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Reply of General Office of National Health and Family Planning Commission of PRC to Issues on
Implementation of Standard for Use of Food Additives (GB 2760- 2014)

National Health and Family Planning Commission of PRC, May 29, 2015

GWBSPH (2015) No. 469

China National Food Industry Association:

Suggestions on Issues Regarding Transition Period of Implementation of National Food Safety Standard Standard for Use of Food Additives (ZGSX (2015) No. 23) has been received by us. With study and agreement with Ministry of Industry and Information Technology, AQSIQ and China Food and Drug Administration, we now reply to the suggestions as follows:

I. National Food Safety Standard Standard for Use of Food Additives(GB 2760- 2014) came into effect on May 24, 2015. Please strengthen propaganda and training and implement the standard in the industry.

II. With regard to the old-version labeling issues caused by modification for names of food additives, with study and the precondition of no impact on food safety, for food produced before June 30, 2016, the labels are allowed to use specified names of food additives, and the food can be sold during their shelf life; since June 30, 2016, food production enterprises shall label foods according to GB 2760- 2014.

It is hereby replied.

General Office, National Health and Family Planning Commission of PRC

May 27, 2015

Address: 1 Xizhimen Wainanlu Road, Xicheng District, Beijing

Postcode: 100044

Mailbox:  Tel: 010-68792114

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Technical Support: Statistics and Information Center, National Health and Family Planning Commission of PRC

Division of Food Safety Standards and Monitoring and Evaluation, National Health and Family Planning
Commission of PRC

Announcement of National Health and Family Planning Commission of PRC Concerning Approval of β -
Galactosidase as A New Variety of Food Additive

(2015 No. 1)

National Health and Family Planning Commission of PRC, January 29, 2015

2015 No.1

It is hereby announced.

According to Food Safety Law of the People's Republic of China and The Measures for Administration of New Varieties Of Food Additives, after examination and verification, now β -galactosidase is approved as a new variety of food additive; 6-Methyloctanal is approved as a new variety of food flavor ingredient; the range and amount of application for 11 food additives, nitrous oxide, arabicgum, monascus yellow, ascorbic acid (vitamin C), rosemary extract, dimethyl dicarbonate, aluminium potassium sulfate/aluminium ammonium sulfate, phosphoric acid, sodium pyrophosphate, sodium hexametaphosphate, rosemary extract, are extended.

It is hereby announced.

National Health and Family Planning Commission of PRC

January 23, 2015

Attachment 1

A Variety of New Food Additive, β -Galactosidase

No.	Enzyme	Source	Donor
1	β -galactosidase	Bifidobacterium bifidum	

A New Variety of Food Flavor Ingredient, 6-Methyloctanal

6-Methyloctanal

English Name: 6-methyloctanal

Function: food flavor ingredient (synthetic)

(I) Amount and Range of Application

It shall meet provisions on the use of food flavor ingredients in Appendix B of GB 2760.

(II) Quality Specification and Requirements

1. Production Process

6-Methyloctanal carrene is produced through catalytic oxidation reaction of the catalyzer 2,2,6,6-tetramethylpiperidinoxy in the solvent dichloromethane. After completion of the reaction, remove the solvent, and carry out vacuum distillation to get the colorless liquid product which is the food additive 6-methyloctanal.

2. Technical Requirements

2.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	colorless	place the samples in comparison tubes, and observe them by eyes.
state	colorless	
aroma	delicate fragrance	GB/T 14454.2

2.2 Physical and chemical indexes: physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
content, w /%	≥ 96	GB/T 11538
relative density (25°C/25°C)	0.825 - 0.829	GB/T 11540
refraction index (20°C)	1.422 - 1.427	GB/T 14454.4
acid value (on the basis of KOH)/ (mg/g)	< 4	GB/T 14455.5

