

Compendium of food additive specifications

Addendum 13

**Joint FAO/WHO Expert Committee
on Food Additives (JECFA)
65th Meeting**

Geneva, Switzerland, 7 — 16 June 2005

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Introduction

This volume contains specifications of identity and purity prepared at the 65th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva, 7 - 16 June 2005. These specifications should be considered only in conjunction with the report of the above meeting which will be printed in the WHO Technical Report Series. Toxicological monographs of the substances considered at the 65th meeting of JECFA will be published in the WHO Food Additives Series.

The general principles applied in the elaboration of specifications established at the earlier JECFA sessions have been published in the Principles for the Safety Assessment of Food Additives and Contaminants in Food, WHO Environmental Health Criteria, No. 70, 1987. The specifications of identity and purity of food additives established by JECFA are meant to identify the substance that has been subject to biological testing, to ensure that the substance is of adequate degree of purity for safe use in food, and to reflect and encourage good manufacturing practices. These principles were last reaffirmed by the 59th session of JECFA in 2002.

The specifications are mainly established for the use of toxicologists and others concerned with the identity and purity of the substance. As agreed by JECFA at its 26th meeting, specifications may also be established prior to the eventual completion of toxicological evaluation, in certain cases, when the available toxicological data are inadequate or incomplete, and do not permit the establishment of full or temporary acceptable daily intakes (ADIs). References are made in individual specifications to some of the criteria that may be of interest in commerce, but they do not necessarily include all the requirements of interest to the commercial user. These specifications are not more stringent than is necessary to accomplish their purpose and should easily be attainable by the producing industries. The report of the 23rd meeting gives the reasons why certain specifications are designated as "tentative".

There were a total of 149 specifications (ten food additives, uses other than flavouring agent; 138 flavouring agents; one nutritional supplement) considered at the 65th meeting: Specifications for 132 compounds were newly adopted, of which three remained tentative. 18 specifications were revised of which seven remained tentative. Specifications for Quillaia Extracts (Type 1) were not reviewed but maintained.

NOTE: Use of (FNP 5) in specifications refers to General Methods (Guide to JECFA Specifications), FAO Food and Nutrition Paper 5/Rev. 2 (1991).

Notes to the reader

On-line edition of the Compendium of food additive specifications

A consolidated edition of the *Compendium of food additive specifications* is now available at FAO's JECFA Web page at http://www.fao.org/es/esn/jecfa/index_en.stm. The edition is divided into two sections: one covering flavouring agents and the other covering all other food additives. Users can search by additive name or number (INS, JECFA No., FEMA, CAS). For additives other than flavouring agents, they can also search by functional use and purity criteria. Searches can also be conducted for all specifications designated as tentative. The analytical methods for food additives which are published as Guide to JECFA Specifications (FAO Food and Nutrition Paper 5/Rev. 2), can be accessed as well, even with a direct link from the single specification.

Limits for heavy metals in food additives

An explanatory note is available at the FAO Joint Secretariat's Web page. It should be noted that the revision of the limit test for heavy metals constitutes a change of the specifications in its own right. For a food additive the valid JECFA specification consists of the specification as originally published plus the modifications introduced by the revision of the heavy metal test. Modified specifications will be republished in a consolidated second edition of the *Compendium of food additive specifications* (FAO Food and Nutrition Paper 52).

Chemical and Technical Assessments (CTA)

The CTAs which were prepared since the 61st meeting are published electronically at FAO's JECFA webpage http://www.fao.org/es/ESN/jecfa/chemical_assessment_en.stm.

Comments and feedback

The FAO Joint Secretariat to JECFA welcomes and encourages any feedback on this volume and the online edition of the Compendium. Suggestions on how the availability of the results of JECFA's work can be improved are welcome. Please send your comments to:

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Joint FAO/WHO Expert Committee on Food Additives, 65th Meeting, Geneva, 7 – 16 June 2005

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Section A: Principles governing the establishment and revision of specifications and other related issues

Compendium of food additive specifications

The current Compendium of Food Additive Specifications was published in 1992, it consolidated all the food additives specifications that had been elaborated by the Committee up to that time. Specifications updated and developed at meetings since 1992 have been published in a series of Addenda, and FAO now plans to consolidate these together with the earlier specifications in a proposed new 2nd Edition of the Compendium.

At the 65th meeting the Committee considered a paper describing a number of issues that had arisen as a result of an exercise to draft a new introduction for the proposed 2nd Edition of the Compendium of Food Additive Specifications. As well as updating the current Introduction and current texts, the new Introduction is intended to serve as the basis for revising those sections of the *Principles for the Safety Assessment of Food Additives and Contaminants in Food* (Environmental Health Criteria 70) dealing with specifications.

The Committee noted that the new Introduction emphasizes that the setting of specifications is an inherent part of the risk assessment process for food additives, and that the safety evaluation of an additive should therefore always be read in conjunction with the specifications of identity and purity that describe the additive. The Committee also discussed the conditions under which the 'tentative' designation is applied to additive specifications and the possible link with the 'temporary' ADI designation. It agreed that although there should always be a clear link between the specifications and the safety assessment, the conditions under which the 'tentative' specifications and 'temporary' ADI designations are used should continue to be judged on a case-by-case basis. The Committee also reaffirmed that these designations should be time-limited.

Use of the terms “Anhydrous” and “Dried Basis” in specifications

In previous evaluations the Committee has used the terms “anhydrous”, “dried basis” and “dry basis” in food additive specifications. The Committee agreed that this has been a source of misunderstanding, especially relating to “dry basis”.

To clarify the position, the Committee agreed to discontinue the use the term “dry basis” and recommended that provisions in future food additive specifications should refer to either “anhydrous” or “dried basis” and agreed to interpret these terms as follows:

Anhydrous basis relates a) to the calculated amount of substance adjusted for the known stoichiometric number of molecules of water of hydration; and b) to the amount of substance adjusted for the measured amount of water as determined by the use of a method such as the Karl Fischer Method described in FNP 5.

Dried basis expresses the amount of substance remaining after subjecting a sample to the stated conditions for loss on drying (duration, temperature, pressure, presence of a desiccant etc.).

Residual solvents

At the 61st meeting, the Committee recognized the need to revise the general method for the determination of residual solvents, which is published in FNP 5, and following that meeting, a tentative general method using headspace capillary gas chromatography with flame ionisation detection for the determination of residual solvents was published in FNP 52 Add. 11.

In reviewing the responses in the call for data for comments on the tentative general method, the Committee noted that the critical parts of the determination are the liberation of the solvent residues from the food additive and their capture through headspace sampling prior to the gas chromatographic step. The Committee decided, therefore, that the critical steps should be included in future individual additive specifications rather than in the general method. The Committee also decided that there was a need to revise the tentative general method to include more solvents. The Committee further recommended that methods for the analysis of many common solvents used in the preparation of food additives should be reviewed during the revision of FNP 5.

Safety evaluation of enzymes produced by Genetically Modified Microorganisms (GMM)

In 1987, the Committee outlined criteria for the safety evaluation of enzymes (Environmental Health Criteria 70, Annex III, 135-136). It was proposed to group enzyme preparations into 5 major groups on the basis of their origin (enzymes obtained from edible tissues of animals commonly used as foods; enzymes obtained from edible portions of plants; enzymes derived from microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods; enzymes derived from non-pathogenic microorganisms commonly found as contaminants of foods; enzymes derived from microorganisms that are less well known). At the same time, the Committee envisaged three cases for the safety assessment of enzymes (added directly to food but not removed, added to food but removed; or immobilized enzyme preparations) and indicated guidelines that are appropriate for evaluation of safety in each case.

In 1987, the case of enzymes produced by genetically modified microorganisms (GMM) was not considered. Since then, the Committee has evaluated several enzymes produced by GMM, for example, laccase from *Myceliophthora*

thermophila expressed in *Aspergillus oryzae* and xylanase from *Thermomyces lanuginosus* expressed in *Fusarium venenatum*. The Committee evaluated the safety of these two enzyme preparations on the basis of toxicological data that included, in both cases, a 90-day study in the rat, a test for reverse mutation *in vitro*, and a test for chromosomal aberration. The committee allocated an ADI 'not specified' to these enzyme preparations.

The present Committee evaluated an enzyme preparation of Phospholipase A1 produced by the same host strain of *A. oryzae* that had been modified to produce other enzymes. However, it could not assess the safety of Phospholipase A1 using the information available on one of the other enzymes produced by this host strain as comparators, and the Committee concluded that guidelines need to be developed for the safety assessment of enzymes produced by GMM. These guidelines should set out what information is essential for different enzyme preparations and what details of molecular characterization of the producing microbial strain are necessary to allow an adequate assessment of safety. Furthermore, the Committee reiterated the view expressed at its 57th meeting that the existing General Specifications and Considerations for Enzyme Preparations used in Food Processing should be revised, together with the elaboration of the guidelines for the safety evaluation of enzyme preparations within the Project to Update the Principles and Methods for the Risk Assessment of Chemicals in Food.

The Committee also recommended that the report from the Joint FAO/WHO Expert Consultation on Safety Assessment of Food Derived from Genetically Modified Microorganisms (2001) should constitute a starting basis for this future task.

Hexane

As used in the food industry, 'hexane' is a mixture of hydrocarbons. Recent changes in environmental regulations have led to a change in composition of hexanes since the original specifications were established. In addition, the composition of hexanes will depend on the region of production, the source of the raw material and the site of production. Therefore, the Committee concluded that the present articles of commerce differ from those previously evaluated by the Committee and that the composition of the residues and their levels in foods may not be the same as those evaluated in the original safety assessment. The Committee also concluded that there was insufficient information available to change the current specifications, and therefore recommended a re-evaluation of hexanes.

Monomagnesium phosphate and trisodium phosphate

As no information was received on these substances, the existing tentative specifications were withdrawn.

Previously evaluated flavourings agents

The Committee noted that seven of the 131 flavouring agents had been evaluated previously by the Committee and had food additive specifications. For such substances, the Committee has previously agreed that the material used for flavouring should comply with the existing food additive specifications:

- two of the substances, Maltol (No.1480) and Ethyl maltol (No. 1481), were believed to have uses in addition to flavouring agent uses. In addition to the prepared new specifications presented in flavouring agent format, the existing food additive specifications were revised;
- five of the substances (Eugenol (No. 1529), Methyl anthranilate (No. 1534), Methyl N-methylantranilate (No. 1545), Ethyl 3-phenylglycidate (No. 1576) and Ethyl methylphenylglycidate (No. 1577)) have no other functional uses than as flavouring agents, and therefore the Committee decided that the specifications presented in flavouring agent format should replace existing food additive specifications.

Flavouring agents with a conditional safety evaluation

The Committee noted that an increasing number of the flavouring agents submitted for evaluation in recent years had no recorded poundage data in either the EU or the USA, and MSDI values could only be calculated on the basis of an annual poundage anticipated by the manufacturer. This was the situation for 60 of 135 flavouring agents on the agenda of the present meeting, and for a number of those evaluated during the 59th, 61st and 63rd meetings. As MSDI estimates based only on anticipated poundage data contain additional uncertainty, the Committee decided that in future either the dietary exposure to such substances should be assessed using an alternative approach, or the assessment should be deferred until actual poundage data were available.

The Committee decided that the Procedure would be applied where appropriate for the safety evaluation of flavouring agents submitted to this meeting, including those where anticipated poundage data were submitted for the USA and/or the EU. The evaluation was made conditional if it was based on an MSDI derived from anticipated poundage estimates, and the Committee decided that the results of the conditional assessments will be revoked if use levels or poundage data

are not provided before the end of 2007.¹ This decision was not unanimous, and two members registered a minority opinion.²

The Committee also requested use levels or poundage data to be provided for the flavouring agents it had previously evaluated using MSDIs calculated from anticipated poundage. This includes any substances where the MSDI based on an anticipated poundage for one region (EU or USA) was higher than the MSDI based on a recorded poundage in the other region. The existing assessments for the following flavourings will be revoked if such data are not forthcoming by the end of 2007:

No.	Flavouring agent
963	Ethyl cyclohexanecarboxylate
986	10-Hydroxymethylene-2-pinene
1063	2,5-Dimethyl-3-furanthiol
1065	Propyl 2-methyl-3-furyl disulfide
1066	Bis(2-methyl-3-furyl) disulfide
1067	Bis(2,5-dimethyl-3-furyl) disulfide
1068	Bis(2-methyl-3-furyl) tetrasulfide
1070	2,5-Dimethyl-3-furan thioisovalerate
1077	Furfuryl isopropyl sulfide
1082	2-Methyl-3,5- or 6-(furfurylthio)pyrazine
1085	3-[(2-Methyl-3-furyl)thio]-4-heptanone
1086	2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone
1087	4-[(2-Methyl-3-furyl)thio]-5-nonanone
1089	2-Methyl-3-thioacetoxo-4,5-dihydrofuran
1157	4-Hydroxy-4-methyl-5-hexenoic acid gamma lactone
1158	(+/-) 3-Methyl-gamma-decalactone
1159	4-Hydroxy-4-methyl-7-cis-decenoic acid gamma lactone
1160	Tuberose lactone
1161	Dihydromintlactone
1162	Mintlactone
1163	Dehydromenthofuroolactone
1164	(+/-)-(2,6,6-Trimethyl-2-hydroxycyclohexylidene)acetic acid gamma-lactone
1167	2-(4-Methyl-2-hydroxyphenyl)propionic acid-gamma-lactone
1174	2,4-Hexadien-1-ol
1176	(E,E)-2,4-Hexadienoic acid
1180	(E,E)-2,4-Octadien-1-ol
1183	2,4-Nonadien-1-ol
1188	(E,Z)-2,6-Nonadien-1-ol acetate
1189	(E,E)-2,4-Decadien-1-ol
1191	Methyl (E)-2-(Z)-4-decadienoate
1193	Ethyl 2,4,7-decatrionoate
1199	(+/-)-2-Methyl-1-butanol
1217	2-Methyl-2-octenal
1218	4-Ethyl-octanoic acid
1226	8-Ocimenyl acetate
1228	3,7,11-Trimethyl-2,6,10-dodecatrional
1229	12-Methyltridecanal

No.	Flavouring agent
1232	1-Ethoxy-3-methyl-2-butene
1236	2,2,6-Trimethyl-6-vinyltetrahydropyran
1239	Cycloionone
1245	2,4-Dimethylanisole
1248	1,2-Dimethoxybenzene
1265	4-Propenyl-2,6-dimethoxyphenol
1289	erythro- and threo-3-Mercapto-2-methylbutan-1-ol
1290	(±)-2-Mercaptomethylpentan-1-ol
1292	3-Mercapto-2-methylpentanal
1293	4-Mercapto-4-methyl-2-pentanone
1296	spiro[2,4-Dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3'-(1'-oxa-2'-methyl)-cyclopentane]
1299	2,3,5-Trithiahexane
1300	Diisopropyl trisulfide
1311	2-(2-Methylpropyl)pyridine
1319	2-Propionylpyrrole
1322	2-Propylpyridine
1334	4-Methylbiphenyl
1342	delta-3-Carene
1343	alpha-Farnesene
1344	1-Methyl-1,3-cyclohexadiene
1367	trans-2-Octen-1-yl acetate
1368	trans-2-Octen-1-yl butanoate
1369	Cis-2-Nonen-1-ol
1370	(E)-2-Octen-1-ol
1371	(E)-2-Butenoic acid
1372	(E)-2-Decenoic acid
1373	(E)-2-Heptenoic acid
1374	(Z)-2-Hexen-1-ol
1375	trans-2-Hexenyl butyrate
1376	(E)-2-Hexenyl formate
1377	trans-2-Hexenyl isovalerate
1378	trans-2-Hexenyl propionate
1379	trans-2-Hexenyl pentanoate
1380	(E)-2-Nonenoic acid
1381	(E)-2-Hexenyl hexanoate
1382	(Z)-3- & (E)-2-Hexenyl propionate
1384	2-Undecen-1-ol
1407	Dihydronootkatone
1409	beta-Ionyl acetate

¹ Flavours for which the safety evaluation was considered to be conditional by the 65th meeting of the Committee, are listed in Section C with a 'C' in the status column JECFA.

² Minority Opinion (Prof Gérard Pascal, Dr Philippe Verger): The minority opinion states that for the 60 flavouring substances submitted to the Committee without reported poundage, the safety evaluation using the normal Procedure should not be performed, even on a conditional basis.

No.	Flavouring agent
1410	alpha-Isomethylionyl acetate
1411	3-(l-Menthoxo)-2-methylpropane-1,2-diol
1412	Bornyl butyrate
1413	D,L-Menthol(+/-)-propylene glycol carbonate
1414	L-Monomenthyl glutarate
1415	L-Menthyl methyl ether
1416	p-Menthane-3,8-diol

No.	Flavouring agent
1435	Taurine
1438	L-Arginine
1439	L-Lysine
1447	Tetrahydrofurfuryl cinnamate
1457	(+/-)-2-(5-Methyl-5-vinyl-tetrahydrofuran-2-yl)propionaldehyde
1475	Ethyl 2-ethyl-3-phenylpropanoate
1478	2-Oxo-3-phenylpropionic acid

Further information required for specifications

Sodium 3-methyl-2-oxobutanoate (No. 631.2), Sodium 3-methyl-2-oxopentanoate (No. 632.2), Sodium 4-methyl-2-oxopentanoate (No. 633.2) and Sodium 2-oxo-3-phenylpropionate (No. 1479)

The existing tentative specifications for these four flavouring agents were revised to include new information on methods of assay. However, the tentative designations of the specifications were maintained, pending more detailed information on these methods. For the first three substances, information on an assay by HPLC using an ion exchange column are required, and for flavouring No. 1479 information on an assay by HPLC is required.

