solution B). Take 2, 4, 6, 8 and 10 ml of solution B and dilute to 25 ml with mobile phase for the standard curves.

Sample solution
Weigh accurately about 50 mg of test sample into a 50-ml volumetric flask. Dissolve and dilute to 50 ml with mobile phase.

Procedure
Inject the standard and sample solutions into the chromatograph and measure the area of the peak.
Note: The retention time of ethyl-N^{α}-lauroyl-L-arginate·HCl is approx. 4.3 min.

Calculate the percentage of ethyl-N^{α}-lauroyl-L-arginate·HCl in the test sample as follows:

$$\% \text{ Ethyl-}N^{\alpha}\text{-lauroyl-L-arginate·HCl} = \frac{C \times 50}{W \times 1000} \times 100$$

where
C is the ethyl-N^{α}-lauroyl-L-arginate·HCl concentration determined (μg/ml);
W is the weight of sample (mg); and
50 is the volume of sample solution (ml).

LECITHIN

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 30th JECFA (1986), published in FNP 37 (1986) and FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI not limited' was established at the 17th JECFA (1973)

SYNONYMS Phosphatides, phospholipids; INS No. 322(i)

DEFINITION Usually prepared from oil-bearing seeds used for food, especially soybeans; may also be prepared from animal sources; a complex mixture of acetone-insoluble phosphatides which consists chiefly of phosphatidyl-choline, phosphatidyl- ethanolamine, and phosphatidyl-inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates; refined grades may contain any of these components in varying proportions and combinations depending on the type of fractionation used; its oil-free forms, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides representing all or certain fractions of the total phosphatide complex.

C.A.S. number 8002-43-5

Assay Not less than 60% of acetone-insoluble matter (phosphatides)

DESCRIPTION Consistency of both natural grades and refined grades may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents; from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached; odourless or has a characteristic, slight nut-like odour. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavour characteristics.

FUNCTIONAL USES Emulsifier, antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Only partially soluble in water; readily hydrates to form emulsions; oil-free phosphatides are soluble in fatty acids, but practically insoluble in fixed oils

Test for phosphorus Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained.

Test for choline To 0.5 g of the sample, add 5 ml of diluted hydrochloric acid (1+1), heat in a water bath for 2 h, and filter. Use this solution as the test solution. Perform

Paper Chromatography with 10 µl of the test solution, using choline chloride solution (1+200) as the control solution and n-butanol-water-acetic acid mixture (4:2:1) as the developing solvent. A red-orange spot corresponding to the spot obtained from the control solution is observed. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent rises about 25 cm, air-dry, spray with Dragendorff TS to develop a colour, and observe in daylight.

Test for fatty acids — Reflux 1 g of the sample for 1 h with 25 ml of 0.5 N ethanolic potassium hydroxide. When cooled to 0^{o}, a precipitate of potassium soap is obtained.

Test for hydrolysis — To a 800 ml beaker add 500 ml of water ($30\text{-}35^{o}$). Then slowly add 50 ml of the sample with constant stirring. Hydrolyzed lecithin will form a homogeneous emulsion. Non-hydrolyzed lecithin will form a distinct mass of about 50 g.

PURITY

Loss on drying (Vol. 4) — Not more than 2% (105º, 1 h)

Acid value — Not more than 36
See description under TESTS

Peroxide value — Not more than 10
See description under TESTS

Toluene-insoluble matter — Not more than 0.3%
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

Acid value — Weigh accurately about 2 g of the well-mixed sample into a 250-ml Erlenmeyer flask. Dissolve in 50 ml of petroleum ether by shaking gently. Then add 50 ml of ethanol, previously neutralized to phenolphthalein with 0.1 N sodium hydroxide, and shake to mix. Add 4 drops of phenolphthalein TS and titrate while shaking with 0.1 N sodium hydroxide until the pink colour persists for 5 sec.

$$Acid\ value = \frac{ml\ 0.1\ N\ NaOH \times 5.6}{weight\ of\ sample\ (g)}$$

Peroxide value — Reagents
- Acetic acid-chloroform solution: Mix 3 volumes of acetic acid with 2

volumes of chloroform.
- Potassium iodide solution, saturated: Dissolve excess potassium iodide in freshly boiled water. Excess solid must remain. Store in the dark. Test daily by adding 0.5 ml to 30 ml of the acetic acid-chloroform solution, then add 2 drops of starch TS. If the solution turns blue, requiring more than 1 drop of 0.1 N sodium thiosulfate to discharge the colour, prepare a fresh solution.