Attachment 3

11 Varieties of Food Additives Including Nitrous Oxide Whose Range and Amount of Application Are Extended

Table 1 11 Variety of Processing Agent for the Food Industry Whose Range of Application and Application amount Are Extended

No.	Chinese Name	English Name	Function	Range of Application
1.	nitrous oxide	nitrous oxide	processing agent	processing for single cream and similar products manufacturing process for single cream (unsalted butter) and similar products

Table 2 10 Other Varieties of Food Additives Whose Ranges and Amount of Application Are Extended

No.	Name	Function	Food Category No.	Food Name	The largest application amount /(g/kg)	Note
1.	arabicgum	other	12.01	salt and its substitutes	use with an appropriate amount depending on production demands	
2.	monascus yellow pigment	colorant	10.02.01	spiced corned egg	use with an appropriate amount depending on production demands	
3.	ascorbic acid (vitamin C)	antioxidant	14.02.01	juice (syrup)	1.5	
4.	rosemary extract	antioxidant	12.10.01	solid compound seasoning	0.7	
5.	dimethyl dicarbonate	preservative	14.04.02.01	drinks for special use (including sports drink and nutrient drinks)	0.25	The application amount of solid drinks is increased according to the dilution factor.
6.	aluminium potassium sulfate, aluminium ammonium sulfate	swelling agent	06.05.02.01	vermicelli	use with an appropriate amount depending on production demands	The residual quantity of aluminium < 200mg/kg (dry sample, calculated on the basis of Al)
7.	phosphoric acid	acid regulator	15.02	compound wine	5.0	The largest application amount is calculated on the basis of phosphate radical (PO ₄ ³⁻).

8.	sodium pyrophosphate	antitackiness agent, humectant	09.04	cooked aquatic products (can be eaten directly)	5.0	It can be used singly or mixed with hexametaphosphate, and the largest application amount is calculated on the basis of phosphate radical (PO ₄ ³⁻).
9.	sodium hexametaphosphate	antitackiness agent, humectant	09.04	cooked aquatic products (can be eaten directly)	5.0	It can be used singly or mixed with hexametaphosphate, and the largest application amount is calculated on the basis of phosphate radical (PO ₄ ³⁻).
10.	rosemary extract (supercritical carbon dioxide extraction)	antioxidant	12.10.01	solid compound seasoning	0.7	
			12.10.02	semi-solid compound seasoning	0.3	
			12.10.03	liquid compound seasoning(12.03 and 12.04 not included)	0.3	

Announcement Concerning New Varieties of Food Additives Including Calcium Alginate

National Health and Family Planning Commission of PRC June 30, 2016

2016 No.8

According to Food Safety Law, the review body has organized experts to review the safety evaluation materials for and approved 10 new varieties of food additives including calcium alginate, extension of the range or amount of application for 19 food additives including L (+)-tartaric acid and 3 new varieties of food nutrient enhancers including magnesium L-threonate and extension of the application amount of the food nutrient enhancer of L-carnitine.

It is hereby announced.

Attachment:

1. 10 New Varieties of Food Additives Including Calcium Alginate
2. Extension of the Range or Amount of Application for 19 Varieties of Food Additives Including L (+)-Tartaric Acid
3. 3 New Varieties of Food Nutrient Enhancers Including Magnesium L-Threonate
4. Extension of the Application Amount of the Food Nutrient Enhancer of L-Carnitine

National Health and Family Planning Commission of PRC

June 15, 2015

Attachment 1

10 New Varieties of Food Additives Including Calcium Alginate

I. Calcium Alginate

English Name: Calcium alginate

Functional classification: thickening agent, stabilizer and coagulant

(I) Amount and Range of Application

Food Category No.	Food Name	the largest application amount/(g/kg)	Note
06.03.02	wheat flour products	5.0	
07.01	bread	5.0	

(II) Quality Specification and Requirements

1 Scope

The quality specification and requirements apply to calcium alginate, a food additive which is extracted from such brown alga plants as laminaria, macrocystis and ascophyllum.