Maltol (No.1480) and Ethyl maltol (No. 1481)

New specifications were prepared for these substances in the flavouring agent format. However both substances are believed to have uses in addition to flavouring agent uses, and the existing specifications in the standard food additive format were revised and made tentative. In both cases information on functional uses other than flavouring uses and on the method of assay is required.

Maltyl isobutyrate (No. 1482), 3-Acetyl-2,5-dimethylfuran (No. 1506) and 2,4,5-Trimethyl-delta-3-oxazoline (No. 1559)

New tentative specifications were prepared for these substances. In each case, further information is required on the reasons why the quoted specific gravity ranges are wider than would be expected given the level of purity of the substances. In addition, further information is required on why the refractive index range for flavouring No. 1559 is wider than would be expected given the level of purity of the substance.

Sucrose esters of fatty acids

The specifications for sucrose esters of fatty acids were revised but maintained as tentative. Information is required on

- a method of analysis for the determination of free sucrose using capillary GC or HPLC;
- an alternative and less toxic solvent than pyridine for preparing the standard and sample solutions for the determinations of free sucrose and propylene glycol; and
- a method of analysis for the determination of dimethyl sulfoxide that does not require a packed column.

The tentative specifications mentioned above will be withdrawn unless the requested information is received before the end of the year 2006.

Section B: Specifications of certain food additives (uses other than as flavouring agents) and other substances

Aspartame-acesulfame salt	7
Beeswax	11
Calcium L-5-methyltetrahydrofolate	15
Candelilla wax	21
Ethyl maltol	23
Laccase from <i>Myceliophthora thermophila</i> expressed in <i>Aspergillus oryzae</i>	25
Maltol	29
Phospholipase A1 from <i>Fusarium venenatum</i> expressed in <i>Aspergillus oryzae</i>	31
Pullulan	35
Quillaia extract (Type 1)	39
Quillaia extract (Type 2)	43
Sucrose esters of fatty acids	47

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 ADI for aspartame (0-40 mg/kg bw) established at the 25th JECFA (1981) and the ADI 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.

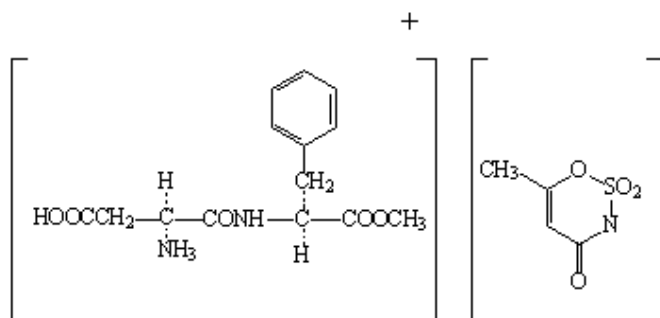
C.A.S. number

106372-55-8

Chemical formula

C₁₈H₂₃O₉N₃S

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 (FNP 5)

Sparingly soluble in water, and slightly soluble in ethanol.

PURITY

Loss on drying (FNP 5)

No more than 0.5% (105°, 4 h)

Transmittance (FNP 5)

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 (FNP 5)	$[\alpha]_D^{20} +14.5$ 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 (FNP 5)	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 FNP 5, "Instrumental Methods."

TESTS

PURITY 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.
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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- μ m 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 μ m 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 μ l portions of the standard and the sample into the chromatograph (set the flow rate of the mobile phase at 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 solution

Prepare a 0.1 M standard solution of tetrabutylammonium hydroxide 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 (V_1) and second (V_2) 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 (ml)
 N = normality of the standard 0.1 M tetrabutylammonium hydroxide
163 = formula weight of acesulfame moiety
294 = formula weight of aspartame moiety
10 = conversion of g to % (m/m)

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 (FNP 5)

Insoluble in water; sparingly soluble in alcohol; very soluble in ether

PURITY

Melting range (FNP 5)

62 – 65°

Acid value (FNP 5)

17 – 24

Peroxide value

Not more than 5
See description under TESTS

Saponification value (FNP 5)

87 -104

Carnauba wax

Passes test
See description under TESTS

Ceresin, paraffins, and certain other waxes

Passes test
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 (FNP 5)	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 FNP 5, "Instrumental Methods".
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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.
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$$\text{Peroxide value} = (a-b) \times N \times 1000/W$$

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 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 carnauba 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°. 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.

CALCIUM L-5-METHYLTETRAHYDROFOLATE

New specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005). At the 65th JECFA (2005) the Committee had no safety concerns for the use of the substance in dry crystalline or microencapsulated form as an alternative to folic acid used in dietary supplements, foods for special dietary uses and other foods.

SYNONYMS

L-5-Methyltetrahydrofolic acid, calcium salt
L-Methyltetrahydrofolate, calcium salt
L-Methylfolate, calcium
L-5-MTHF-Ca

DEFINITION

Calcium L-5-methyltetrahydrofolate is the calcium salt of L-5-methyltetrahydrofolic acid, which is the predominant, naturally occurring form of folate. It is synthesized by reduction of folic acid to tetrahydrofolic acid followed by methylation and diastereoselective crystallization (in water) of L-5-methyltetrahydrofolic acid as its calcium salt. The product contains variable amounts of water of crystallization.

Chemical name

N-{4-[[[(6S)-2-amino-3,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridiny]methyl]amino]benzoyl}-L-glutamic acid, calcium salt

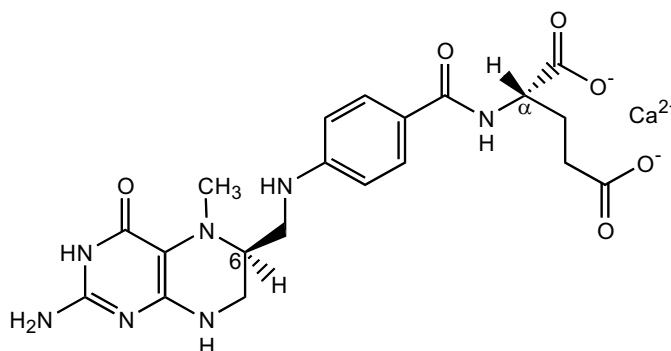
C.A.S. number

151533-22-1

Chemical formula

C₂₀H₂₃CaN₇O₆ (anhydrous form)

Structural formula



(anhydrous form)

Formula weight

497.5 (anhydrous form)

Assay

95.0 – 102.0% (anhydrous basis)

DESCRIPTION

White to light yellowish, almost odourless, crystalline powder

FUNCTIONAL USES

Nutritional supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (FNP 5)

Sparingly soluble in water and very slightly soluble or insoluble in most organic solvents; soluble in alkaline solutions

Infrared absorption

The infrared absorption spectrum of a potassium bromide dispersion of the sample corresponds to that of a Calcium L-5-methyltetrahydrofolate standard (see Appendix).

Calcium	Dilute 30 g of acetic acid (glacial) to 100 ml with water. Dissolve 5.3 g of $K_4Fe(CN)_6$ in 100 ml of water. To 5 ml of the acetic acid solution, add 20 mg of the sample and then 0.5 ml of the potassium ferrocyanide solution. Mix and add 50 mg of ammonium chloride. A white crystalline precipitate is formed.
Liquid chromatography	Retention time matches that of a reference standard (see under TESTS)
PURITY	
Water (FNP 5)	Not more than 17.0% (Karl Fischer method) <i>Note:</i> Allow sufficient time (15 min) for release of bound water.
Calcium	7.0 - 8.5% (anhydrous basis) Accurately weigh 500 mg of sample and transfer to a 500-ml conical flask. Add 150 ml of water to dissolve the sample and 20 ml of a pH 10 buffer (NH_3/NH_4Cl). Using eriochrome black T as indicator, titrate (continuous stirring) with standardized 0.1 M EDTA until the colour changes from violet to blue/green. Each 1.0 ml of 0.1 M EDTA corresponds to 4.008 mg of calcium. Calculate the calcium content on the anhydrous basis.
Other folates and related substances	Not more than 2.5% See description under TESTS
D-5-Methylfolate	Not more than 1.0% See description under TESTS
Total viable aerobic count (FNP 5)	Not more than 1000 CFU/g
Lead (FNP 5)	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 FNP 5, "Instrumental methods".

TESTS

PURITY TESTS

Other folates and related substances	<u>Principle</u> Using a reference standard for L-5-Methyltetrahydrofolic acid, calcium salt, Other folates and related substances are quantitated by HPLC. The suitability of the applied HPLC system is checked daily by a system suitability test. <u>Reference standard solution</u> Accurately weigh 50 mg of L-5-methyltetrahydrofolic acid, calcium salt (Merck Eprova AG, CH-8200 Schaffhausen, Switzerland) into a 100-ml volumetric flask. Dissolve in a small quantity of water and dilute to volume. <u>Sample solution</u> Prepare as for the reference standard using 50 mg of the sample. <u>Mobile phase solutions</u> <i>A:</i> Dissolve 7.80 g of $NaH_2PO_4 \cdot 2H_2O$ (0.05 mol) in 1000 ml of water and adjust the pH to 6.5 with 32% NaOH. Filter and degas the solution. <i>B:</i> Dissolve 5.07 g of $NaH_2PO_4 \cdot 2H_2O$ (0.03 mol) in 650 ml of water and 350 ml of methanol (chromatography grade) and adjust the pH to 8.0 with 32% NaOH. Filter and degas the solution.
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Chromatography conditions

<i>Column:</i>	Hypersil-ODS, 5 µm; 250 x 4 mm (Thermo Hypersil Keystone or equivalent)			
<i>Flow rate:</i>	1.1 ml/min			
<i>Gradient:</i>	<i>Time (min)</i>	<i>% Mobile phase A</i>	<i>% Mobile phase B</i>	<i>Remark</i>
	0	100	0	Start
	0 - 14	100 - 45	0 - 55	Linear gradient
	14 - 17	45 - 0	100	Linear gradient
	17 - 22	0	100	Hold
	22 - 31	100	0	Reconditioning
<i>Temperature:</i>	Room temperature			
<i>Injection volume:</i>	10 µl			
<i>Detection:</i>	UV (280 nm)			
<i>Run time:</i>	22 min			

Retention times given below are approximate:

<i>Folates and related substances</i>	<i>Retention time (min)</i>
4-Aminobenzoylglutamic acid (ABGA)	3.1
4α-Hydroxy-5-methyltetrahydrofolic acid (HOMeTHFA)	4.3
D-Pyrazino-s-triazine derivative (D-Mefox)	6.1
L-Pyrazino-s-triazine derivative (L-Mefox)	6.3
Tetrahydrofolic acid (THFA)	8.5
7,8-Dihydrofolic acid (DHFA)	11.2
Folic acid (FA)	11.4
5,10-Methylenetetrahydrofolic acid (CH ₂ THFA)	11.7
5-Methyltetrahydrofolic acid (5-MTHF)	13.6
5-Methyltetrahydropteroic acid (MeTHPA)	15.1
N ² -Methylamino-5-methyltetrahydrofolic acid (DiMeTHFA)	17.6

Sample analysis

Inject the reference standard solution and the sample solutions immediately after preparation, using the conditions described above. (*Note:* After analysis, flush the column with methanol/water 85:15 and store it under the same conditions.) Calculate the content of each folate (other than 5-MTHF) and related substance, X_i (%), listed in the above table according to the following formula:

$$X_i (\%) = A_i \times W_s \times S \times (RF)_i / A_s \times W$$

where

A _i	= the peak area for each folate (other than 5-MTHF) and related substance
A _s	= the peak area for the L-5-MTHF-Ca Standard
W _s	= the weight (mg) of L-5-MTHF-Ca Standard
W	= the weight (mg) of the sample
S	= the percent of L-5-MTHF in the L-5-MTHF-Ca Standard, calculated as free acid
(RF) _i	= Response Factor for the i-th substance (absorbance at 280 nm in the applied eluent system relative to that of L-5-MTHF) as follows:
<i>Folates and related substances</i>	<i>RF</i>
ABGA	0.93
HOMeTHFA	1.11
L-Mefox and D-Mefox	1.11
DHFA	0.98
FA	0.86
MeTHPA	0.68
THFA	1.00
CH ₂ THFA	1.00
DiMeTHFA	1.00
If there are any unidentified impurities, apply a RF of 1.00	

Calculate the total amount of "Other folates and related substances" by summing the X_i for all impurities.

System suitability test

Mixed folates solution: Weigh 25 mg each of ABGA, HOMeTHFA, L-Mefox, DHFA, FA and MeTHPA (all available from Merck Eprova AG) into a 100-ml volumetric flask. Add a small quantity of water to dissolve the mixture; add some sodium hydrogen carbonate and sodium carbonate to aid the dissolution, and fill to the mark with water.

System suitability test solution (SST solution): Weigh accurately 50 mg of a L-5-MTHF-Ca sample containing DiMeTHFA into a 100-ml volumetric flask. (Available from Merck Eprova AG). Add 1 ml of the Mixed folates solution and a small quantity of water to dissolve, mix and dilute to volume with water.

Procedure: Inject 10 μ l of the SST solution immediately. The resolution between L-5-MTHF and MeTHPA must be at least 5.

D-5-Methylfolate

Principle

D-5-Methylfolate is quantitated by HPLC using a chromatographic system which allows separation of the D- from the L-stereoisomer. The suitability of the applied HPLC system is checked daily by a "system suitability test".

Sample preparation

Accurately weigh 50 mg of the sample into a 100 ml volumetric flask. Dissolve in water and dilute to volume with water.

Mobile phase

Mix 970 ml of 0.03 M NaH_2PO_4 (obtained by dissolving 4.68 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in water and diluting with water to 1000 ml) with 30 ml of acetonitrile (chromatography grade) and adjust the pH to 6.8 with 32% NaOH. Filter and degas the solution.

Chromatography Conditions

Column: Chiral Protein HSA, 5 μ m, 150 x 4 mm (ChromTech or equivalent)
Flow rate: 1 ml/min
Temperature: 40°
Injection volume: 10 μ l
Detection: UV (280 nm)
Run time: 22 min
Solvent: Water

Sample analysis

Inject the sample solution immediately after preparation using the conditions described above. Determine the areas under peak for L-5-MTHF (retention time: ca. 11 min) and D-5-MTHF (retention time: ca. 15 min).

Calculation

Determine the ratio of the peak area for the D-isomer (A_D) to the sum of the peak areas for the D- and L-isomers (A_T), and calculate the D-5-MTHF content as follows:

$$\text{D-5-MTHF (\%)} = 100A_D/A_T$$

System suitability test

System suitability test solution (SST solution): Weigh and transfer into a 200-ml volumetric flask the following: 1.0 mg of HOMeTHFA, 1.5 mg ABGA, 2.0 mg each of L-Mefox and MeTHPA, 3.0 mg of FA, 4.0 mg of DHFA, 10 mg of L,D-5-MTHF and D,D-5-MTHF (L-5-MTHF and D-5-MTHF carrying D-glutamic acid substitution), and 50 mg of racemic 5-MTHF-Ca (L-5-MTHF and D-5-MTHF carrying L-glutamic acid substitution) (all available from Merck Eprova

AG). Add a small quantity of water to dissolve the mixture; add some sodium hydrogen carbonate to aid the dissolution, and fill to the mark with water. Immediately inject into the HPLC system. The resolution between L-5-MTHF and D-5-MTHF must be at least 2.