Procedure

Weigh accurately about 5 g of the sample into a 250-ml Erlenmeyer flask. Add 30 ml of the acetic acid-chloroform solution and swirl to dissolve. Add 0.5 ml of the saturated potassium iodide solution, allow to stand with occasional shaking for 1 min, and add 30 ml of water. Slowly titrate with 0.01 N sodium thiosulfate with vigorous shaking until the yellow colour is almost gone. Add about 0.5 ml of starch TS, and continue the titration, shaking vigorously to release all the iodine from the chloroform layer, until the blue colour disappears.

Perform a blank determination and make any necessary correction.

$$\text{Peroxide value} = \frac{S \times N \times 1000}{W}$$

where
S = ml of N sodium thiosulfate
N = normality of sodium thiosulfate
W = weight of the sample (g)

Toluene-insoluble matter

Weigh 10 g of the well-mixed sample into a 250-ml flask. Add 100 ml of toluene and shake until dissolved. Filter through a tared filter funnel G3 or equivalent with a porosity of 16-40 µm. Wash the flask with 25-ml portions of toluene and pour the washings through the funnel. Place the funnel in a forced-draft oven and dry at 105^{o} for 1 h. Weigh dried funnel and subtract tare to determine weight of toluene insoluble residue:

$$\frac{\textit{weight of residue (g)}}{\textit{weight of sample (g)}} \times 100\ \%$$

METHOD OF ASSAY

Purification of phosphatides

Wash about 10 g of the sample 3 times well with each 100 ml of acetone. The insoluble residue (phosphatides) is used. Residues (phosphatides) obtained from assays carried out previously can also be used. Dissolve 5 g of these phosphatides in 10 ml of petroleum ether, and add 25 ml of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-ml centrifuge tubes using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 ml with acetone, stir again, chill for 15 min in an ice bath, stir again, and then centrifuge for 5 min. Decant the acetone, stir, chill, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the phosphatide-acetone solution. To saturate about 16 litres of acetone, 5 g of the purified phosphatides are required.

Phosphatide acetone solution

Add a quantity of purified phosphatides to sufficient acetone, previously cooled to a temperature of about 5°, to form a saturated solution, and maintain the mixture at this temperature for 2 h., shaking it vigorously at 15-min. intervals. Decant the solution through a rapid filter paper, avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°).

Procedure

If lecithin is plastic or semisolid, soften a portion of the sample by warming it in a water bath at a temperature not exceeding 60° and then mixing it thoroughly. Transfer about 2 g of a well-mixed sample, accurately weighed, into a previously tared 40-ml centrifuge tube, containing a glass stirring rod, and add 15 ml of Phosphatide-Acetone Solution from a buret. Warm the mixture in a water bath until the lecithin melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, and then transfer the tube into an ice bath, chill for 5 min, remove from the ice bath, and add about one half of the required volume of Phosphatide-Acetone Solution, previously chilled for 5 min in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 ml with chilled Phosphatide-Acetone Solution (5°), again stir, and return the tube and contents to the ice bath for 15 min. At the end of the 15-min chilling period, stir again while still in the ice bath, remove the stirring rod, temporarily supporting it in a vertical upside-down position, and centrifuge the mixture immediately at about 2000 rpm for 5 min. Decant the supernatant liquid from the centrifuge tube, crush the centrifuged solids with the same stirring rod previously used, and refill the tube to the 40-ml mark with chilled (5°) Phosphatide-Acetone Solution and repeat the chilling, stirring, centrifugation, and decantation procedure previously followed. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the assigned stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105° for 45 min in a forced-draft oven, cool, and weigh.

Calculate the percentage of acetone-insoluble matter by the formula (100R/S) - B, in which R is the weight of residue, S is the weight of the sample, and B is the percentage of toluene-insoluble matter (see TESTS).