2 Molecular Formula



3 Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	white to yellow	Place appropriate amount of samples on a clean, dry, white porcelain plate in a well-lighted environment with no odor, and observe their color, luster and state.
state	fibrous or granular powder	

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
calcium alginate content (on the basis of calcium oxide, and on a dry basis), w/%	8.0 ~ 13.0	A.3 in Appendix A
dry reduction, W/%	≤ 15.0	GB 5009.3 direct drying method
ash content (on dry basis), w/%	10.0 ~ 20.0	GB 5009.4 a

lead (Pb) /(mg/kg)	≤	4.0	GB 5009.12
arsenic (on the basis of As)/(mg/kg)	≤	2.0	GB 5009.11
^a The burning temperature is 700°C~800°C			

Appendix A

Test Method

A.1 General Provisions

Unless otherwise specified, under the quality specification and requirements, purity of all reagents shall be higher than that of the analytical reagent, and standard titration solutions in the test and standard solution,s preparations and products for for determination of impurities shall be prepared according to GB/T601, GB/T602 and GB/T603, and the test water shall meet provisions for Grade III water in GB/T 6682. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.2 Identification Test

A.2.1 Reagents and Materials

A.2.1.1 ethanol solution of 1,3-Dihydroxynaphthalene (10 g/L): Weigh 1g 1,3-Dihydroxynaphthalene, dissolve it in 100mL of absolute ethyl alcohol, mix them (use it right after it is ready).

A.2.1.2 hydrochloric acid

A.2.1.3 isopropyl ether

A.2.2 Identification

A.2.2.1 Solubility Test

The product is insoluble in water or organic solvents and soluble in alkaline solution or solution of a substance combined with calcium.

A.2.2.2 Identification of Alginate

Place 5mg sample in a test tube, add 5mL water to the tube, add 1mL newly prepared ethanol solution of 1,3-Dihydroxynaphthalene and 5mL concentrated hydrochloric acid to the tube, and shake up them. Heat the said mixture until it boils, slightly boils it for 3 min, cool it to a temperature of about 15°C, transfer it into a 30mL separating funnel, use 5mL water to wash the vessel, and put the cleaning water into the separating funnel. Add 5mL isopropyl ether, shake it, extract the ether layer, prepare blank control, and compare the isopropyl ether layer of the sample tube with that of the control tube. The isopropyl ether layer of the sample tube shall be dark purple.

A.3 Determination of Calcium Alginate Content

A.3.1 Method Abstract

Make incinerated calcium alginate react with acid to form soluble calcium salt, titrate calcium ions with the standard titration solution of ethylenediaminetetraacetic acid disodium salt and convert it into the content of calcium alginate (on the basis of calcium oxide, and on a dry basis)

A.3.2 Reagents and Materials

A.3.2.1 Potassium hydrate solution(2mol/L): precisely weigh 112g potassium hydrate, place it in 1000mL water, dissolve it and mix them.

A.3.2.2 Digestive liquid of mixed acids: mix nitric acid and perchloric acid with a mass ratio of 4:1 for use in the future.

A.3.2.3 triethanolamine solution(10%): measure 10mL triethanolamine, place it in 90mL water, and mix them.

A.3.2.4 Calcon-carboxylic acid (1%): Weigh 1g calcon-carboxylic acid (C21O7N2SH14), add 99g solid sodium chloride, grind it in a mortar, and place it in a brown wide-mouth bottle for future use.