METHOD OF ASSAY

Calculate the percentage of L-5-MTHF-Ca in the sample from the content of 5-MTHF-Ca (L- and D-diastereoisomers), determined in the test for "Other folates and related substances", and the content of D-5-MTHF-Ca, determined in the test for D-5-Methylfolate, and correcting for water content, as follows:

$$\text{L-5-MTHF-Ca (\%)} = 100 \times A_T \times W_S \times S \times (100 - D) \times 1.083 / A_S \times W \times (100 - \%H_2O)$$

where

A_T is taken from the calculation for the D-5-Methylfolate analysis

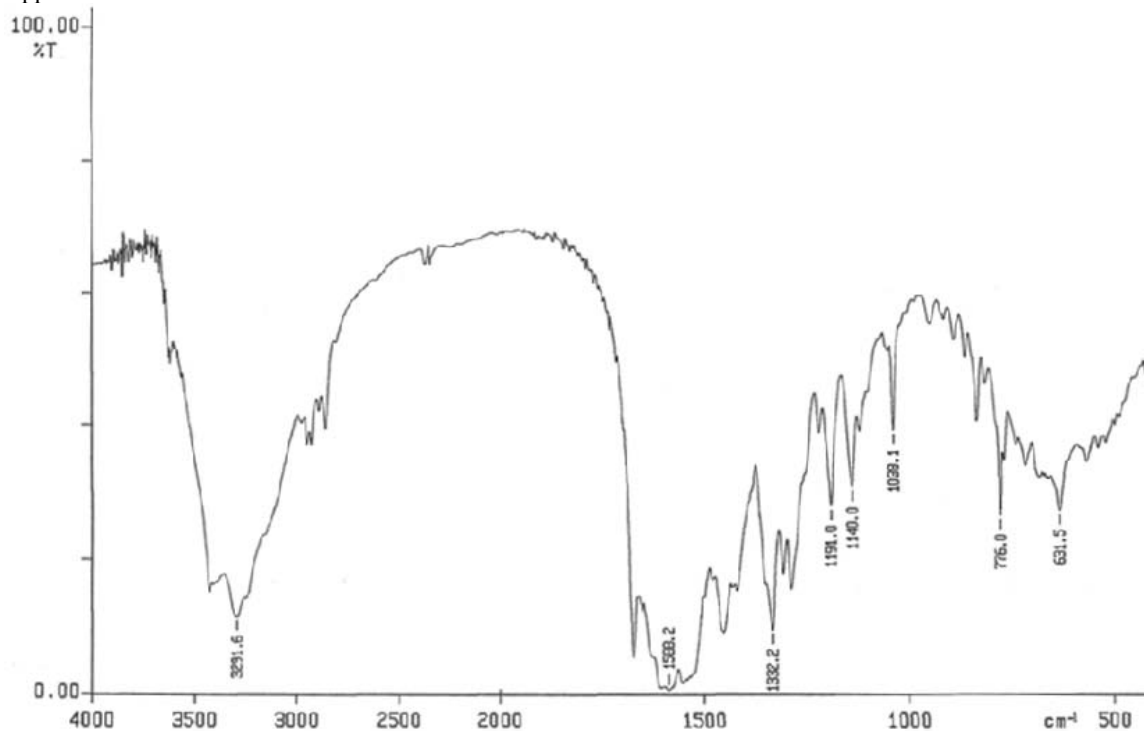
D = the percentage of D-5-Methylfolate in the sample

A_S , W, W_S , and S are taken from the determination of Other folates and related substances

$\%H_2O$ = water content (%)

1.083 is the ratio of the formula weight of 5-MTHF-Ca to that of 5-MTHF.

Appendix



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 (C₂₉ to C₃₃), together with esters of acids and alcohols with even-numbered carbon chains (C₂₈ to C₃₄). 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 (FNP 5)

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 (FNP 5)

68.5 - 72.5°

Acid value (FNP 5)

Between 12 and 22

Saponification value (FNP 5)

Between 43 and 65

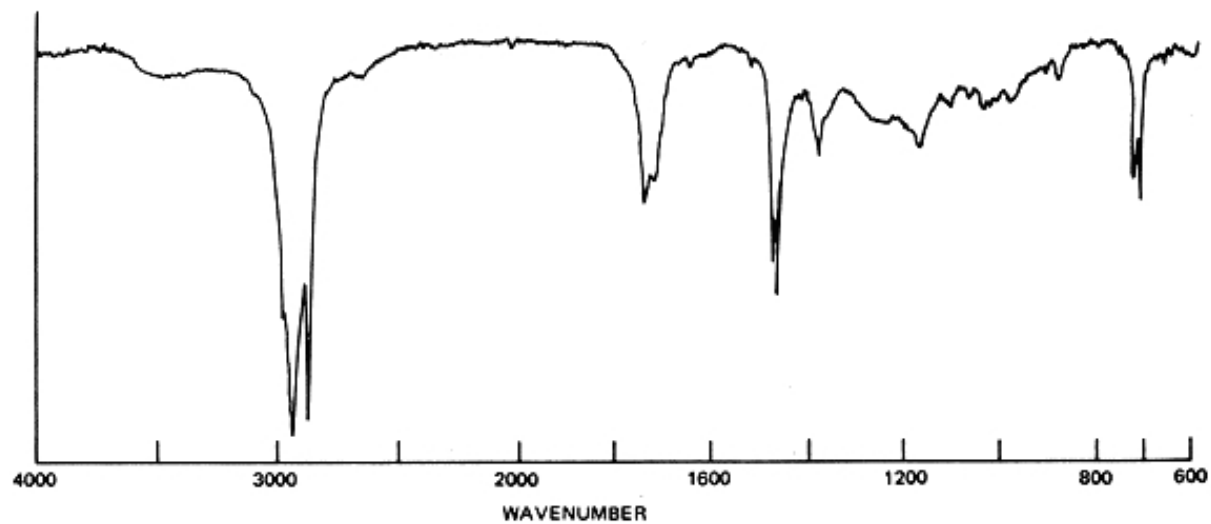
Lead (FNP 5)

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 FNP 5, "Instrumental Methods".

APPENDIX

Infrared spectrum of candelilla wax



**ETHYL MALTOL
(Tentative)**

Information on functional uses and method of assay required

Revised tentative specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2006), superseding specifications prepared at the 14th JECFA (1970) and published in NMRS 48B (1971) and in FNP 52 (1992), and incorporating the decisions on metals and arsenic specifications agreed at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001). An ADI of 0-2 mg/kg bw was established at the 18th JECFA (1974)

SYNONYMS

INS No. 637

DEFINITION

Chemical names

2-Ethyl-3-hydroxy-4-pyrone

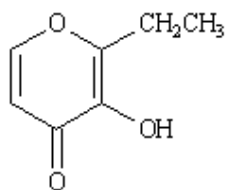
C.A.S. number

4940-11-8

Chemical formula

C₇H₈O₃

Structural formula



Formula weight

140.14

Assay

Not less than 98%

DESCRIPTION

White, crystalline powder having a characteristic odour

FUNCTIONAL USES

Flavour enhancer, stabilizer, flavouring agent (See 'Flavouring agents' monograph No. 1481)

CHARACTERISTICS

IDENTIFICATION

Solubility

Sparingly soluble in water; soluble in alcohol

Melting range

89 - 93°

PURITY

Water (FNP 5)

Not more than 0.5 % w/w (Karl Fischer Method)

Sulfated ash (FNP5)

Not more than 0.2 % w/w

Lead (FNP 5)

Not more than 1mg/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 FNP 5, "Instrumental Methods".

METHOD OF ASSAY

Prepare a solution of ethyl maltol in 0.1 N hydrochloric acid containing 10 µg/ml, and determine the extinction at 276 nm. E (1%, 1 cm): 276 nm: 655-675

Calculation

$$\text{Ethyl maltol (\%)} = 100 \times E(\text{sample})/E(\text{standard})$$

where

$$E = E (1\%, 1 \text{ cm})$$

**LACCASE FROM *MYCELIOPHTHORA THERMOPHILA*
EXPRESSED IN *ASPERGILLUS ORYZAE***

Revised specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005), superseding specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). An ADI “not specified” was established at the 61st JECFA (2003).

SOURCES

Produced by submerged fed-batch pure culture fermentation of a genetically modified strain of *Aspergillus oryzae* containing the laccase gene derived from *Myceliophthora thermophila*, using recombinant DNA techniques and traditional mutagenesis. The enzyme is isolated from the fermentation broth by filtration to remove the biomass and concentrated by ultrafiltration and/or evaporation. Residual production microorganisms are removed from the enzyme concentrate by germ filtration. The final product is formulated using food-grade stabilizing and preserving agents.

ACTIVE PRINCIPLES

Laccase (synonyms: urishiol oxidase; p-diphenol oxidase)

SYSTEMATIC NAMES AND NUMBERS

Benzenediol:oxygen oxidoreductase; EC 1.10.3.2; CAS No. 80498-15-3

REACTIONS CATALYSED

Oxidation of a range of phenolic substances with concomitant reduction of oxygen to water.

DESCRIPTION

Brown liquid

FUNCTIONAL USES

Enzyme preparation.
Used in the brewing of beer to prevent the formation of off-flavour compounds such as trans-2-nonenal. Scavenges oxygen that otherwise would react with fatty acids, amino acids, proteins, and alcohols to form off-flavour precursors.

GENERAL SPECIFICATIONS

Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations used in Food Processing,

CHARACTERISTICS

IDENTIFICATION

Laccase activity

The sample shows laccase activity
See description under TESTS

TESTS

Laccase activity

Principle

Laccase catalyses the oxidation of syringaldazine to tetramethoxy-azo-bis (methylene quinone) that is measured spectrophotometrically at 530 nm. Laccase activity is expressed in Laccase Myceliophthora Units (LAMU). One LAMU is defined as the amount of enzyme that oxidizes 1 micromole of syringaldazine per minute under standard conditions (pH 7.5; 30°).

(Note: The method can be adapted for manual execution; any suitable spectrophotometer may be used in place of a centrifugal analyser.)

Apparatus

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

Reagents

(Note: Use only deionised water)

Laccase standard (available from Novozymes A/S)

TRIS (Tris(hydroxymethyl)aminomethane)

Maleic acid

Triton X-100 (polyethylene glycol tert-octylphenyl ether)

Ethanol 96%

Syringaldazine

PEG 6000

Glucose

Glycine

Reagent Solutions

TRIS, 1 M stock solution: Dissolve 121.1 g of TRIS in approximately 900 ml of water in a 1-litre volumetric flask. Make to volume and mix.

Maleic acid, 1 M solution: Dissolve 23.2 g of maleic acid in approximately 150 ml water in a 200-ml volumetric flask. Make to volume and mix.

Triton X-100, 10% stock solution: In a beaker, add 25.0 g of Triton X-100 to approximately 200 ml water; stir to dissolve. Transfer to a 250-ml volumetric flask and make up to volume with water.

TRIS buffer, 25 mM (pH 7.50): Add 5 ml of 1 M TRIS, 2 ml of 1 M maleic acid solution, and 1 ml of Triton X-100 10% solution to a 200-ml volumetric flask. Add 150 ml water and adjust pH to 7.50 ± 0.05 using 1 M maleic acid solution. Make up to volume with water. (Note: do not adjust the pH with hydrochloric acid because chloride inhibits laccase activity.)

PEG 6000, 50 g/l solution: Weigh 250 g of PEG 6000 in a beaker, transfer to a 5000 ml volumetric flask, add water and stir until dissolved. Add water to volume.

Syringaldazine, 0.56 mM stock solution: Rinse a 50-ml volumetric flask with water and ethanol to remove any soapy residues. Weigh 10.0 mg of syringaldazine in a weighing boat and transfer to the volumetric flask. Add 96% ethanol to the mark and stir until the syringaldazine is dissolved (approximately 3 h). The solution must be stored in a dark bottle in a refrigerator.

Syringaldazine, 0.22 mM working solution: Rinse a 10-ml volumetric flask with water and ethanol to remove any soapy residues. Transfer 4.0 ml of syringaldazine stock solution to the flask and add water to volume. The solution can be kept in a dark bottle for up to two hours at room temperature.

Glycine buffer, 1.5%: Dissolve 75 g glycine, 150 g glucose, and 250 g PEG 6000 in approximately 4.5 litre water in a 5-l volumetric flask. Adjust pH to 9.20 ± 0.05 using NaOH or 1 M maleic acid. Add water to volume. (Note: Do not adjust pH with hydrochloric acid because chloride inhibits laccase activity.)

Standard and sample solutions

Laccase standard stock solution: Weigh the amount of laccase standard needed to obtain a laccase activity of 0.350 LAMU/ml and transfer the laccase to a 500-ml volumetric flask. Add 300 ml PEG 6000 solution and stir on a magnetic stirrer for 15 min to dissolve the laccase. Add PEG 6000 solution to volume. The laccase stock solution should be prepared on the day of the experiment.

Laccase working standard solutions (for the construction of the standard curve): Prepare six solutions by diluting the laccase stock solution with PEG 6000 solution as shown in the table below. Use the diluter and vials compatible with the centrifugal analyser.

Sample No.	Dilution factor	Laccase stock solution (µl)	PEG 6000 solution (µl)	Activity, LAMU/ml
1	30	20	580	0.01167
2	24	25	575	0.01458
3	20	30	570	0.01750
4	15	40	560	0.02333
5	12	50	550	0.02917
6	10	60	540	0.03500

Laccase control sample: Use a laccase preparation with known activity.

Accurately weigh the amount of the preparation sufficient to obtain laccase activity of approximately 0.70 LAMU/ml in a 200-ml volumetric flask. Place the preparation in the flask and add the PEG 6000 solution to volume. Stir on the magnetic stirrer for 15 min. This is a stock solution. It should be prepared daily. Dilute the stock solution with the PEG 6000 solution 30 times using diluter. Place the diluted solution in a vial.

Analyse the control sample in each run to test the method's performance. A result within 8 percent of the nominal activity is acceptable.

Test samples: Dilute test samples on the basis of the anticipated enzyme content to obtain activity between 0.0117 and 0.0350 LAMU/ml.

Example: accurately weigh 0.6 g sample and dissolve in the PEG 6000 solution in a 250 ml volumetric flask. Stir the solution for 15 min on the magnetic stirrer. If necessary, dilute the sample solution again with the PEG 6000 solution. Place the solutions in vials.

Procedure

1. Pour the syringaldazine working solution (0.22 mM) into a 4-ml reagent container placed in the reagent rack of the centrifugal analyser.
2. Pour the TRIS buffer into a 15-ml reagent container placed in the reagent rack.
3. Place the vials containing standard solutions and the control sample in the calibration rack.
4. Place the vials containing the test samples in the sample rack.
5. Set up the analysis program and start the analysis.

Analysis

The analysis is performed automatically by the centrifugal analyser. The empty rotor of the analyser rotates until the temperature in the cuvette container reaches 30°. Twenty five microliters of the standard solution, control sample or test sample, 20 microliters of water, and 325 microliters of glycine buffer are pipetted into cavities in the rotor. The rotor accelerates and centrifuges and mixes the buffer and samples in the cuvettes. Subsequently, 30 microliters of the substrate is pipetted into each cuvette. The rotor accelerates and centrifuges and mixes the substrate with samples in the cuvettes. The first absorbance reading is taken five seconds later. A total of 25 readings are taken from each cuvette at 5-second intervals. Readings 12 to 24 are used to calculate the increase of absorbance per minute ($\Delta\text{Abs}/\text{min}$).

Calculations

The analyser creates a standard curve and uses it to convert the $\Delta\text{Abs}/\text{min}$ from each cuvette containing the test sample into activity expressed in LAMU/ml. The activity of test samples expressed in LAMU/g is then calculated using the following formula:

$$\text{LAMU} / \text{g} = \frac{A \times \text{Vol} \times D}{W}$$

Where

- A = $\Delta\text{Abs}/\text{min}$ converted to activity (LAMU/ml)
 Vol = volume of the volumetric flask used to dilute the test sample (ml)
 D = additional dilution of the sample (ml/ml)
 W = weight of the sample (g)

MALTOL
(Tentative)

Information on functional uses and method of assay required

Revised specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add13 (2005), superseding specifications prepared at the 25th JECFA (1981) and published in FNP 52 (1992). An ADI of 0-1 mg/kg bw was established at the 25th JECFA (1981)

SYNONYMS

INS No. 636

DEFINITION

Chemical names

3-Hydroxy-2-methyl-4-pyrone

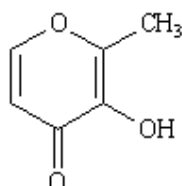
C.A.S. number

118-71-8

Chemical formula

C₆H₆O₃

Structural formula



Formula weight

126.11

Assay

Not less than 99%

DESCRIPTION

White to off-white crystalline powder having a characteristic caramel-butterscotch odour

FUNCTIONAL USES

Flavour enhancer, stabilizer, flavouring agent (see Flavouring agents monograph No. 1480)

CHARACTERISTICS

IDENTIFICATION

Solubility (FNP 5)

Soluble in water and ethanol

Melting range (FNP 5)

160 - 164°

Test for phenol

Dissolve 0.1 g of the sample in 10 ml of ethanol and add 3 drops of ferric chloride TS. A reddish violet colour is produced.

Precipitation test

Dissolve 0.5 g of the sample in 10 ml of sodium hydroxide TS and pass carbon dioxide through the solution. White crystals are formed; collect and recrystallize from dilute ethanol. The crystals melt between 160 - 164°

Iodoform reaction

Dissolve 0.1 g of the sample in 5 ml dioxane, add 1 ml of sodium hydroxide TS, and add sufficiently iodine-potassium iodide TS (Iodine TS) with shaking until the colour remains. Heat on a water bath for 5 min. Yellow crystals are formed.

PURITY

Lead (FNP 5)

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 methods described in FNP 5, "Instrumental Methods".

METHOD OF ASSAY

Standard Solution

Transfer about 50 mg of Maltol Reference Standard (available from the United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, Md. 20852, USA), accurately weighed, into a 250-ml flask, dilute to volume with 0.1 N hydrochloric acid, and mix. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Assay Solution

Transfer about 50 mg of the sample, accurately weighed, into a 250-ml flask, dilute to volume with 0.1 N hydrochloric acid. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Procedure

Determine the absorbance of each solution in a 1-cm quartz cell at 274 nm using 0.1 N hydrochloric acid as the blank.

Calculate the percent of Maltol in the sample by the formula:

$$\% \text{ of Maltol} = 100 \times W_S \times A_A / A_S \times W_A$$

where

A_A = absorbance of the Assay Solution

A_S = absorbance of the Reference Standard Solution

W_A = weight in mg of the Assay solution (sample)

W_S = weight in mg of the Reference Standard

**PHOSPHOLIPASE A1 FROM *FUSARIUM VENENATUM* EXPRESSED
IN *ASPERGILLUS ORYZAE***

New specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005). The 65th JECFA (2005) was unable to assess the safety of the additive.