LIPASE FROM ANIMAL

Prepared at the 15th JECFA (1971), published in NMRS 50B (1972) and in FNP 52 (1992). An ADI 'not limited' was established at the 15th JECFA (1971)

SYNONYMS Lipase, triglycerin lipase, tributyrase; INS No.1104

SOURCES Obtained from two primary sources, 1) edible forestomach of calves, kids and lambs and 2) animal pancreatic tissue. These preparations may be partially purified edible tissue preparations or they may be aqueous extracts.

Active principles: Triacylglycerol lipase

Systematic names and numbers: Triacylglycerol acylhydrolase (EC 3.1.1.3)

Reactions catalyzed: The enzyme preparations hydrolyze triglycerides or simple fatty acid esters yielding di- or monoglycerides plus free fatty acids.

DESCRIPTION Dispersible in water and insoluble in ethanol

FUNCTIONAL USES Enzyme preparation
Used in cheese making and modifications of lipids

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume 1, Introduction)

CHARACTERISTICS

IDENTIFICATION

Pregastric esterase activity: The sample shows pregastric esterase activity
See description under TESTS

Esterase activity: The sample shows esterase activity
See description under TESTS

TESTS

IDENTIFICATION TESTS

Pregastric esterase activity: According to Richardson, G.H. and J.H. Nelson, Assay and Characterization of Pregastric Esterase, J. Dairy Sci., 50, 1061-1065, (1967).

Principle
The action of an enzyme inoculum on the assay substrate is measured by a recording pH stat.
One lipase unit is defined as 1/250th of chart width or 0.01 ml of base.

Instrument

Equip a sargent recording pH stat (E.H. Sargent & Co., Chicago, Ill) with a 2.5 ml burette and 7 ml reaction vessels. Set the stat at v/5 vol with stirrer at 8 throughout the work. Adjust temperature and pH indicated. Fill the burette with 0.025 N NaOH. Use 1 ml of a 1:100 dilution of the sample as the inoculum.

Preparation of Enzyme Inoculum

Prepare the enzyme dilution by adding 0.200-1,000 g powder into a dry 100 ml volumetric flask. Add approximately 75-80 ml 0.5 M NaCl solution, and place the flask on a rotary shaker (120 rpm) for 19.0 min. Remove the flask, make up to 100 ml with 0.5 M NaCl and mix well. Remove the sample and inject so that inoculation occurs after a rehydration time of exactly 20 min.

Preparation of Substrate

Place 95 ml distilled water in a half-pint glass freezer jar suitable for attachment to an osterizer; add 2,600 g casein (Sheftone C 2 Soluble Casein Flour, Sheffield Chemical Co., Norwich, N.Y.), 0.5 ml 10% lecithin solution and 5.0 ml n-tributyrin. Tighten an osterizer head atop the jar. Homogenize the substrate mixture at low speed for 1 min. Cover spare substrate and temper to 42^{o} in a water bath until used.

Assay Procedure

Charge the small pH stat reaction vessel with 5 ml substrate and a magnetic stirring bar. To begin an assay, transfer a reaction vessel to the pH stat, and turn on the temperature control and stirring control. Turn the pH stat function control to Run. The unit adjusts to the control pH of 6.20. Then inoculate 1 ml of enzyme dilution.

Use the slope developed over the first 5 min following instrument pH adjustment to calculate activity. Prepare standard curves and compare with colorimetric assay for standardization of esterase activity.

Relative Precision

A comparison of the precision of the colorimetric vs. the pH stat method favoured the latter. Between substrates, as prepared from one day to the next and using the same source of enzyme powder, the pH stat method had a relative standard deviation of 8.4% compared with 17.2% for the colorimetric assay.

Esterase activity

According to Ramsay, H.A., Photometric Procedure for Determining Esterase Activity, J. Clin. Chem., 3, 185 (1957).

Principle

Fatty acid esters of 2-naphthol are employed as substrates. The 2-naphthol liberated by hydrolysis is coupled with a diazonium salt to form an azo dye, the concentration of which is estimated photometrically.