A.3.2.5 the standard titration solution of ethylenediaminetetraacetic acid disodium salt (0.01mol/L): is prepared according to 4.15 of GB/T601-2002

A.3.3 Instrument and Equipment

A.3.3.1 Base buret (50mL)

A.3.3.2 Universal resistance furnace

A.3.4 Analysis Steps

A.3.4.1 Sample Digestion

Take a crucible together with its residue under the ash determination item, carefully add 25mL±5mL mixed acid digestive liquid in it, place it on a universal resistance furnace in a draught cupboard, heat it, and repeatedly add a small amount of mixed acid digestive liquid to it in the case of too less acid liquid until the digestive liquid is colorless and transparent. This moment there may be residual acid digestive liquid, so we shall continue to heat the digestive liquid to evaporate it; if there is less digestive liquid, we may repeatedly add 10mL±5mL to it and heat it slowly, and take it down when there is no white smoke rising any more; when it is cooled, transfer the digestive liquid in the crucible to a 250mL volumetric flask, repeatedly rinse the crucible with a small amount of water, continuously test it with PH test paper until the cleaning water is not acidic obviously any more, and merge the cleaning water into the volumetric flask and produce the constant volume.

Take mixed acid digestive liquid with the same amount with that of digestive test sample, and conduct reagent blank test according to the above operation.

A.3.4.2 Determination

A.3.4.2.1 Test Sample and Blank Titration

Take 5mL sample digestive liquid and blank into a 250mL triangular flask, add 50mL distilled water into the flask, mix them, add 5mL 2mol/L potassium hydrate solution into it, add 1mL 10% triethanolamine into it, add 0.1g calred, and shake up them. Make the solution mixed uniformly, with continuous shaking, titrate with 0.01 mol/L standard titration solution of ethylenediaminetetraacetic acid disodium salt; when the claret solution turns into solid blue it is the end point.

A.3.4.2.2 Result Calculation

The mass fraction of calcium alginate content (on the basis of calcium oxide, and on a dry basis) is calculated according to Formula (A.1):

$$w_2 = \frac{c \times (V - V_0) \times M \times 250}{m \times 50 \times 1000(1 - w_1)} \times 100\% \dots\dots\dots(A.1)$$

Where:

m- the mass of sample, with a unit of gram (g);

C- concentration of the standard titration solution of ethylenediaminetetraacetic acid disodium salt, in terms of liter/mol (mol/L);

C- concentration of the standard titration solution of ethylenediaminetetraacetic acid disodium salt, in terms of liter/mol (mol/L);

V- the volume of the standard titration solution of ethylenediaminetetraacetic acid disodium salt consumed by titration test sample, in terms of milliliter (mL);

V- the volume of the standard titration solution of ethylenediaminetetraacetic acid disodium salt consumed by titration sample, in terms of milliliter (mL);

V_0 - the volume of the standard titration solution of ethylenediaminetetraacetic acid disodium salt consumed by titration blank, in terms of milliliter (mL);

V_0 - the volume of the standard solution of ethylenediaminetetraacetic acid disodium salt consumed by titration blank, in terms of milliliter (mL);

250 - the volume of volumetric flask, in terms of milliliter (mL);

250 - volume of volumetric flask, in terms of milliliter (mL);

5- volume of removed sample digestive liquid, in terms of milliliter (mL);

M- the molar mass of calcium oxide, in terms of gram/mol (g/mol) [$M(\text{CaO})=56.08$];

1000- conversion factor;

W_1 – the mass fraction of dry reduction of test sample, in terms of percentage (%).

W_1 – mass fraction of dry reduction of sample, in terms of percentage (%).

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 3.0% of the arithmetic mean value.

II. Quillaia Extract

English name: quillaia extract

Functional classification: emulsifier

(I) Amount and Range of Application

Food Category No.	Food Name	Largest Application amount (g/kg)	Note
14.02.03	drinks of fruit and vegetable juice (syrup)	0.05	it is calculated on the basis of saponin, and the application amount of solid drinks is increased according to the dilution factor.
14.03	protein drinks	0.05	it is calculated on the basis of saponin, and the application amount of solid drinks is increased according to the dilution factor.
14.04	sodas	0.05	it is calculated on the basis of saponin, and the application amount of solid drinks is increased according to the dilution factor.
14.07	drinks for special use	0.05	it is calculated on the basis of saponin, and the application amount of solid drinks is increased according to the dilution factor.
14.08	flavored drinks	0.05	it is calculated on the basis of saponin, and the application amount of solid drinks is increased according to the dilution factor.