SYNONYMS	Phospholipase A1
SOURCES	Produced by submerged fed-batch pure culture fermentation of a genetically modified strain of <i>Aspergillus oryzae</i> containing the phospholipase A1 gene derived from <i>Fusarium venenatum</i> . The enzyme is isolated from the fermentation broth by filtration to remove the biomass and concentrated by ultrafiltration and/or evaporation. Residual production microorganisms are removed from the enzyme concentrate by germ filtration. The final product is formulated using food-grade stabilizing and preserving agents.
ACTIVE PRINCIPLES	Phospholipase A1
SYSTEMATIC NAMES AND NUMBERS	Phosphatidylcholine 1-acylhydrolase; EC 3.1.1.32; CAS No. 9043-29-2
REACTIONS CATALYSED	Hydrolysis of the sn-1 ester bond of diacylphospholipids to form 2-acyl-1-lysophospholipids and free fatty acids
DESCRIPTION	Brown liquid.
FUNCTIONAL USES	Enzyme preparation. Used in cheese production to reduce the loss of fat and milk solids and increase cheese yield.
GENERAL SPECIFICATIONS	Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations used in Food Processing
CHARACTERISTICS	
IDENTIFICATION	
Phospholipase A1 activity	The sample shows phospholipase A1 activity See description under TESTS
TESTS	
Phospholipase A1 activity	<u>Principle</u> Phospholipase A1 activity is measured relative to a phospholipase standard using lecithin as a substrate. Phospholipase A1 catalyses the hydrolysis of lecithin to lyso-lecithin and a free fatty acid. The liberated fatty acid is titrated with 0.1 N sodium hydroxide under standard conditions (pH=8.0; 40° ±0.5). The activity of phospholipase A1 is determined as the rate of sodium hydroxide consumption during neutralization of the fatty acid and is expressed in Lecitase units (LEU) relative to a Lecitase (phospholipase) standard. 1 LEU is defined as the amount of enzyme that under standard conditions (pH=8.0; 40° ±0.5) results in the same rate of sodium hydroxide consumption (in microeq/min) as the Lecitase standard diluted to a nominal activity of 1 LEU/g. The quantification limit of the method is approximately 1.5 LEU/ml. (Note: The method can be carried out using either an automated system or standard laboratory equipment for carrying out titration experiments. Procedures and calculations for both the automated and manual versions are described.)

Automated method

Apparatus

Printer

Computer

pH-Stat Titration Manager (analytical robot; Novo Nordisk Engineering A/S), consisting of the following elements:

- PHM290 pH-Stat Controller (Radiometer)
- ABU901 Autoburette (Radiometer)
- Liquid Handler (Gilson)
- Temperature Regulator (Gilson)
- Syringe Pump (Gilson)
- Silverson Homogenizer L4R
- pH electrode (Radiometer)

Reagents and solutions

(Note: use only deionized water)

Titrant, sodium hydroxide 0.1 N: Use NaOH solution standardized for preparation of 1000 ml of 0.1 N NaOH, for example, one ampoule of Merck Titrisol No. 1.09959 or equivalent. Transfer quantitatively the NaOH solution into a 1000-ml volumetric flask containing approximately 500 ml of water. Add water to volume and mix. The solution is stable for up to 2 months at room temperature.

Calcium chloride, 0.32 M: Weigh 4.70 g calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Merck 2382 or equivalent). Dissolve in water in a 100 ml volumetric flask. Add water to volume and mix. The solution is stable for up to one week at room temperature.

Sodium deoxycholate, 0.016 M: Weigh 6.7 g sodium deoxycholate ($\text{C}_{24}\text{H}_{39}\text{NaO}_4$). Dissolve in water in a 1000-ml volumetric flask. Add water to volume and mix. The solution is stable for up to one week at room temperature.

Lecithin substrate: Use lecithin (L- α -phosphatidylcholine) Sigma P-5638 or equivalent. Mix lecithin with a spoon and weigh exactly 20.0 g. Transfer to a 1000-ml beaker, add 400 ml of water and stir until the lecithin is dissolved. Add 20 ml of 0.32 M calcium chloride and stir for 1-2 min until calcium chloride is dissolved. Add 200 ml of 0.016 M sodium deoxycholate and 400 ml of water. Stir for about 0.5 hour and homogenize for 10 min on a Silverson L4R homogenizer. The solution is stable for up to one day at room temperature.

Hydrochloric acid, 1 N: Use HCl solution standardized for preparation of 1000 ml of 1 N HCl, for example, one ampoule of Merck Titrisol No. 1.09970 or equivalent. Transfer the HCl solution quantitatively to a 1000-ml volumetric flask. Add water to volume. The solution is stable for up to 6 months at room temperature.

Hydrochloric acid, 0.001 N: Transfer 1 ml of 1 N HCl to a 1000-ml volumetric flask. Add water to volume. The solution is stable for up to one week at room temperature.

Standard and sample solutions

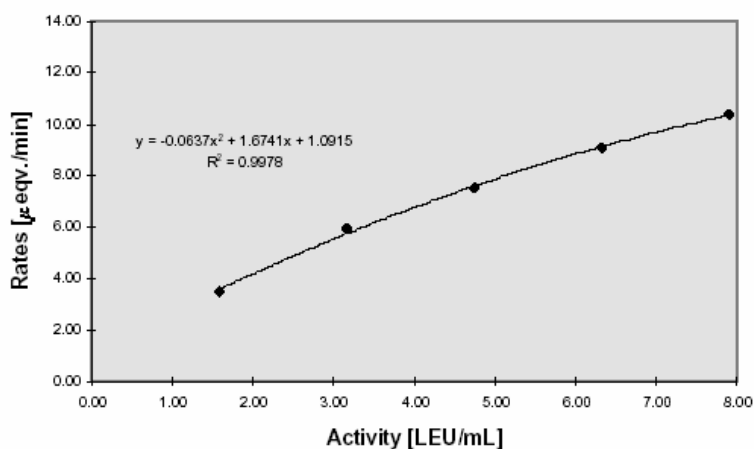
Standard stock solution: Use a phospholipase standard (Lecitase standard from Novozymes A/S or equivalent) with known activity, for example, 103160 LEU/g. Accurately weigh (to 4 decimal places) approximately 0.73 g of the standard. Transfer to a 250-ml volumetric flask and add 0.001 N HCl to volume. Stir for approximately 30 min. The solution can be stored for 3 weeks at 5°.

Standard working solutions: Transfer quantitatively 5 ml of the standard stock solution to a 200-ml volumetric flask. Add 0.001 N HCl to volume. This solution contains approximately 7.5 LEU/ml and is referred to as 100% standard. Using the 100% standard, prepare additional standard solutions in 25-ml volumetric flasks according to the following table:

Standard solution	Approximate strength (LEU/ml)	Volume of 100% standard (ml)	Volume of 0.001 N HCl
80%	6.0	20	5
60%	4.5	15	10
40%	3.0	10	15
20%	1.5	5	20

The standard solutions are used by the automated system to calculate a 2nd degree polynomial standard curve. An example of the standard (calibration) curve is shown below.

Calibration Curve



Control sample: A phospholipase product with known activity, for example, 10500 LEU/ml, is used to prepare the control sample. Transfer 1 ml of the control solution to a 50-ml volumetric flask and add 0.001 N HCl to volume to obtain a stock solution. Transfer 1 ml of the stock solution to a 50-ml volumetric flask and add 0.001 N HCl to volume. Analyse the control sample in each run. A result within 10 percent of the nominal activity is acceptable.

Blank sample: Use 0.001 N HCl as the blank sample.

Test samples: Remove the test sample from a refrigerator or freezer and keep it at room temperature for approximately 2 h before analysis. Accurately weigh the sample into a measuring flask and add 0.001 N HCl to volume. Repeat dilution if necessary to obtain the activity of approximately 6 LEU/ml. The dilution should be performed within one hour of the analysis. Enter the sample weight (in grams) and the dilution volume (in milliliters) into the calculation program for the automated method or into the calculation formula for the manual method.

Procedure

The method uses a pH-stat Titration Manager at pH=8.0 and $40^{\circ} \pm 0.5$. The pH-stat Titration Manager automatically runs a two-point calibration of the pH electrode and then analyses the blank, the standard solutions, the control sample and the test samples, each in duplicate.

Start the reaction when 800 μl of the sample is added to 20 μl of the lecithin substrate. Titrate the liberated fatty acid with 0.1 N sodium hydroxide under standard conditions (pH=8.0; $40^{\circ} \pm 0.5$). Record the rate of sodium hydroxide consumption (microeq/min) over 2 min, starting 90 sec and ending 210 sec from the start of the reaction and use to calculate the mean slope of the titration curve. The mean slopes of the titration curves for the control sample, standard solutions, and test samples are automatically transferred to the calculation program.

Calculations

All results are calculated automatically by the calculation program. A 2nd degree polynomial standard curve is calculated based on the mean slopes (microeq/min) for the standard solutions. Based on the standard curve, the calculation program calculates the results for the control and test samples in Lecitase activity units per 1 ml (LEU/ml). Subsequently, the program calculates the activity of the test samples in Lecitase activity units per one gram of the phospholipase A1 preparation (LEU/g).

Manual method

Procedure

The method is carried out using a titrator that measures the titrant consumption rate as a function of time (e.g., TitraLab 854 from Radiometer). The titrator must be programmed to maintain pH=8 and measure the NaOH consumption rate in microequivalents per minute (microeq/min). The following procedure is followed:

Calibrate the pH electrode at pH 7 and 10. Transfer 20 ml of the lecithin substrate to a beaker and place in a water bath at $40^{\circ} \pm 0.5$. Adjust the substrate to pH 8.0 using 0.1 N NaOH and start the titration by the addition of either 0.8 ml of the standard solution, the test sample, or the control sample. Measure the NaOH consumption rate for 4 min. Determine the NaOH consumption rate between 90 and 120 sec from the reaction start and use this information to construct a standard curve and for activity calculations.

Calculation

Construct a standard curve by plotting the NaOH consumption rate (in microeq/min) against the enzyme activity (in LEU/ml). The activity of the control sample and test samples are read from the calibration curve (in LEU/ml).

Note: a 2nd degree polynomial standard curve can be plotted using suitable software, and the activity of the test sample and control sample can be calculated from the standard curve using the same software.

The activity of the test samples in LEU/g is calculated according to the following equation:

$$\text{Activity(LEU/g)} = \frac{\text{Activity(LEU/ml)} \times V(\text{ml})}{W(\text{g})}$$

where: W(g) is the sample weight and

V(ml) is the volume of the volumetric flask in which the sample was diluted. For example, if the sample is weighed into a 50 ml volumetric flask and diluted to volume, V=50 ml.

PULLULAN

New specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005). An ADI "not specified" was established at the 65th JECFA (2005).

SYNONYMS

INS No. 1204

DEFINITION

Linear, neutral glucan consisting mainly of maltotriose units connected by α -1,6 glycosidic bonds. It is produced by fermentation from a food grade hydrolysed starch using a non-toxin producing strain of *Aureobasidium pullulans*. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilized and pigments and other impurities are removed by adsorption and ion exchange chromatography.

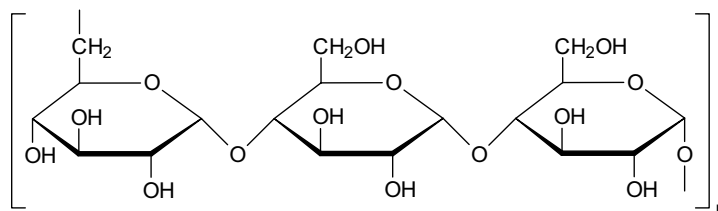
C.A.S. number

9057-02-7

Chemical formula

$(C_6H_{10}O_5)_x$

Structural formula



Assay

Not less than 90% of glucan on the dried basis

DESCRIPTION

White to off-white odourless powder

FUNCTIONAL USES

Glazing agent, film-forming agent, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (FNP 5)

Soluble in water, practically insoluble in ethanol

pH (FNP 5)

5.0 - 7.0 (10% solution)

Precipitation with polyethylene glycol 600

Add 2 ml of polyethylene glycol 600 to 10 ml of a 2% aqueous solution of pullulan. A white precipitate is formed.

Depolymerisation with pullulanase

Prepare two test tubes each with 10 ml of a 10% pullulan solution. Add 0.1 ml pullulanase solution having activity 10 units/g (refer to pullulanase activity, under Methods for enzyme preparations in FNP 5) to one test tube, and 0.1 ml water to the other. After incubation at about 25° for 20 min, the viscosity of the pullulanase-treated solution is visibly lower than that of the untreated solution.

PURITY

Loss on drying (FNP 5)

Not more than 6% (90°, pressure not more than 50 mm Hg, 6 h)

Mono-, di- and oligosaccharides

Not more than 10% (expressed as glucose)
See description under TESTS

Viscosity

100-180 mm²/s (10% w/w aqueous solution at 30°)
See description under TESTS

Lead (FNP 5)	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 methods described in FNP 5, "Instrumental Methods".
Microbiological criteria (FNP 5)	Yeast and moulds: Not more than 100 CFU/g Coliforms: Negative in 25 g Salmonella: Negative in 25 g

TESTS

PURITY TESTS

Mono-, di- and oligosaccharides	<u>Principle</u> The soluble mono-, di- and oligosaccharides of pullulan are measured using the anthrone-sulfuric acid method after pullulan has been precipitated with methanol and KCl. <u>Equipment</u> Spectrophotometer capable of measuring absorbance at 620 nm <u>Procedure</u> <i>Preparation of standard:</i> Weigh accurately 0.2 g glucose, dissolve in water and make up to 1 l. <i>Measurement of mono-, di- and oligosaccharides:</i> Weigh accurately 0.8 g sample and dissolve in water to make 100 ml (stock solution). Place 1 ml of the stock solution in a centrifuge tube. Add 0.1 ml saturated potassium chloride solution. Add 3 ml methanol and mix vigorously for 20 sec. Centrifuge at 11000 rpm for 10 min. Add 0.2 ml of the supernatant to 5 ml modified anthrone solution (0.2 g anthrone in 100 g 75% (v/v) sulfuric acid, freshly prepared). Add 0.2 ml of glucose standard solution and 0.2 ml water (blank control) to separate 5 ml portions of modified anthrone solution. Mix rapidly. Place samples in a 90° water bath and incubate for 15 min. Measure absorbance of the test solution at 620 nm. Calculate the percent of mono-, di- and oligosaccharides expressed as glucose, C, in the sample: $C(\%) = [(A_t - A_b) \times 0.41 \times G \times 100] / (A_s - A_b) \times W$ where A_t is absorbance of the test solution A_b is absorbance of the water blank A_s is absorbance of the standard solution G is weight of the glucose (g) W is weight of the sample (g)
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Viscosity	Dry the sample for 6 h at 90° under reduced pressure (50 mm Hg). Weigh 10.0 g of the sample and dissolve in water to yield 100 g of solution. Use an Ubbelohde-type (falling-ball) viscometer. Charge the viscometer with sample in the manner dictated by the design of the instrument. Immerse the viscometer vertically in the thermostatic tank at $30 \pm 0.1^\circ$ and allow to stand for 20 min so that the sample equilibrates with the temperature in the tank. Adjust the meniscus of the column of liquid in the capillary tube to a position about 5 mm above of the first mark. With the sample flowing freely, measure, in seconds, the time required for the meniscus to pass from the first to the second mark. Calculate the viscosity, V:
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$$V (\text{mm}^2/\text{s}) = C \times t$$

where

C = calibration constant of the viscometer (mm^2/s^2)
 t = flow time (s)

METHOD OF ASSAY

Calculate the percentage of pullulan on dried basis, P , as the difference between 100% and the sum of the percentages of known impurities (mono-, di- and oligosaccharides and water).

$$P(\%) = 100 - (L + C)$$

where

L is loss on drying

C is taken from the calculation for mono-, di- and oligosaccharides

QUILLAIA EXTRACT (TYPE 1)

Specifications prepared at the 61st JECFA (2003), published in FNP 52 Add 11 (2003) and republished in FNP 52 Add 13 (2005). A group ADI of 0.1 mg quillaia saponins per kg bw was established at the 65th JECFA (2005) for Quillaia Extract (Type 1) and Quillaia Extract (Type 2).

SYNONYMS	Quillaja extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract; INS No. 999
DEFINITION	Quillaia extract (Type 1) is obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of Quillaja saponaria Molina (family Rosaceae). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are major components and some sugars and calcium oxalate will be present. Quillaia extract (Type 1) is available commercially as liquid product or as spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.
C.A.S. number	68990-67-0
Formula weight	Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues
ASSAY	Saponin content: not less than 20 % and not more than 26 % on the dried basis
DESCRIPTION	Red-brownish liquid or light brown powder with a pink tinge
FUNCTIONAL USES	Emulsifier, foaming agent
CHARACTERISTICS	
IDENTIFICATION	
Solubility (FNP 5)	Very soluble in water, insoluble in ethanol, acetone, methanol and butanol
Foam	Dissolve 0.5 g of powder extract in 9.5 g of water or 1 ml of liquid extract in 9 ml of water. Add 1 ml of this mixture to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam level (ml) after 30 min. Typical values are 150 ml of foam
Chromatography	Determine as in METHOD OF ASSAY. The retention time of major peak of the sample corresponds to the major saponin peak (QS-18) of the standard.
Colour and turbidity	Powder form only: Dissolve 0.5 g in 9.5 g of water. The solution is not turbid. Determine the absorbance of the solution against water at 520 nm. The absorbance is less than 1.2.
PURITY	
Water (FNP 5)	Powder form: not more than 6% (Karl Fischer Method)
Loss on drying (FNP 5)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (FNP 5)	3.7 - 5.5 (4 % solution)
Ash (FNP 5)	Not more than 14% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from loss on drying)
Tannins	Not more than 8% on a dried basis See description under TESTS

Lead (FNP 5)

Not more 2 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 FNP 5, "Instrumental Methods".