Reagents

- Stock solution of 2-naphthyl ester, 2.96×10^{-2} M: To a 10 ml volumetric flask add successively 3.75 g of melted Brij 35 (Brij 35, a polyoxyethylene lauryl alcohol, is a colourless, nonionic surfactant

having a melting range of approximately 40-44^{o} and is manufactured by Atlas Powder Company, Wilmington 99, Del), 0.296 M of a 2-naphthylester and approximately 5 ml of redistilled 1,4-dioxane, reagent grade. Mix the contents of the flask, warming slightly if necessary to obtain solution, and dilute to volume with additional dioxane. The ester should contain no free 2-naphthol or, at the most, only traces. Stock solutions of the caprylate and the palmitate esters are stable for at least several months if refrigerated.
- Phosphate or citrate buffer, 0.067 M. The choice of buffer will depend upon the pH optimum of the esterase.
- Phosphate buffer, 1.0 M, pH 6.8
- 4-Sulfamoylbenzenediazonium chloride solution: Prepare this reagent by mixing equal volumes of a solution of sulfanilamide, 0.035M in 0.48 N hydrochloric acid, and a solution of sodium nitrite, 0.042 M. Let this mixture stand for several min at room temperature, and then place it in an ice bath. When kept ice-cold, this reagent is stable for at least 6 to 8 h.
- Hydrochloric acid, 1.6 N
- Sodium hydroxide, 3.3 N

Note: 2-naphthyl caprylate and 2-naphthyl palmitate may be synthesized according to the general procedure of Nachlas and Seligman (Nachlas, M.M. and A.M. Seligman, J. Biol. Chem., 181, 343, (1949)) or of Gomori (Gomori, G., J. Lab. and Clin. Med., 42, 445, (1953)); they possess melting points at 43.0-44.0^{o} and 69.0-70.5^{o}, respectively.

Assay Procedure

Prepare a buffered solution of substrate in the following manner immediately prior to its use. All reagents should be at room temperature. Into a gently agitated mixture of 40 ml of 0.067 M phosphate or citrate buffer and approximately 50 ml of water, slowly add 1 ml of the stock solution of ester with a pipette, the tip of which is held beneath the surface of the mixture. Dilute to 100 ml with additional water.
Transfer 5 ml of the buffered solution of substrate to a 25 x 200 mm Pyrex test tube calibrated to contain 25 ml. Place the tube in a constant temperature water bath. After equalization of temperature, add 1 ml of an esterase preparation, and mix the contents of the tube thoroughly. Following incubation, the length of which is determined primarily by the rate at which the substrate is hydrolyzed, add 5 ml of 1.0 M phosphate buffer, pH 6.8. Immediately add 0.5 ml of 4-sulfamoylbenzenediazonium chloride solution. Wait exactly 1 min for the development of colour, and add 5 ml of 1.6 N hydrochloric acid. Place the tube in water which is kept at a rolling boil for 20 min. Cool the contents of the tube to room temperature. Add 5 ml of 3.3 N sodium hydroxide, letting it flow down the wall of the tube. Without undue agitation, dilute the contents of the tube to 25 ml with water and mix thoroughly. Measure the optical density of this solution at 460 nm using as a reference blank the solution from a concomitant control in which the esterase was inactivated prior to incubation by heating.
Many esterase preparations may be assayed simultaneously by staggering the addition of enzyme to the buffered solution of substrate at 2 min intervals. In order that all samples will be incubated for the same length of time, the sequential addition of 1.0 M phosphate buffer, 4-

sulfamoylbenzenediazonium chloride solution and 1.6 N hydrochloric acid, all of which requires approximately 1.5 min for a single tube, is staggered also at 2 min intervals. At this stage, i.e., after addition of the hydrochloric acid, subsequent steps do not need be performed immediately because the colour is stable if the mixture is not exposed to sunlight.

Standard Curve

From stock solutions of 2-naphthol in 1,4-dioxane and Brij 35, prepare buffered solutions of this compound in the same way that the buffered solution of substrate is prepared.