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive quillaia extract which is derived from barks, trunks or branches of *Quillajasaponaria* Molina which are grinded, extracted with hydrosolvent and then subject to processing procedures of clarification and refinement. Commercialized quillaia extract products can be liquid or powder, and powdery products can contain such carriers as lactose, maltol, maltodextrin, dextrin and dextran. Liquid products can be stored with sodium benzoate and ethanol.

2. Product classification

Quillaia extract can be classified into two product types, Type 1 and Type 2, depending on the range of saponin content.

3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Index	Test Method
state	liquid or powdery	place an appropriate amount of test samples on a clean, dry, white porcelain plate in natural light, observe their color, luster and state and smell them.
color and luster	light brown to brown	
odor	odor specific to quillaia extract	
impurity	no visible foreign impurities	

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index		Test Method	
	Type 1	Type 2		
water content, W/% (limited to powder products)	≤	6		GB 5009.3 Karl Fjscher method
loss on drying, w/% (limited to liquid products)		50~80	50~90	GB 5009.3 direct drying method
pH		3.7~5.5		A.3 in Appendix A
ash content (on dry basis), w/%	≤	14	5	GB 5009.4 ^a

tannic acid (on a dry basis), w/%	≤	8		A.4 in Appendix A
saponin content(on a dry basis), w/%		20~26	65~90	A.5 in Appendix A
lead (Pb) / (mg/kg)	≤	2.0		GB 5009.12
<p>a in the case of powdery sample, use 1.0 g; in the case of liquid sample, use the residues after loss on drying.</p>				

Appendix A

Test Method

A.1 General Provisions

Unless otherwise specified, under the quality specification and requirements, chromatographic pure reagents and Grade I water specified in GB/T 6682 shall be used in chromatographic analysis, purity of all agents in other tests are consistent with that of analytical reagent, and the test water shall meet provisions for Grade III water in GB/T 6682.

A.2 Identification Test

A.2.1 The test sample shall have strong water solubility and are not soluble in ethanol, acetone, methanol or butanol.

A.2.2 Weigh 0.5g powdery sample, dissolve it in 9.5g water (or measure 1mL liquid sample and dissolve it in 9mL water), add it into a 1000mL graduated cylinder with 350mL water, cover the graduated cylinder, shake the cylinder for 30 times, then keep the cylinder still, record the foam volume (mL) 30min later, and the volume of foam shall reach 150mL.

A.2.3 According to the high performance liquid chromatography for saponin content (A.5), the retention time of main peak shall be consistent with that of the main peak of main constituents of saponin standard (QS-18).

A.2.4 Observe the solution of powdery sample in A.2.2, and there shall be no turbidity in the solution. Place the solution in the 520nm wavelength, determine the absorbance which shall be lower than 1.2 (the method is only limited to powdery sample).

A.3 Determination of pH

A.3.1 Instrument and Equipment

pH meter

A.3.2 Operation Steps

A.3.2.1 Adjustment of pH meter

Debug and calibrate the pH meter according to the pH meter instructions.

A.3.2.2 Determination

Weigh an appropriate amount of test sample, and prepare it into 4% (W%) quillaia extract solution for test with water. Rinse the electrode probe with water, absorb the water on the probe with filter paper, insert the probe into the solution for test, adjust the temperature adjustor, make the indicating temperature of the instrument identical to that of the solution, read the data after it is stable.