TESTS

PURITY TESTS

Tannins

Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (Wi). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at 800 × g. Recover the supernatant and dry this solution at 105° (5 h). Weigh the recovered solid (Wf, in g). The percentage of tannins in the sample is:

$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle

The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 1).

Sample preparation

Powders: Weigh 0.5 g of sample and dissolve in 9.5 g of water. Filter through a 0.2 µm filter.

Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 g of water. Filter through a 0.2 µm filter.

In either case, the sample volume is ca. 10 ml.

Standard preparation

Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 µm filter.

High performance liquid chromatography (HPLC)

HPLC conditions

Column:	Vydac 214TP54 (4.6 x 250 mm length, 5 µm pore) or equivalent		
Column temperature:	room temperature		
Pump:	gradient		
Solvent A:	0.15% trifluoroacetic acid in HPLC-grade water.		
Solvent B:	0.15% trifluoroacetic acid in HPLC-grade acetonitrile.		
Gradient:	Time(min)	% solvent A	% solvent B
	0	70	30
	40	55	45
	45	70	30
Flow rate:	1 ml/min		
Detection wavelength:	220 nm		
Injection volume:	20 µl		

Calculation

The concentration of saponins, C_{sap} , in mg/ml, in the solution prepared as directed under sample preparation is:

$$C_{\text{sap}} = (A_{\text{sample}}/A_{\text{standard}}) \times C_{\text{standard}}$$

where C_{standard} (mg/ml) is the saponins concentration of the standard injected (e.g., $C_{\text{standard}} = 13.5$ mg/ml if the saponin content of 1.5 g of standard sample is 90 %) and A_{sample} and A_{standard} are the sums of the peak areas attributed to the four principle saponins in the sample preparation and in the standard preparation, respectively, as noted in the figure. (Tannins and Polyphenols will elute before the saponins. The peaks due to the saponins will appear after the major peak due to the polyphenols - see figure.)

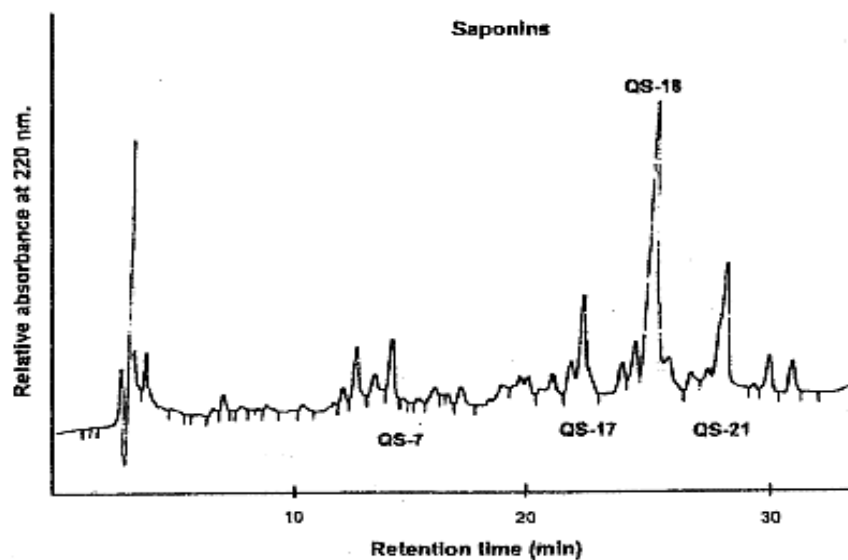
The percentage of saponins in the test sample is:

$$\% \text{ Saponins} = 100 \times C_{\text{sap}} / (0.1 W_{\text{sample}})$$

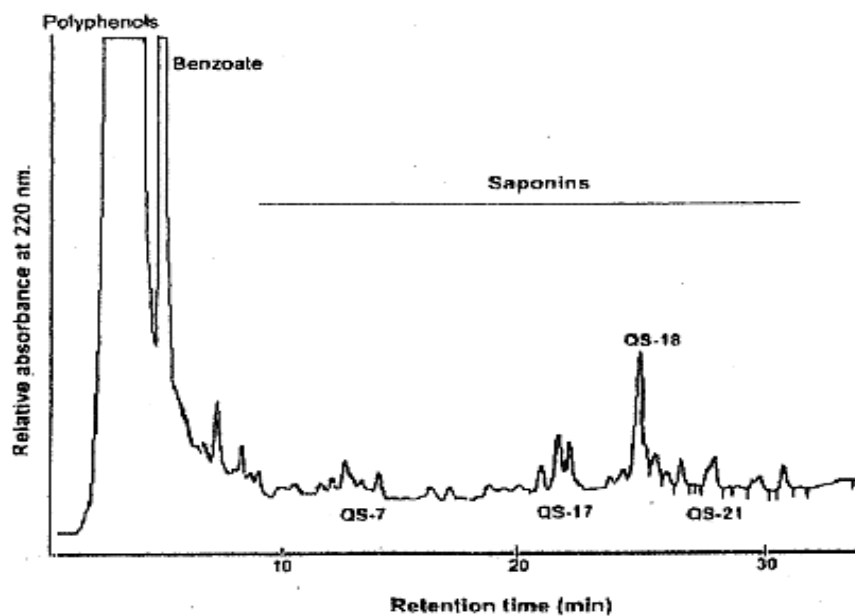
where W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

Appendix

Chromatogram of Standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).



Chromatogram of Quillaia extract (Type 1) (55 mg solids/ml)



QUILLAIA EXTRACT (TYPE 2)

Revised specifications prepared at the 65th JECFA and published in FNP 52 Add 13 (2005), superseding specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). A group ADI of 0.1 mg quillaia saponins per kg bw was established at the 65th JECFA (2005) for Quillaia Extract (Type 1) and Quillaia Extract (Type 2).

SYNONYMS	Quillaia extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract
DEFINITION	Quillaia extract (Type 2) is obtained either by chromatographic separation or ultrafiltration of the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of <i>Quillaja saponaria</i> Molina (family <i>Rosaceae</i>). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are minor components. Some sugars and calcium oxalate will also be present. Quillaia extract (Type 2) is available commercially as a liquid product or as a spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.
C.A.S. number	68990-67-0
Formula weight	Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues
Assay	Saponin content: not less than 65% and not more than 90% on the dried basis
DESCRIPTION	Light red-brownish liquid or powder
FUNCTIONAL USES	Emulsifier, foaming agent
CHARACTERISTICS	
IDENTIFICATION	
Solubility (FNP 5)	Very soluble in water, insoluble in ethanol, acetone, methanol, and butanol
Foam	Dissolve 0.5 g of the powder form in 9.5 ml of water or 1 ml of the liquid form in 9 ml of water. Add 1 ml of this solution to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam volume (ml) after 30 min. Typical volumes are about 260 ml.
Chromatography	Determine as in METHOD OF ASSAY. The retention time of major sample peak corresponds to the major saponin peak (QS-18) of the standard.
Colour and turbidity	Powder form only: Dissolve 0.5 g in 9.5 ml of water. The solution shall not turbid. Determine the absorbance of the solution against water at 520 nm. The absorbance shall be less than 0.7.
PURITY	
Water (FNP 5)	Powder form: not more than 6% (Karl Fischer Method)
Loss on drying (FNP 5)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (FNP 5)	3.7 - 5.5 (4 % solution)
Ash (FNP 5)	Not more than 5% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from Loss on drying)

Tannins	Not more than 8% on a dried basis See description under TESTS
Lead (FNP 5)	Not more 2 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 FNP 5, "Instrumental Methods".

TESTS

PURITY TESTS

Tannins	Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (W_i). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at $800 \times g$. Recover the supernatant and dry this solution at 105° (5 h). Weigh the recovered solid (W_f , in g). The percentage of tannins in the sample is:
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$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle:

The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 2).

Sample preparation:

Powders: Weigh 0.5 g of sample and dissolve in 9.5 ml of water. Filter through a 0.2 μ m filter.

Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 ml of water. Filter through a 0.2 μ m filter.

In each case, the sample volume is ca. 10 ml.

Standard preparation:

Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 μ m filter.

High performance liquid chromatography (HPLC):

HPLC conditions:

Column:	Vydac 214TP54 (4.6 x 250 mm length, 5 μ m particle size) or equivalent		
Column temperature:	Room temperature		
Pump:	Gradient		
Solvent A:	0.15% trifluoroacetic acid in HPLC-grade water.		
Solvent B:	0.15% trifluoroacetic acid in HPLC-grade acetonitrile.		
Gradient:	Time(min)	% solvent A	% solvent B
	0	70	30
	40	55	45
	45	70	30
Flow rate:	1 ml/min		
Detection wavelength:	220 nm		
Injection volume:	20 μ l		

Calculation:

The concentration of saponins, C_{sap} , in mg/ml, in the solution prepared as directed under sample preparation is:

$$C_{\text{sap}} = (A_{\text{sample}}/A_{\text{standard}})C_{\text{Standard}}$$

where C_{Standard} (mg/ml) is the saponins concentration of the standard injected (e.g., $C_{\text{Standard}} = 13.5$ mg/ml if the saponin content of 1.5 g of standard sample is 90 %) and A_{sample} and A_{standard} are the sums of the peak areas attributed to the four principle saponins in the sample preparation and in the standard preparation, respectively, as noted in the figure. (Tannins and polyphenols will elute before the saponins. The peaks corresponding to the saponins will appear after the major peak corresponding to the polyphenols)

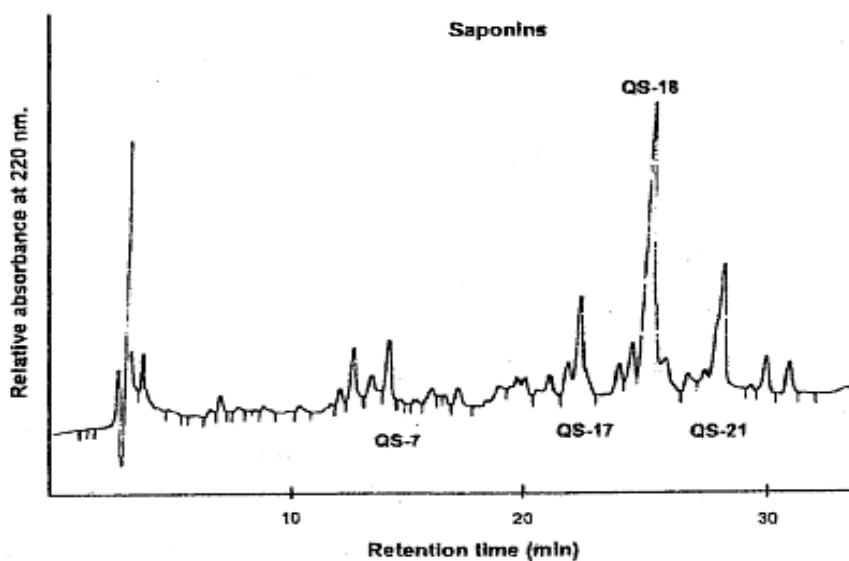
The percentage of saponins in the test sample is:

$$\% \text{ Saponins} = 100 \times C_{\text{sap}}/(0.1W_{\text{sample}})$$

where W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

Appendix

Chromatogram of standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).



SUCROSE ESTERS OF FATTY ACIDS (Tentative)

Information required on

- **method of analysis for the determination of free sucrose using capillary GC or HPLC**
- **an alternative and less toxic solvent than pyridine for preparing the standard and sample solutions for the determinations of free sucrose and propylene glycol**
- **method of analysis for the determination of dimethyl sulfoxide that does not require a packed column**

Note: The tentative specifications will be withdrawn unless the requested information is received before the end of the year 2006.

Tentative specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005), superseding the specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). An ADI of 0-30 mg/kg bw for this substance together with sucroglycerides was established at the 49th JECFA (1997).

SYNONYMS

Sucrose fatty acid esters, INS No. 473

DEFINITION

Mono-, di- and tri-esters of sucrose with food fatty acids, prepared from sucrose and methyl and ethyl esters of food fatty acids by esterification in the presence of a catalyst or by extraction from sucroglycerides. Only the following solvents may be used for the production: dimethylformamide, dimethyl sulfoxide, ethyl acetate, isopropanol, propylene glycol, isobutanol and methyl ethyl ketone.

Assay

Not less than 80% of sucrose esters

DESCRIPTION

Stiff gels, soft solids or white to slightly greyish white powders

FUNCTIONAL USES

Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (FNP 5)

Sparingly soluble in water, soluble in ethanol

Fatty acids

Add 1 ml of ethanol to 0.1 g of the sample, dissolve by warming, add 5 ml of dilute sulfuric acid TS, heat in a water bath for 30 min and cool. A yellowish white solid or oil is formed, which has no odour of isobutyric acid, and which dissolves when 3 ml of diethyl ether are added. Use the aqueous layer separated from the diethyl ether in the Test for sugars.

Sugars

To 2 ml of the aqueous layer separated from the diethyl ether in the test for fatty acids, carefully add 1 ml of anthrone TS down the inside of a test tube; the boundary surface of the two layers turns blue or green.

PURITY

Sulfated ash (FNP 5)

Not more than 2%
Test 1 g of the sample (Method I)

Acid value (FNP 5)

Not more than 6

Free sucrose

Not more than 5%
See description under TESTS

Dimethylformamide	Not more than 1 mg/kg See description under TESTS
Dimethyl sulfoxide	Not more than 2 mg/kg See description under TESTS
Ethyl acetate, isopropanol and propylene glycol	Not more than 350 mg/kg, singly or in combination See description under TESTS
Isobutanol	Not more than 10 mg/kg See description under TESTS
Methanol	Not more than 10 mg/kg See description under TESTS
Methyl ethyl ketone	Not more than 10 mg/kg See description under TESTS
Lead (FNP 5)	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 FNP 5, "Instrumental methods".

TESTS

PURITY TESTS

Free sucrose Determine by gas liquid chromatography (FNP 5).

Standard solutions

Prepare a stock solution containing 5.0 mg/ml of sucrose in N,N-dimethylformamide. Prepare a range of standard solutions containing 0.5, 1.25 and 2.5 mg/ml of sucrose by dilutions of the stock solution with pyridine.

Internal standard solution

Weigh accurately 0.25 g of octacosane into a 50-ml volumetric flask, add 25 ml of tetrahydrofuran to dissolve the octacosane, and add pyridine to the mark.

Chromatography conditions

Column: 2% Dexsil 300GC on Uniport HP 80/100 mesh (slightly polar, 2.1 m x 3.2 mm i.d.) or equivalent
Carrier gas: Nitrogen
Flow rate: 40 ml/min
Detector: FID
Temperatures: - injection: 280°
- column: Hold for 1 min at 160°, then 160-300° at 15°/min, hold for 60 min at 300°
- detector: 320°

The retention times of free sucrose and octacosane measured under the above conditions are approx. 8.2 and 9.8 min, respectively.

Procedure

Weigh accurately 20-50 mg of the sample into a centrifugation tube, add 1 ml internal standard solution, 1 ml pyridine, 0.4 ml of N,O-bis(trimethylsilyl)acetamide (BSA) and 0.2 ml trimethylchlorosilane (TMCS). After sealing the tube, shake and let stand for 5 min at room temperature. Inject 1 µl into the gas liquid chromatograph.

Standard curve

Prepare silylate standard solutions following the same procedure using 1 ml each of the standard solutions in place of the sample and pyridine. Draw a standard curve by plotting amount of sucrose (mg) in 1 ml of the standard solution (X-axis) vs. ratio of peak area of sucrose/internal standard (Y-axis).

Measure the peak areas for sucrose and internal standard. Calculate the ratio of their peak areas, and obtain the amount of sucrose in sample from the standard curve.

Calculate the percentage of free sucrose from:

$$\% \text{ free sucrose} = \frac{\text{amount of sucrose detected (mg)}}{\text{weight of sample (mg)}} \times 100$$

Dimethylformamide

Determine by gas liquid chromatography (FNP 5).

Standard solutions

Prepare a stock solution containing 1.00 mg/ml of dimethylformamide in tetrahydrofuran. Prepare a range of standard solutions containing 0.05, 0.1 and 0.2 µg/ml of dimethylformamide by diluting the stock solution with tetrahydrofuran.

Chromatography conditions

Column: Polyethylene glycol (30 m x 0.32 mm i.d. with a 0.5 µm film)
Carrier gas: Helium
Pressure: 150 kPa (constant pressure)
Detector: Nitrogen phosphorus detector (NPD) (synonym: Flame thermoionic detector (FTD))
Temperatures: - injection: 180°
- column: Hold for 2 min at 40°, then 40-160° at 20°/min, hold for 2 min at 160°
- detector: 325°
Injection method: Splitless injection of 1.0 µl with auto-injector, followed by start of purge after 1.0 min.

The retention time of dimethylformamide measured under the above conditions is approx. 6.4 min.

Procedure

Weigh accurately 2 g of sample into a 20-ml volumetric flask, add 10 ml of tetrahydrofuran to dissolve the sample, add tetrahydrofuran to the mark, and mix the solution well. Inject 1.0 µl of the sample solution into the chromatograph.