Take 5 ml samples of these buffered solutions, and, except for incubating them with esterase, subject them to the same procedure outlined in the assay technique. When measuring the optical densities of the final solutions, use as a reference blank the solution of a concomitant control without the 2-naphthol. The stock solutions of 2-naphthol are somewhat less stable under refrigeration than are those of the 2-naphthyl esters and, therefore, should be prepared immediately prior to their use. The standard curve is reproducible, thus obviating the preparation of standards for every series of assays.

LYSOZYME HYDROCHLORIDE

Prepared at the 39th JECFA (1992), published in FNP 52 Add 1 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). Acceptable for use in food processing in accordance with GMP established at the 39th JECFA (1992)

SYNONYMS Lysozyme, INS No. 1105

DEFINITION A polypeptide obtained from hen's egg whites consisting of 129 amino acids and having a molecular weight of about 14,000 and an isoelectric point of 10.7; possesses enzymatic activity in its ability to hydrolyze the ß(1-4) linkages between N-acetylmuramic acid and N-acetylglucosamine in the outer membranes of bacterial species, in particular Gram-positive organisms; usually obtained in the hydrochloride form for food use; must conform to the *General Specifications for Enzyme Preparations used in Food Processing*.

C.A.S. number 9066059-5

Assay Not less than 950 µg/mg, as lysozyme hydrochloride, calculated on the anhydrous basis

DESCRIPTION White, odourless powder

FUNCTIONAL USES Preservative (mainly to prevent the late blowing of cheese caused by *Clostridium tyrobutyricum*)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, insoluble in organic solvents and in concentrated saline solutions

pH (Vol. 4) 3.0 - 3.6 (2 in 100 soln)

Spectrophotometry (Vol. 4) An aqueous solution containing 25 mg/100 ml shows an ultraviolet absorbance maximum at 281 nm and a minimum at 252 nm

PURITY

Water (Vol. 4) Not more than 6% (Karl Fischer Method)

Residue on ignition (Vol. 4) Not more than 1.5%

Nitrogen (Vol. 4) Between 16.8 and 17.8%

Chlorides Between 3.2 and 4.2%
See description under TESTS

Sodium Not more than 0.6%

See description under TESTS

Microbiological criteria (Vol. 4)

Total bacterial count: not more than 5 x 10^4 col/g
Salmonella spp.: absent in 25 g
Staphylococcus aureus: absent in 1 g
E. coli: absent in 1 g

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

PURITY TESTS

Chlorides

Accurately weigh about 0.5 g of the sample and dissolve in 20 ml of water. Adjust the pH to 2.5 with 0.1 N nitric acid, add 20 ml of water and 15 drops of indicator (dissolve 0.125 g of diphenylcarbazone and 0.0125 g of bromophenol blue in 25 ml of 95% dry ethyl alcohol) and titrate with 0.025 N mercuric nitrate solution until a violet colour is produced. Prepare a blank and titrate as described above. Calculate the chloride content from

$$Cl = \frac{(mlx - mlb) \times 35.45 \times 0.025 \times 100}{W}$$

where
mlx = ml of 0.025 N mercuric nitrate solutions used for sample
mlb = ml of 0.025 N mercuric nitrate solutions used for blank
W = weight of the sample (mg)

Sodium

Determine the sodium content of a 1.0% solution of the sample by means of an ion analyzer equipped with a sodium detection electrode. Calibrate the apparatus using solutions of sodium chloride containing exactly 100 mg/l, 50 mg/l and 10 mg/l of sodium. Read the concentration of sodium in the sample solution from the calibration curve or directly from the digital display of the apparatus in units of mg/kg. The value found for the sample solution is not more than 60 mg/kg, equivalent to not more than 0.6% of sodium.

METHOD OF ASSAY

Turbidimetric determination

- The method is based on the changes in turbidity of a suspension of *Micrococcus luteus* ATCC 4698 induced by the lytic action of lysozyme. In appropriate experimental conditions these changes are proportional to the amount of lysozyme in the medium.