Standard curve

Prepare daily by injecting 1.0 µl of each of the standard solutions into the chromatograph.

Calculate the concentration CDFA of dimethylformamide from:

$$\text{CDFA (mg/kg)} = [C (\mu\text{g/ml}) \times 20 (\text{ml})] / W (\text{g})$$

where

C = dimethylformamide concentration detected (µg/ml)
W = weight of sample (g)

Note: The nitrogen phosphorus detector is insensitive to components that do not contain nitrogen or phosphorus. As a consequence, the capillary column can become obstructed with compounds of low volatility, although the baseline of the chromatogram is stable. Accordingly, the column must be reconditioned frequently. Overnight reconditioning (flow carrier gas in the reverse direction at 180°) is required after about every 15 samples.

Dimethyl sulfoxide

Determine by gas liquid chromatography (FNP 5).

Standard solutions

Prepare a 0.25 mg/ml stock solution of dimethyl sulfoxide in tetrahydrofuran.

Prepare a range of solutions containing 0.5, 1 and 5 µg/ml of dimethyl sulfoxide by dilutions of the stock solution with tetrahydrofuran.

Chromatography conditions

Column: 10% PEG 20M and 3% KOH on Gas Chrom Z (2 m x 3 mm i.d.) or equivalent. Raise the oven temperature to 180° at a rate of 10°/min and let stabilize for 24 to 48 h with 30 to 40 ml/min of nitrogen for conditioning

Carrier gas: Nitrogen

Flow rate: 50 ml/min

Detector: Flame photometric detector (using 394 nm sulfur filter)

Temperatures - injection: 210°

- column: 160°

The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3.4 min.

Procedure

Weigh accurately 5 g of the sample into a 25-ml volumetric flask, add 10 ml of tetrahydrofuran to dissolve the sample, add tetrahydrofuran to the mark, and mix the solution well. Inject 3 µl of the sample solution into the chromatograph.

Standard curve

Prepare daily by injecting 3 µl of each of the standard solutions into the chromatograph.

Calculate the concentration CDMSO of dimethyl sulfoxide in mg/kg from:

$$\text{CDMSO (mg/kg)} = [\text{C (}\mu\text{g/ml)} \times 25 \text{ (ml)}] / \text{W (g)}$$

where

C = dimethyl sulfoxide concentration determined (µg/ml)

W = weight of sample (g)

Propylene glycol

Determine by gas liquid chromatography (FNP 5).

Internal standard solution

Prepare a 500 µg/ml solution of ethylene glycol in pyridine.

Standard solution

Prepare a 50 µg/ml solution of propylene glycol in pyridine.

Chromatography conditions

Column: Polydimethylsiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)

Carrier gas: Helium

Flow rate: 1.5 ml/min (Constant flow)

Detector: FID

Temperatures - injection: 230°

- column: Hold for 5 min at 60°, then 60-250° at 20°/min, hold for 5 min at 250°

- detector: 250°

The retention times of ethylene glycol and propylene glycol derivatives are approx. 7.7 min and 7.9 min, respectively.

Procedure

Weigh accurately 1 g of the sample in a 10-ml volumetric flask, and add 100 µl of the internal standard solution. Dissolve and make to volume with pyridine. Take 0.5 ml of sample solution in a centrifugation tube, and add 0.25 ml of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 0.1 ml of trimethylchlorosilane (TMCS). After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 µl of this centrifugal supernatant into the chromatograph.

Standard curve

Prepare following the same procedure using 0.05, 0.2, 0.5 and 1 ml of the standard solution in place of the sample.

Calculate the concentration CPG of propylene glycol in mg/kg from:

$$\text{CPG (mg/kg)} = [C (\mu\text{g/ml}) \times 10 (\text{ml})] / W (\text{g})$$

where

C = polyethylene glycol concentration determined (µg/ml)

W = weight of sample (g)

Note: It will be necessary to clean the injection port and to recondition column at 300° after about every 20 samples, because of contamination of the column.

Methanol, isopropanol,
isobutanol, ethyl acetate and
methyl ethyl ketone

Determined by gas chromatography with a head space sampler.

Standard solutions

Prepare standard solution A containing 4000 mg/l each of methanol, isopropanol, isobutanol, ethyl acetate and methyl ethyl ketone by weighing accurately 0.2 g of each solvent into a 50-ml volumetric flask containing approx. 20 ml of water, then adding water to volume. By dilutions of this solution, prepare solutions containing 2000 mg/l (standard solution B) and 1000 mg/l (standard solution C).

Procedure

Weigh accurately 1 g of the sample in each of four sample vials. To one vial add 5 µl of water, to the second, third and fourth, add, respectively, standard solutions A, B and C, and seal them quickly with a septum. (The concentrations of each solvent after adding 5 µl of standard solutions A, B and C to 1 g of the sample are equal to 20, 10 and 5 mg/kg of sample, respectively). Place the sample vials in a head space sampler and analyse using the following conditions:

Column: 100% Polydimethylsiloxane (30 m x 0.53 mm i.d. with 1.5 µm film, for example DB-1 manufactured by J&W Co. Ltd.)

Carrier gas: Nitrogen

Flow rate: 3.5 ml/min

Detector: FID

Temperatures - injection: 110°

- column: 40°

- detector: 110°

Head space sampler:

- sample heat insulating temperature: 80°

- sample heat insulating period: 40 min

- syringe temperature: 85°

- sample gas injection: 1.0 ml

Calculation

Plot the relationship between the added amount against the peak area for each solvent using the analytical results. The relationship should be linear ($R^2 > 0.99$). Extrapolate and determine the x-intercept, w_i , and calculate the solvent concentrations C_i in mg/kg in the sample from:

$$C_i = w_i / W$$

where

w_i = x-intercept of relationship line using the standard addition method (μg)

W = weight of sample (g)

METHOD OF ASSAY

Determine by HPLC using the following conditions:

Procedure

Accurately weigh 250 mg of the sample and transfer to a 50-ml volumetric flask. Dilute to volume with tetrahydrofuran and mix. Filter through a 0.5 μm membrane filter. Inject 100 μl of the sample into the pre-stabilized chromatograph.

Chromatography conditions

Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G2000 (Tosoh) or equivalent)

Mobile phase: HPLC-grade degassed tetrahydrofuran

Flow rate: 0.7 ml/min

Detector: RI

Temperatures: - Column: 38°
- Detector: 38°

Record the chromatogram for about 90 min.

Calculate the percentage of sucrose ester content in the sample from:

$$\% \text{ sucrose ester} = 100 A/T$$

where

A = the sum of peak areas for the three main components, the mono-, di- and tri-esters, eluting at about 65, 68 and 73 min, respectively

T = the sum of all peak areas eluting within 90 min

Section C: Specifications of certain flavouring agents

At its 44th meeting JECFA considered a new approach to the safety evaluation of flavouring agents. This approach incorporates a series of criteria whose use enables the evaluation of a large number of these agents in a consistent and timely manner. At the current meeting of the Committee specifications of identity and purity were prepared or revised for 138 flavouring agents.

Information on specifications for flavouring agents is given on the following tables under the following headings, most of which are self-explanatory:

Name; Chemical name (Systematic name); Synonyms; Flavour and Extract Manufacturers' Association of the United States (FEMA) No; FLAVIS (FL) No; Chemical Abstract Service Registry (CAS) No; Molecular weight; Chemical formula; Physical form/odour; Solubility; Solubility in ethanol, Boiling point (for information only); Identification test (ID); Assay min% (Gas chromatographic (GC) assay of flavouring agents); Acid value max; Refractive index (at 20°, if not otherwise stated); Specific gravity (at 25°, if not otherwise stated)

The field called "Other requirements" contains three types of entry:

- Items in normal type are additional requirements, such as further purity criteria or other tests
- Items contained in square brackets are provided for information, for example the typical isomer composition of the flavouring agent. These are not considered to be requirements.
- Substances listed after "SC:" are secondary constituents which have been taken into account in the safety evaluation of the named flavouring agent. If the commercial product contains less than 95% of the named compound, it is a requirement that the major part of the product (i.e. not less than 95%) is accounted for by the sum of the named compound and one or more of the secondary constituents.

The field called "JECFA" contains the number of the meeting at which the specifications were prepared and the status of the specification. R means "specifications revised", S means "existing specifications maintained", S,T means "existing tentative specifications maintained, (further information required)", N means "new specifications", N,T means "new tentative specifications, (further information required)", and N,C means "new specifications for a flavouring agent with a conditional safety evaluation". In Section A of this document further information on the conditional safety evaluation and its implications are given.

The last column indicates the data (information) required for the tentative specifications. Abbreviations for "data required" are as follows: A=assay minimum, A(e)=equivalence factor for assay, A(m)=details of assay method, BP=boiling point, ID=identity test, Low assay=quantitative information on by-products, RI=refractive index, SG=specific gravity. A(e) is an equivalence factor to convert an assay obtained by aldehyde / ketone determination, ester determination, total alcohols determination, or titration determination to the % value which would be obtained if GC had been used for the assay (FNP 5).

The infrared and other spectra, used for identification and comparison purposes, are provided from page 71 onwards (copies for certain spectra may be obtained from the FAO Joint Secretariat in Rome or from FEMA, Suite 925, 1620 I Street, N.W., Washington DC, USA).

A comprehensive index listing all names, chemical names, and synonyms is added on page 90.

Note on spectra: only spectra which were submitted to the 65th meeting are reproduced in this volume. The reader is referred to previous Addenda for copies of spectra of revised specifications.

No	Name chemical name / synonyms	FEMA / FL / CAS numbers	Formula M.W.	Physical form / odour	Solubility / solubility in ethanol	B.P. (°C)	ID test / assay min	A.V.	Ref. index / Sp. gravity	Other requirements	JECFA	Data required
631.2.3-Methyl-2-oxobutanoic acid, sodium salt	Sodium 3-methyl-2-oxobutyrate Sodium, alpha-ketoisovalerate; Butanoic acid, 3-methyl-2-oxo-, sodium salt; Sodium 3-methyl-2-oxobutanoate	3869 08.051 3715-29-6	C5 H7 O3 Na 138.10	-	-	n/a	NMR 99	-	n/a n/a	Assay by hplc using an ion exchange column	65th/R,T	A(m)
632.2.3-Methyl-2-oxopentanoic acid, sodium salt	Sodium 3-methyl-2-oxovalerate Valeric acid, 3-methyl-2-oxo-, sodium salt	3870 08.093 3715-31-9	C6 H9 O3 Na 152.10	-	-	n/a	NMR 99	-	n/a n/a	Assay by hplc using an ion exchange column	65th/R,T	A(m)
633.2.4-Methyl-2-oxopentanoic acid, sodium salt	Sodium 4-methyl-2-oxovalerate Valeric acid, 4-methyl-2-oxo, sodium salt; Sodium 4-methyl-2-ketopentanoate; Sodium 4-methyl-2-oxopentanoate	3871 08.052 4502-00-5	C6 H9 O3 Na 152.10	-	-	n/a	NMR 99	-	n/a n/a	Assay by hplc using an ion exchange column	65th/R,T	A(m)
1479 Sodium 2-oxo-3-phenylpropionate	Sodium 2-oxo-3-phenylpropionate Sodium 3-phenylpyruvate	3892 114-76-1	C9 H7 O3 Na 188.14	-	-	n/a	NMR 97	-	n/a n/a	Assay by hplc	65th/R,T	A(m)
1480 Maltol	3-Hydroxy-2-methyl-4-pyrone 2-Methylpyroneconic acid; 3-Hydroxy-2-methyl-4-pyrone; Larixinic acid; INS No.636	2656 07.014 118-71-8	C6 H6 O3 126.11	White crystalline powder Caramel-butterscotch aroma	Very slightly soluble in water, ether Soluble	-	NMR 98%	-	-	mp: 159-162°; Complies with purity criteria for Maltol in FNP52	65th/N	
1481 Ethyl maltol	2-Ethyl-3-hydroxy-4-pyrone 2-Ethyl-3-hydroxy-4-pyrone; 2-Ethyl-3-hydroxy-4H-pyran-4-one; 2-Ethylpyroneconic acid; ; INS No.637	3487 07.047 4940-11-8	C7 H8 O3 140.14	White crystalline powder Sweet fruit-like aroma	Soluble in water, propylene glycol. Slightly soluble in glycerine Soluble	-	NMR 99%	-	-	mp: 89-93°; Complies with purity criteria for Ethyl maltol in FNP52	65th/N	
1482 Maltol isobutyrate	2-Methyl-4H-pyran-4-one-3-yl 2-methylpropanoate 2-Methyl-4-oxo-4H-pyran-3-yl isobutyrate; Maltol 2-methylpropanoate; 2-Methyl-4-pyran-3-yl 2-methylpropanoate	3462 09.525 65416-14-0	C10 H12 O4 196.20	Colourless to yellow liquid Strawberry aroma	Insoluble in water; Soluble in propylene glycol and fixed oils Soluble	100° (0.01 mm Hg)	IR 96%	10.0	1.493-1.501 1.140-1.153		65th/N,T	SG range

No	Name chemical name / synonyms	FEMA / FL / CAS numbers	Formula M.W.	Physical form / odour	Solubility / solubility in ethanol	B.P. (°C)	ID test / assay min	A.V.	Ref. index / Sp. gravity	Other requirements	JECFA	Data required
1483 2-Methyl-3-(1-oxopropoxy)-4H-pyran-4-one	2-Methyl-4H-pyran-4-one-3-yl propionate Maltol propionate; Valtol propionate	3941 68555-63-5	C9 H10 O4 182.17	White crystalline solid Caramel aroma	Insoluble in water; Soluble in fats Soluble	-	NMR IR 98%	-	-	mp: 42-45°	65th/N,C	
1484 2-Butyl-5- or 6-keto-1,4-dioxane		2204 13.028 65504-95-2	C8 H14 O3 158.20	Colourless liquid Powerful fruity aroma	Slightly soluble in water Soluble	98-99° (13 mm Hg)	NMR 97%	-	1.472-1.478 1.292-1.296	[Lactone undergoes ring opening in basic solution]	65th/N	
1485 2-Amyl-5 or 6-keto-1,4-dioxane	2-Butyl-5 or 6-keto-1,4-dioxane 5(or 6)-Butyl-1,4-dioxan-2-one	2076 13.027 65504-96-3	C9 H16 O3 172.22	Colourless liquid Fruity-winey aroma	Slightly soluble in water Soluble	101-103° (15 mm Hg)	NMR 97%	-	1.480-1.486 1.288-1.294	[Lactone undergoes ring opening in basic solution]	65th/N	
1486 2-Hexyl-5 or 6-keto-1,4-dioxane	2-Hexyl-5 or 6-keto-1,4-dioxane 5(or 6)-Pentyl-1,4-dioxan-2-one	2574 65504-97-4	C10 H18 O3 186.25	Colourless or very pale yellow liquid Powerful sweet, nut-like aroma	Slightly soluble in water; Soluble in oil Soluble	104° (15 mm Hg)	NMR 97%	-	1.486-1.492 1.280-1.286	[Lactone undergoes ring opening in basic solution]	65th/N	
1487 2-Methylfuran	2-Methylfuran alpha-Methylfuran; 5-Methylfuran	4179 13.030 534-22-5	C5 H6 O 82.10	Colourless liquid Spicy smoky aroma	Slightly soluble in water Soluble	64°	NMR IR MS 97%	1.0	1.431-1.437 0.908-0.917		65th/N	
1488 2,5-Dimethylfuran	2,5-Dimethylfuran	4106 625-86-5	C6 H8 O 96.13	Colourless liquid Spicy smoky aroma	Slightly soluble in water Soluble	93°	NMR IR MS 95%	1.0	1.437-1.443 0.892-0.898		65th/N	
1489 2-Ethylfuran	2-Ethylfuran 2-Ethylfuran	3673 13.092 3208-16-0	C6 H8 O 96.13	Colourless liquid Powerful, smoky burnt aroma	Insoluble in water; Soluble in oils Soluble	92-93°	NMR 95%	1.0	1.444-1.450 0.909-0.915		65th/N	
1490 2-Butylfuran	2-Butylfuran	4081 13.103 4466-24-4	C8 H12 O 124.18	Colourless liquid Spicy aroma	Insoluble in water Soluble	139°	MS 95%	1.0	1.444-1.450 0.884-0.890		65th/N	
1491 2-Pentylfuran	2-Pentylfuran 2-Amylfuran	3317 13.059 3777-69-3	C9 H14 O 138.21	Colourless liquid Fruity aroma	Slightly soluble in water Soluble	58-60° (10 mm Hg)	NMR 99%	1.0	1.443-1.449 0.886-0.893		65th/N	
1492 2-Heptylfuran	2-Heptylfuran	3401 13.069 3777-71-7	C11 H18 O 166.26	Colourless to yellowish liquid Nutty, coffee-like aroma	Insoluble in water Soluble	209-210°	NMR 99%	1.0	1.446-1.452 0.860-0.866		65th/N	

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1493 2-Decylfuran 2-Decylfuran		4090 13.106 83469-85-6	C14 H24 O 208.34	Colourless solid Spicy, fatty aroma	Insoluble in water Soluble	-	NMR (13C) 95%	-	-	mp: 30°	65th/N	
1494 3-Methyl-2-(3-methylbut-2-enyl)-furan 3-Methyl-2-(3-methylbut-2-enyl)furan 2-(3-Methyl-2-butenyl)-3-methylfuran; Rosefuran		4174 13.148 15186-51-3	C10 H14 O 150.22	Colourless liquid Caramel aroma	Slightly soluble in water Soluble	70° (11 mm Hg)	MS 98%	1.0	1.473-1.479 0.998-1.004		65th/N	
1495 2,3-Dimethylbenzofuran 2,3-Dimethylbenzofuran		3535 13.074 3782-00-1	C10 H10 O 146.19	Clear to yellow liquid Nutty spicy aroma	Insoluble in water, Soluble in fats Soluble	96-98° (15 mm Hg)	NMR 97%	1.0	1.554-1.563 1.031-1.037		65th/N	
1496 2,4-Difurfurylfuran 2,4-Difurfurylfuran		4095 13.107 64280-32-6	C14 H14 O3 228.24	Colourless solid Floral, fruity aroma	Insoluble in water Soluble	-	NMR (13C) 95%	-	-	mp: 153°	65th/N	
1497 3-(2-Furyl)acrolein 3-(2-Furyl)prop-2-enal 2-Furanacrolein; Furylacrolein; 3-(2-Furyl)-2-propenal; 3-(2-Furyl)acrylaldehyde		2494 13.034 623-30-3	C7 H6 O2 122.12	White or yellow needles Cooked spicy-herb aroma	Insoluble in water Soluble	-	NMR 97%	3.0	-	mp: 49-52°	65th/N	
1498 2-Methyl-3(2-furyl)acrolein 3-(2-Furyl)-2-methylprop-2-enal Furfurylidene-2-propanal; alpha-Methylfurylacroleine; 2-Methyl-3-(2-furyl)propenal		2704 13.046 874-66-8	C8 H8 O2 136.15	Pale yellowish liquid Mild, warm, cinnamon- like aroma	Insoluble in water; Soluble in oils Soluble	225°	NMR 96%	3.0	1.567-1.573 1.097-1.103		65th/N	
1499 3-(5-Methyl-2-furyl)prop-2-enal 3-(5-Methyl-2-furyl)prop-2-enal (5-Methylfuryl)acrolein; 1-(5-Methyl-2-furanyl)-1-propen-3-yl; 3-(5-Methyl-2-furanyl)-2-propenal; 5-Methyl-2-furanacrolein		4175 13.150 5555-90-8	C8 H8 O2 136.15	Colourless liquid Sweet spicy aroma	Slightly soluble in water Soluble	101° (5 mm Hg)	NMR (13C) 95%	3.0	1.006-1.012 0.998-1.004		65th/N	
1500 3-(5-Methyl-2-furyl)-butanal 3-(5-Methyl-2-furyl) butanal 3-(5-Methyl-2-furyl)-butylaldehyde		3307 13.058 31704-80-0	C9 H12 O2 152.19	Colourless liquid Vegetable, fruity aroma	Insoluble in water; Soluble in oils Soluble	88-91° (12 mm Hg)	NMR 98%	3.0	1.575-1.581 1.006-1.012		65th/N	

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1526	O-Ethyl S-(2-furylmethyl)thiocarbonate O-Ethyl S-(2-furylmethyl)thiocarbonate O-Ethyl S-(furan-2-ylmethyl) thiocarbonate; O-Ethyl S-(2-furanylmethyl)thiocarbonate; Ethoxy carbonyl furylthiol	4043 376595-42-5	C8 H10 O3 S 186.23	Colourless liquid Spicy, floral aroma	Practically insoluble in water; Soluble in diether ether, ethyl acetate Soluble	130-135°	NMR IR MS 99%	5.0	1.504-1.510 1.167-1.173		65th/N	
1527	4-Allylphenol 4-Allylphenol 4-(2-Propenyl)phenol; p-Hydroxyallylbenzene; Chavicol	4075 04.058 501-92-8	C9 H10 O 134.18	Colourless liquid Medicinal phenolic aroma	Insoluble in water Soluble	235°	NMR 95%	1.0	1.542-1.548 1.017-1.023		65th/N,C	
1528	2-Methoxy-6-(2-propenyl)phenol 2-Allyl-6-methoxyphenol o-Eugenol	579-60-2	C10 H12 O2 164.20	Colourless to pale yellow liquid Spicy aroma	Slightly soluble in water; Soluble in ether, most fixed oils Soluble	119-121° (12 mm Hg)	NMR 98%	1.0	1.535-1.541 1.065-1.071		65th/N,C	
1529	Eugenol 4-Allyl-2-methoxyphenol 1-Hydroxy-2-methoxy-4-propenylbenzene; 2-Methoxy-4-allylphenol; Allylguaiacol	2467 04.003 97-53-0	C10 H12 O2 164.20	Colourless to pale yellow liquid Aroma of cloves	Slightly soluble in water; Soluble in ether, most fixed oils Soluble	256°	IR 98%	1.0	1.540-1.542 1.064-1.070		65th/R	
1530	Eugenyl formate 4-Allyl-2-methoxyphenyl formate Eugenol formate; 4-(2-Propen-1-yl)-2-methoxyphenyl formate	2473 09.088 10031-96-6	C11 H12 O3 192.21	Colourless to pale-yellowish oily liquid Warm, woody, dry aroma	Insoluble in water; Soluble in oils Soluble	270°	NMR 94%	1.0	1.524-1.526 1.115-1.125	SC: Eugenol	65th/N	
1531	Eugenyl acetate 4-Allyl-2-methoxyphenyl acetate Acetyl eugenol; Eugenol acetate	2469 09.020 93-28-7	C12 H14 O3 206.24	Fused solid, melts at warm room temperature to a pale yellow liquid Mild clove aroma	Insoluble in water; Soluble in ether; fixed oils Soluble	282°	IR 98%	1.0	1.514-1.522 (supercooled liquid) 1.077-1.082 (supercooled liquid)	mp: 25°	65th/N	
1532	Eugenyl isovalerate 4-Allyl-2-methoxyphenyl 3-methylbutanoate 4-Allyl-2-methoxyphenyl isovalerate	4118 09.878 61114-24-7	C15 H20 O3 248.32	White solid Fruity clove-like aroma	Insoluble in water Soluble	-	NMR 99%	-	-	mp: 85°	65th/N,C	
1533	Eugenyl benzoate 4-Allyl-2-methoxyphenyl benzoate Benzoyl eugenol; Eugenol benzoate	2471 09.766 531-26-0	C17 H16 O3 268.31	Colourless crystalline solid Balsamic aroma with and undertone reminiscent of clove	Insoluble in water; Soluble in oils Soluble	-	NMR 97%	-	-	mp: 69-70°	65th/N	

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1534 Methyl anthranilate Methyl 2-aminobenzoate o-Amino methyl benzoate; Methyl o-aminobenzoate		2682 09.715 134-20-3	C8 H9 N O2 151.17	Colourless to pale yellow liquid or crystals with bluish fluorescence Grape-like or orange aroma	Soluble in oils and propylene glycol; Insoluble in glycerol; Slightly soluble in water Soluble	256°	IR 98%	1.0	1.581-1.585 (supercooled liquid) 1.161-1.169		65th/R	
1535 Ethyl anthranilate Ethyl 2-aminobenzoate Ethyl o-aminobenzoate		2421 09.716 87-25-2	C9 H11 N O2 165.19	Colourless to light yellow liquid Pleasant aroma resembling orange	Slightly soluble in water; Soluble in propylene glycol and fixed oils Soluble	267°	IR 96%	1.0	1.563-1.566 1.115-1.120		65th/N	
1536 Butyl anthranilate Butyl 2-aminobenzoate Butyl o-aminobenzoate; n-Butyl anthranilate		2181 09.717 7756-96-9	C11 H15 N O2 193.25	A colourless or very pale straw-Coloured liquid Mild sweet, fruity, floral aroma	Insoluble in water; Soluble in oils Soluble	303°	NMR 96%	1.0	1.539-1.545 1.067-1.073		65th/N	
1537 Isobutyl anthranilate 2-Methylpropyl 2-aminobenzoate Isobutyl 2-aminobenzoate; Isobutyl o-aminobenzoate		2182 09.718 7779-77-3	C11 H15 N O2 193.25	Colourless to pale yellow liquid Faint orange blossom-like aroma	Insoluble in water; Soluble in oils Soluble	169-170° (13.5 mm Hg)	NMR 96%	1.0	1.534-1.540 1.057-1.063		65th/N	
1538 cis-3-Hexenyl anthranilate (Z)-3-Hexenyl 2-aminobenzoate		3925 65405-76-7	C13 H17 N O2 219.29	Colourless liquid Fruity aroma reminiscent of grape	Insoluble in water; Soluble in fats Soluble	160° (5 mm Hg)	NMR IR 98%	2.0	1.545-1.554 1.047-1.054		65th/N,C	
1539 Citronellyl anthranilate 3,7-Dimethyloct-6-enyl 2-aminobenzoate		4086 68555-57-7	C17 H25 N O2 275.40	Colourless liquid Rose, fruity aroma	Insoluble in water Soluble	160° (0.1 mm Hg)	NMR 96%	1.0	1.531-1.537 1.001-1.007		65th/N,C	
1540 Linalyl anthranilate 1,5-Dimethyl-1-vinylhex-4-enyl 2-aminobenzoate 3,7-Dimethyl-1,6-octadien-3-yl anthranilate; Linalyl 2-aminobenzoate; Linalyl o-aminobenzoate		2637 09.721 7149-26-0	C17 H23 N O2 273.38	Pale straw-coloured oily liquid Gardenia-like aroma	Insoluble in water; Soluble in dimethyl sulphoxide Soluble	370-371°	NMR 95%	2.0	1.516-1.522 1.052-1.058 (15.5°)		65th/N	
1541 Cyclohexyl anthranilate Cyclohexyl 2-aminobenzoate		2350 09.722 7779-16-0	C13 H17 N O2 219.29	Pale yellow liquid Faint, fruity, orangeblossom-like aroma	Insoluble in water Soluble	318°	NMR 97%	1.0	1.571-1.577 1.015-1.021		65th/N	
1542 beta-Terpinyl anthranilate p-Menth-1-en-8-yl 2-aminobenzoate p-Menth-1-en-8-yl anthranilate; p-Menth-1-en-8-yl 2-aminobenzoate; Terpinyl 2-aminobenzoate		3048 09.724 14481-52-8	C17 H23 N O2 273.38	Colourless to yellow liquid Complex fruity aroma	Insoluble in water; Soluble in fats Soluble	365°	NMR 95%	2.0	1.480-1.486 1.052-1.058 (15.5°)		65th/N	

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1543	Phenylethyl anthranilate 2-Phenylethyl 2-aminobenzoate 2-Phenylethyl anthranilate; beta-Phenethyl o-aminobenzoate; Phenethyl anthranilate	2859 09.723 133-18-6	C15 H15 N O2 241.29	Fused, colourless to pale yellow-amber crystalline mass Neroli, grape undertone	Insoluble in water Soluble	-	IR 98%	-	-	mp: 40°	65th/N	
1544	beta-Naphthyl anthranilate Naph-2-yl 2-aminobenzoate 2-Naphthyl o-aminobenzoate; 2-Naphthyl anthranilate	2767 09.801 63449-68-3	C17 H13 N O2 263.30	Colourless to pale straw Coloured liquid Mild floral aroma	Insoluble in water Soluble	340°	NMR 98%	2.0	1.531-1.539 1.300-1.308		65th/N	
1545	Methyl N-methylantranilate Methyl N-methyl-2-aminobenzoate Dimethyl anthranilate; 2-Methylamino methyl benzoate; Methyl 2-methylaminobenzoate	2718 09.781 85-91-6	C9 H11 N O2 165.19	Pale yellow liquid with bluish fluorescence Grape-like aroma	Insoluble in water; Slightly soluble in glycerol; Soluble in oils Soluble	256°	NMR 98%	1.0	1.577-1.583 1.124-1.132		65th/R	
1546	Ethyl N-methylantranilate Ethyl N-methyl-2-aminobenzoate Ethyl 2-(methylamino)benzoate	4116 09.765 35472-56-1	C10 H13 N O2 179.22	Yellowish solid Fruity mandarin type aroma	Very slightly soluble in water Soluble	-	NMR 95%	1.0	-	mp: 36°	65th/N,C	
1547	Ethyl N-ethylantranilate Ethyl N-ethyl-2-aminobenzoate Ethyl o-(ethylamino)benzoate	4115 09.764 38446-21-8	C11 H15 N O2 193.25	Yellowish solid Fruity grape aroma	Very Slightly soluble in water Soluble	-	NMR 95%	1.0	-	mp: 74°	65th/N,C	
1548	Isobutyl N-methylantranilate 2-Methylpropyl N-methyl-2-aminobenzoate Isobutyl 2-(methylamino)benzoate	4149 09.769 65505-24-0	C12 H17 N O2 207.27	Yellowish solid Fruity grapefruit aroma	Very slightly soluble in water Soluble	-	NMR 95%	1.0	-	mp: 70°	65th/N,C	
1549	Methyl N-formylantranilate Methyl N-formyl-2-aminobenzoate Methyl 2-(formylamino)benzoate	4171 09.650 41270-80-8	C9 H9 N O3 179.18	Yellowish solid Fruity grape type aroma	Slightly soluble in water Soluble	-	NMR 95%	2.0	-	mp: 53°	65th/N,C	
1550	Methyl N-acetylantranilate Methyl N-acetyl-2-aminobenzoate Methyl 2-(acetylamino)benzoate; Methyl 2- acetamidobenzoate; o-Acetilamidobenzoic acid methyl ester	4170 09.649 2719-08-6	C10 H11 N O3 193.20	White to light yellow crystals Fruity grape aroma	Slightly soluble in water Soluble	-	NMR 95%	-	-	mp: 98-101°	65th/N,C	
1551	Methyl N,N-dimethylantranilate Methyl N,N-dimethyl-2-aminobenzoate Methyl 2-(dimethylamino)benzoate	4169 09.648 10072-05-6	C10 H13 N O2 179.22	Yellowish liquid to solid Fruity orange leaf type aroma	Very slightly soluble in water Soluble	141° (15 mm Hg)	NMR 95%	1.0	1.551-1.557 1.093-1.099	mp: 18°	65th/N,C	

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1552	N-Benzoylanthraniilic acid	4078	C14 H11 N O3	White solid	Slightly soluble in water	-	NMR IR MS	3.0	-	mp: 183°	65th/N,C	
	2-(Benzo[amino]benzoic acid	579-93-1	241.25	fruity aroma	Soluble		99%		-			
	2-Benzoylamino benzoic acid; Dianthramid B;											
	2-Carboxybenzanilide; N-(2-Carboxyphenyl)benzamide											
1553	Trimethylloxazole	13.169	C6 H9 N O	Yellowish liquid	Insoluble in water	134°	NMR	-	1.438-1.446		65th/N,C	
	2,4,5-Trimethyloxazole	20662-84-4	111.14	Boiled beef aroma	Soluble		95%		0.956-0.964			
1554	2,5-Dimethyl-4-ethyloxazole	13.118	C7 H11 N O	Yellowish liquid	Insoluble in water	150°	NMR	-	1.441-1.447		65th/N,C	
	2,5-Dimethyl-4-ethyloxazole	30408-61-8	125.17	Burnt roasted aroma	Soluble		95%		0.957-0.963			
1555	2-Ethyl-4,5-dimethyloxazole	3672	C7 H11 N O	Colourless liquid	Insoluble in water; Slightly soluble in oils	137° (732 mm Hg)	NMR	-	1.454-1.460		65th/N,C	
	4,5-Dimethyl-2-ethyloxazole	13.091	125.17	Burnt roasted aroma	Soluble		99%		1.474-1.480			
1556	2-Isobutyl-4,5-dimethyl-oxazole	26131-91-9	C9 H15 N O	Yellowish liquid	Insoluble in water	227°	NMR	-	1.439-1.447		65th/N,C	
	2-Isobutyl-4,5-dimethyloxazole		153.22	Burnt roasted aroma	Soluble		95%		0.910-0.916			
1557	2-Methyl-4,5-benzo-oxazole	13.154	C8 H7 N O	Yellowish liquid	Insoluble in water	199°	NMR	-	1.544-1.550		65th/N,C	
	2-Methyl-4,5-benzo-oxazole	95-21-6	133.15	Roast burnt aroma	Soluble		95%		1.109-1.115			
1558	2-Methylbenzoxazol											
	2,4-Dimethyl-3-oxazoline	13.115	C5 H9 N O	Yellowish liquid	Insoluble in water	141°	NMR	-	1.431-1.437		65th/N,C	
1559	2,4,5-Trimethyl-delta-3-oxazoline	77311-02-5	99.13	Boiled beef aroma	Soluble		95%		1.002-1.008			
	2,4,5-Trimethyl-4delta-3-oxazoline	3525	C6 H11 N O	Yellow orange liquid	Soluble in water, propylene glycol; Insoluble in most fixed oils	96-97°	NMR	-	1.414-1.435	SC: Trimethyloxazole	65th/N,T	RI range; SG range
1560	Allyl isothiocyanate	13.039	113.16	Powerful, musty, slight green, wood, nut aroma	Soluble		94%		0.911-0.932			
	Allyl isosulfocyanate; Allyl thiocarbonimide; 2-Propenyl isothiocyante	22694-96-8										
1561	Butyl isothiocyanate	2034	C4 H5 N S	Colourless or pale yellow liquid	Slightly soluble in water; Soluble in ether	150-151°	IR	1.0	1.524-1.531		65th/N	
	Butyl mustard oil	12.025	99.15	Very pungent, irritating aroma	Soluble		98%		1.013-1.020			
	Butyl isothiocyanate	57-06-7										
	Butyl isothiocyanate											
1561	Butyl isothiocyanate	4082	C5 H9 N S	Yellowish liquid	Insoluble in water	167°	NMR	1.0	1.492-1.498		65th/N,C	
	Butyl mustard oil	12.107	115.20	Strong irritating green aroma	Soluble		95%		0.952-0.958			