Substrate

- Suspend a suitable quantity (40-60 mg) of dry powdered *Micrococcus luteus* ATCC 4698 (Boehringer) in a few ml of M/15 pH 6.6±0.1 phosphate buffer solution to obtain a homogeneous suspension and dilute to 100 ml with the same buffer (use manual agitation or ultrasonic bath; do not use electromagnetic agitator). (The exact quantity of *Micrococcus* to be used

depends on the type of spectrophotometer available). Prepare a control consisting of 5 ml of buffer solution and of 5 ml of M. luteus suspension and measure the absorbance of this suspension with a suitable spectrophotometer at 450 nm against a blank consisting of phosphate buffer. The reading should be not less than 0.800. If the reading does not correspond, adjust the initial suspension so as to obtain the desired absorbance (The absorbance values 0.800 -0.900 of the solution prepared as mentioned before, are obtained using a spectrophotometer with suitable sensitivity. Apparatuses with lower sensitivity give lower absorbances even when using the same suspension (0.500 - 0.600). In this case it is not correct to increase the quantity of substrate to obtain the initial absorbance values 0.800 - 0.900 as the reproducibility and linearity of the dosage could be unreliable).

Standard solution

- Dissolve about 50 mg of lysozyme hydrochloride working standard (Wst) (available from Federation International Pharmacetique, International Commission on Pharmaceutical Enzymes, Centre for Standards, Harelbekestraat 72, B-9000 Ghent, Belgium), accurately weighed, in water and dilute to 100 ml in a volumetric flask.

Dilute 5 ml of this solution to 50 ml in a volumetric flask with water and then 2 ml of the last solution to 100 ml in a volumetric flask with M/15 phosphate buffer to obtain a solution containing 1 µg/ml of lysozyme (standard solution).

Sample solution

- Proceed as for the standard solution.

Procedure

In 180 x 80 nm test-tubes, prepare the following solutions:

Standard soln.	M/15 buffer	Lysozyme conc.
2.0 ml	3.0 ml	0.4 µg/ml
2.8 ml	2.2 ml	0.56 µg/ml
4.0 ml	1.0 ml	0.8 µg/ml

It is advisable to prepare three replications for each dilution of the standard and of the sample solutions.

Separately prepare two test-tubes with 5 ml of buffer as control of the *Micrococcus* suspension. Use the first tube of the control at the beginning and the second at the end of the assay.

At exactly 30 sec intervals, to each test-tube, randomly add 5 ml of the suspension of *Micrococcus luteus* (maintained under manual agitation to avoid decantation). Agitate rapidly and place the test-tubes to incubate in a water bath at 37±0.5^{o} for exactly 12 min. The final quantities of lysozyme contained in the test-tubes are therefore 0.2 - 0.28 - 0.4 µg/ml.

After incubation, remove the test-tubes from the water bath in the same order as they were put it and at 30 sec intervals. Agitate and read the absorbances with a suitable spectrophotometer at 450 nm against a blank of buffer. Normally the assay is acceptable when the difference between the absorbances of the two control tubes is not more than 5%.

Calculate the result of the assay by standard statistical methods or by the following calculation: prepare the standard curve plotting on a graph the average of values of absorbance obtained for each dilution (on the ordinates) against the concentrations of lysozyme (on the abscissa) in logarithmic scale. Do the same with the solutions of the sample.
Draw a straight line through the points obtained for the standard and another straight line through the points obtained for the sample. The two lines must be parallel otherwise the dosage is not valid. Then draw a line parallel to the axis of the abscissa so as to intersect the two lines at about half-way between the end limits of the dosage. At the two intersection points correspond two concentration on the abscissa (C_s the concentration of the standard curve and C_x the concentration of the sample curve).
Calculate the microbiological assay (potency) of the sample under test as follows:

$$Potency\ (\mu g / kg) = \frac{C_s \times W_{st} \times P_{st}}{C_x \times W_x}$$

where
C_s = concentration of the lysozyme hydrochloride working standard
C_x = concentration of the sample
P_{st} = potency of the lysozyme hydrochloride working standard
W_{st} = weight in mg of the lysozyme hydrochloride working standard
W_x = weight in mg of the sample under test

Calculate the potency of the anhydrous basis as follows:

$$\frac{Potency\ (\mu g / kg) \times 100}{100 - water}$$

where
water = determined as described above

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