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1571	beta-Ionone epoxide 4-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)but-3-en-2-one beta-Ionone 5,6-epoxide; 4-(1,2-Oxido-2,6,6-trimethylcyclohexyl)-3-buten-2-one; 4-(2,6,6-Trimethyl-1,2-epoxycyclohexyl)-3-buten-2-one	4144 07.170 23267-57-4	C13 H20 O2 208.30	Colourless solid Sweet berry aroma	Insoluble in water Soluble	-	NMR MS 95%	-	-	mp: 48°	65th/N,C	
1572	cis-Carvone-5,6-oxide 5,6-Epoxy-p-menth-8-en-2-one cis-Carvone oxide	4084 16.042 18383-49-8	C10 H14 O2 166.22	Colourless liquid Sweet spicy aroma	Insoluble in water Soluble	105° (5 mm Hg)	NMR MS 95%	1.0	1.481-1.487 1.027-1.033		65th/N	
1573	Epoxyoxophorone 2,3-Epoxy-2,6,6-trimethyl-1,4-cyclohexanedione 3,5,5-Trimethyl-2,3-epoxycyclohexane-1,4-dione	4109 16.051 38284-11-6	C9 H12 O3 168.19	Colourless solid Sweet camphourous aroma	Insoluble in water Soluble	-	NMR 95%	-	-	mp: 157°	65th/N,C	
1574	Piperitenone oxide 1,2-Epoxy-p-menth-4(8)-en-3-one (1S)-Lippione; (+)-Rotundifolone	4199 16.044 35178-55-3	C9 H14 O2 166.22	Colourless solid Herbaceous minty aroma	Soluble in water Soluble	-	NMR MS 95%	1.0	-	mp: 25°	65th/N	
1575	beta-Caryophyllene oxide 4,5-Epoxy-4,12,12-trimethyl-8-methylene-bicyclo[8.2.0]dodecane Caryophyllene oxide; Caryophyllene epoxide	4085 16.043 1139-30-6	C15 H24 O 220.36	Colourless solid Sweet fruity aroma	Insoluble in water Soluble	-	NMR MS 95%	-	-	mp: 61°	65th/N	
1576	Ethyl 3-phenylglycidate Ethyl 3-phenyl-2,3-epoxypropionate Ethyl phenylglycidate; Ethyl alpha,beta-epoxy-beta-phenylpropionate	2454 16.018 121-39-1	C11 H12 O3 192.21	Clear, Colourless to pale yellow liquid Strong fruity aroma suggestive of strawberry	Insoluble in water; Soluble in ether, oils	96° (0.5 mm Hg)	IR 98%	2.0	1.516-1.521 1.120-1.125		65th/R	
1577	Ethyl methylphenylglycidate Ethyl 2,3-epoxy-3-methyl-3-phenylbutanoate Ethyl 3-methyl-3-phenylglycidate; Strawberry aldehyde	2444 16.015 77-83-8	C12 H14 O3 206.24	Colourless to pale yellow liquid Strong fruity aroma suggestive of strawberry	Insoluble in water, glycerol; Soluble in propylene glycol, fixed oils	272-275°	IR 98%	2.0	1.504-1.513 1.086-1.096		65th/R	
1578	Ethyl methyl-p-tolylglycidate Ethyl 2,3-epoxy-3-(4-methylphenyl)butanoate Ethyl methyl-p-methylphenylglycidate	3757 16.040 74367-97-8	C13 H16 O3 220.27	Colourless or pale straw Coloured viscous liquid Mild deep fruity, slightly floral aroma	Insoluble in water; Soluble in ether, oils	123-125°	NMR 96%	2.0	1.523-1.529 1.081-1.087 (20°)		65th/N	

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1579 Ethylamine Ethylamine 1-Aminoethane		4236 11.015 75-04-7	C2 H7 N 45.08	Colourless to yellow gas Ammonia fishy aroma	Soluble in water Soluble	17°	MS 95%	-	- 0.682-0.686 (10°)	mp: -81°	65th/N,C	
1580 Propylamine Propylamine 1-Aminopropane; Monopropylamine		4237 11.004 107-10-8	C3 H9 N 59.11	Colourless to yellow liquid Aggressive ammonia aroma	Soluble in water Soluble	48°	MS 95%	-	1.384-1.390 0.714-0.720		65th/N,C	
1581 Isopropylamine 2-Aminopropane 1-Amino-2-methylethane; 1-Methylethylamine; 2-Propylamine		4238 11.018 75-31-0	C3 H9 N 59.11	Colourless to yellow liquid Fishy ammonia aroma	Soluble in water Soluble	34°	MS 95%	-	1.367-1.373 0.687-0.693		65th/N,C	
1582 Butylamine Butylamine 1-Aminobutane; N-Butylamine		3130 11.003 109-73-9	C4 H11 N 73.14	Colourless liquid, tends to yellow on standing Ammoniacal aroma	Soluble in water Soluble	78°	NMR 99%	-	1.398-1.404 0.732-0.740		65th/N	
1583 Isobutylamine 2-Methylpropylamine 1-Amino-2-methylpropane		4239 11.002 78-81-9	C4 H11 N 73.14	Colourless to yellow liquid Fishy cheesy aroma	Soluble in water Soluble	68°	MS 95%	-	1.391-1.397 0.731-0.737		65th/N,C	
1584 sec-Butylamine 1-Methylpropylamine 2-Aminobutane; 2-Butyl amine		4240 11.005 13952-84-6	C4 H11 N 73.14	Colourless to yellow liquid Fishy ammonia aroma	Soluble in water Soluble	63°	MS 95%	-	1.387-1.393 0.715-0.721		65th/N,C	
1585 Pentylamine Pentylamine 1-Aminopentane; Amylamine; Norleucamine		4242 11.021 110-58-7	C5 H13 N 87.16	Colourless to yellow liquid Fishy aroma	Soluble in water Soluble	103°	MS 95%	-	1.418-1.424 0.750-0.759		65th/N,C	
1586 2-Methylbutylamine 2-Methylbutylamine 1-Amino-2-methylbutane; 2-Methyl-1- butylamine; 2-Ethylpropylamine		4241 11.020 96-15-1	C5 H13 N 87.16	Colourless to yellowish liquid Fishy aroma	Soluble in water Soluble	96°	MS 95%	-	1.417-1.423 0.777-0.779		65th/N,C	
1587 Isopentylamine 3-Methylbutylamine I-iso-Aminopentane; iso-Amylamine; Isobutyl Carbamine; 3-Methylbutylamine		3219 11.001 107-85-7	C5 H13 N 87.16	Colourless or very pale straw-Coloured mobile liquid Ammoniacal aroma	Soluble in water, propylene glycol, glycerin, oils Soluble	95-97°	NMR 98%	-	1.405-1.411 0.747-0.753		65th/N	
1588 Hexylamine Hexylamine 1-Aminohexane		4243 11.016 111-26-2	C6 H15 N 101.19	Colourless to yellow liquid Fishy aroma	Soluble in water Soluble	130°	MS 95%	-	1.415-1.421 0.761-0.767		65th/N,C	

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1589 Phenethylamine	Phenethylamine beta-Phenylethylamine; 2-Aminoethylbenzene; 1-Amino-2-phenylethane	3220 11.006 64-04-0	C8 H11 N 121.18	Colourless to pale, slightly yellow liquid Fishy aroma	Soluble in water, ether Soluble	194-195°	NMR 95%	-	1.526-1.532 (25°) 0.961-0.967		65th/N	
1590 2-(4-Hydroxyphenyl)ethyl-amine	2-(4-Hydroxyphenyl)ethylamine 4-(2-Aminoethyl)phenol; p-(2-Aminoethyl)phenol; Tyramine	4215 11.007 51-67-2	C8 H11 N O 137.18	Colourless to yellow solid Sweet meaty aroma	Soluble in water Soluble	-	MS 95%	-	-	mp: 165°	65th/N,C	
1591 1-Amino-2-propanol	1-Amino-2-propanol Isopropanol amine; alpha-Aminoisopropyl alcohol; 1-Methyl-2-aminoethanol; 2-Hydroxy- 1-methylethanol	3965 78-96-6	C3 H9 N O 75.13	Colourless to faint yellow liquid Fishy aroma	Soluble in water Soluble	160°	NMR 95%	1.0	1.445-1.451 0.970-0.976		65th/N,C	
1593 Butyramide	Butyramide Butylamide	4252 16.049 541-35-5	C4 H9 N O 87.12	Yellowish solid Nuffy aroma	Soluble in water Soluble	-	NMR 95%	-	-	mp: 115°	65th/N,C	
1594 1,6-Hexalactam	1,6-Hexalactam 2-Azacycloheptanone; Caprolactam; Hexahydro-2H-azepin-2-one	4235 16.052 105-60-2	C6 H11 N O 113.16	Yellowish solid Amine, spicy aroma	Soluble in water Soluble	-	NMR 95%	-	-	mp: 70°	65th/N,C	
1595 2-Isopropyl-N,2,3-trimethylbutyramide	2-Isopropyl- N,2,3-trimethylbutanamide N,2,3-Trimethyl-2-isopropylbutanamide; Methyl diisopropyl propionamide; 6-Caprolactam	3804 16.053 51115-67-4	C10 H21 N O 171.28	White crystalline solid Cool minty aroma	Insoluble in water and fats; Soluble in diethyl ether, and hydrocarbons Soluble	-	NMR 99%	1.0	-	mp: 56-64°	65th/N,C	
1596 N-Ethyl (E)-2,(Z)-6-nonadienamide	N-Ethyl (2E,6Z)-nonadienamide	4113 608514-56-3	C11 H19 N O 181.28	Pale yellow to yellow viscous liquid Meaty spicy aroma	Sparsingly soluble in water Soluble	120° (0.6 mm Hg)	NMR IR MS 96%	3.0	1.484-1.493 0.910-0.920		65th/N,C	
1597 N-Cyclopropyl (E)-2,(Z)-6-nonadienamide	N-Cyclopropyl (2E,6Z)-nonadienamide	4087 608514-55-2	C12 H19 N O 193.29	Pale yellow low melting solid Meaty, Herb-like aroma	Sparsingly soluble in water Soluble	-	NMR IR 95%	-	-	mp: 33-37°	65th/N,C	

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1598	N-Isobutyl (E,E)-2,4-decadienamide	4148 18836-52-7	C ₁₄ H ₂₅ N O 223.36	Colourless off-white to yellow solid Spicy herb-type aroma	Insoluble in water Soluble	-	NMR IR MS 95%	5.0	- -	mp: 82-90°	65th/N,C	
	N-(2-Methylpropyl)-(2E,6Z)-nonadienamide (E,E)-N-(2-Methylpropyl)-2,4-decadienamide; Pellitorin; Pellitorine											
	Nonanoyl 4-hydroxy-3-methoxybenzylamide	2787 16.006 2444-46-4	C ₁₇ H ₂₇ N O ₃ 293.41	White powder or crystals odourless	Slightly soluble in water Soluble	-	NMR 96%	1.0	- -	mp 124-128°	65th/N	
	N-(4-Hydroxy-3-methoxybenzyl) nonanamide; Pelargonyl vanillylamide; N-Nonanoyl vanillylamide											
1600	Piperine	2909 14.003 94-62-2	C ₁₇ H ₁₉ N O ₃ 285.34	Virtually colourless white crystals Aroma reminiscent of pepper	Very slightly soluble in water; Soluble in ether, oils Soluble	-	NMR 97%	-	- -	mp: 128-130°	65th/N	
	1-(5-(3,4-Methylenedioxyphenyl)-1-oxo-2,4-pentadienyl)piperidine Piperoylpiperidine											
1601	N-Ethyl 2-isopropyl-5-methyl-cyclohexanecarboxamide	3455 16.013 39711-79-0	C ₁₃ H ₂₅ N O 211.35	White solid Slight menthol like cooling effect	Insoluble in water Soluble	-	NMR 98%	-	- -	mp: 91-93°	65th/N	
	N-Ethyl-2-isopropyl-5-methylcyclohexane carboxamide											
	N-Ethyl-p-menthane-3-carboxamide; Ethyl menthane carboxamide											
1602 (+/-),N,N-Dimethyl menthyl succinamide	N,N-Dimethyl N'-(3-menthyl) succinamide	4231 544714-08-1	C ₁₆ H ₃₀ N ₂ O ₂ 282.43	Clear yellow to orange liquid Cool minty aroma	Slightly soluble in water Soluble	380°	IR NMR 95%	3.0	1.522-1.530 0.965-0.975		65th/N,C	
	1-Pyrroline	3898 5724-81-2	C ₄ H ₇ N 69.10	Colourless liquid Pungent ammoniacal odour	Soluble in water Soluble	87-89°	NMR 99%	1.0	1.440-1.446 0.849-0.855		65th/N,C	
1603 1-Pyrroline	3,4-Dihydro-(2H)-pyrroline; Isopyrroline; 3,4-Dihydro-2H-pyrrole	4249 14.080 99583-29-6	C ₆ H ₉ N O 111.14	Colourless to yellow solid Fishy aroma	Soluble in water Soluble	-	MS 95%	-	- -	mp: 19°	65th/N,C	
	2-Acetyl-1-pyrroline											
1605 2-Propionylpyrroline	2-Propionyln-1-pyrroline 1-(3,4-dihydro-2H-pyrrol-5-yl)-1-propanone	4063 133447-37-7	C ₇ H ₁₁ N O 125.17	Yellowish liquid Fisly aroma	Soluble in heptane, triacetin Soluble	89-90° (1 mm Hg)	NMR IR MS 95%	-	1.446-1.452 0.979-0.985	[Material is provided as a 1% solution in vegetable oil triglyceride]	65th/N,C	

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1606 Isopentylidene isopentylamine	N-(3-Methylbutylidene)-3-methyl-1-butylamine N-Isoamylidene-isoamylamine; N-Isopentylideneisopentylamine	3990 11.017 35448-31-8	C10 H21 N 155.29	Clear, Colourless liquid Fishy aroma	Insoluble in water; Soluble in heptane, triacetin Soluble	145-148°	NMR IR MS 93%	-	1.422-1.428 0.768-0.774	SC: Diisopentylamine; 3- Methylbutylaldehyde	65th/N,C	
1607 Piperidine	Piperidine Hexahydropyridine; Hexazane; Pentamethylenimine	2908 14.010 110-89-4	C5 H11 N 85.15	Colourless to pale yellow liquid Ammoniacal, fishy, nauseating aroma	Soluble in water, ether Soluble	106°	IR 98%	-	1.450-1.454 0.858-0.862		65th/N	
1608 2-Methylpiperidine	2-Methylpiperidine 2-Pipecoline	4244 14.133 109-05-7	C6 H13 N 99.18	Colourless to yellow liquid Pepper-like aroma	Soluble in water Soluble	118°	MS 95%	-	1.442-1.448 0.838-0.844		65th/N,C	
1609 Pyrrolidine	Tetrahydropyrrole Tetramethylenamine; Azacyclopentane; Butylenimine	3523 14.064 123-75-1	C4 H9 N 71.12	Colourless liquid Penetrating amine-type aroma	Soluble in water, fats Soluble	87-89°	NMR IR 95%	-	1.440-1.446 0.847-0.853		65th/N	
1610 Trimethylamine	Trimethylamine	3241 11.009 75-50-3	C3 H9 N 59.11	Colourless gas Pungent fishy odour at low concentration	Soluble in water, ether Soluble	3-4°	NMR 98%	-	- 0.667-0.675 (4°)		65th/N	
1611 Triethylamine	Triethylamine	4246 11.023 121-44-8	C6 H15 N 101.19	Colourless to yellowish liquid Fishy aroma	Soluble in water Soluble	88°	MS 95%	-	1.395-1.401 0.724-0.730		65th/N,C	
1612 Tripropylamine	Tripropylamine Propyldi-n-propylamine; Tri-n-propylamine	4247 11.026 102-69-2	C9 H21 N 143.27	Colourless to fishy liquid Mild fishy aroma	Soluble in water Soluble	156°	MS 95%	-	1.411-1.417 0.754-0.760		65th/N,C	
1613 N,N-Dimethylphenethylamine	N,N-Dimethylphenethylamine (R)-N,N-[alpha]-Trimethylbenzylamine; (R)- N,N-Dimethyl- α -phenylethylamine	4248 11.014 19342-01-9	C10 H15 N 149.24	Colourless to yellow liquid Sweet fishy aroma	Soluble in water Soluble	183°	MS 95%	-	1.500-1.506 0.898-0.904		65th/N,C	
1614 Trimethylamine oxide	Trimethylamine oxide Trimethylamine N-oxide dihydrate	4245 11.025 1184-78-7	C3 H9 N O 75.11	Colourless to yellow solid Odourless	Soluble in water Soluble	-	MS 95%	-	- -	mp: 213°	65th/N,C	