A.4 Determination of Tannic Acid

A.4.1 Reagents and Materials

A.4.1.1 Acetic acid

A.4.1.2 Polyvinylpyrrolidone

A.4.2 Instrument and Equipment

A.4.2.1 Electrothermal Constant-temperature Dry Box

A.4.2.2 Centrifuge

A.4.3 Analysis Steps

Weigh 3.0g powdery sample (or liquid sample containing 3.0g solid substance (converted with the value of loss on drying)), with a precise of 0.01 g, dissolve it in 250mL water, adjust the pH to 3.5 with acetic acid, weigh 25mL solution, dry it for 5h at 105°C, cool it, weight it (m_1). Measure 50mL solution, mix it with 360mg polyvinylpyrrolidone, stir them for 30min at the room

temperature, and then centrifugate at the rotation rate of 3000 rpm. Collect the clear liquid of the upper layer, dry it at 105°C, cool it, weigh it (m₂).

A.4.4 Result Calculation

The mass fraction of tannic acid (on a dry basis), W, is calculated according to Formula (A.1):

$$w = \frac{m_1 - \frac{m_2}{2}}{m_1} \times 100\% \dots\dots\dots(A.1)$$

Where:

m₁- mass of the solution after being dried before addition of polyvinylpyrrolidone, in terms of gram (g);

m₂- mass of the solution after being dried after addition of polyvinylpyrrolidone, in terms of gram (g);

2- conversion factor.

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 5.0%.

A.5 Determination of Saponin Content

A.5.1 Principle of Determination Method

Separate saponin's main constituents QS-7, QS-17, QS-18 and QS-21 with high performance liquid chromatography, and the total level of saponin in quillaia extract is calculated on the basis of the total amount of QS-7, QS-17, QS-18 and QS-21.

A.5.2 Reagents and Materials

A.5.2.1 Saponin standard (or similar to saponin standard with known saponin content)

A.5.2.2 Trifluoroacetic acid

A.5.2.3 HPLC-level acetonitrile

A.5.2.4 filter membrane with 0.2µm pore diameter

A.5.3 Instrument and Equipment

High performance liquid chromatograph: is equipped with UV detector

A.5.4 Reference Chromatographic Conditions

A.5.4.1 chromatographic column: C4 bonded silica chromatographic column (length: 4.6x250 mm, pore diameter: 300Å, particle diameter: 5µm) or other equivalent chromatographic column

A.5.4.2 Column temperature: room temperature

A.5.4.3 Sample injection mode: gradient injection

A.5.4.4 Mobile phase:

mobile phase A: dissolve 0.15% trifluoroacetic acid in HPLC-level water

mobile phase B: dissolve 0.15% trifluoroacetic acid in HPLC-level acetonitrile

A.5.4.5 Flow rate: 1.0 mL/min

A.5.4.6 Detection wavelength: 220nm

A.5.4.7 For elution gradient conditions, see Table A.1.

Table A.1 Elution Gradient Conditions

time (min)	mobile phase A%	mobile phase B%	flow rate (mL/min)
0	70	30	1.0
40	55	45	1.0
45	70	30	1.0

A.5.4.8 Injection volume: 20μL

A.5.5 Analysis Steps

A.5.5.1 Preparation of Sample Solutions

A.5.5.1.1 powdery sample

Weigh 0.5g test sample with a precision of 0.001g, dissolve it in 9.5g water and filter it with a filter membrane with the pore diameter of 0.2 μm to obtain the prepared test sample solution of about 10mL.

A.5.5.1.2 Liquid Sample

Weigh 1.0g test sample with a precision of 0.001g, dilute it with 9mL water and filter it with a filter membrane with the pore diameter of 0.2 μm to obtain the prepared test sample solution of about 10mL.

A.5.5.2 Preparation of Standard Solution

Weigh 1.5g saponin standard with a precision of 0.001g, dissolve them in 100mL water and filter them with a filter membrane with the pore diameter of 0.2 μm.

A.5.6 Result Calculation

A.5.6.1 The saponin content in the solution prepared with the above sample preparation method, C_{sap} , in terms of milligram/milliliter (mg/mL), is calculated according to Formula (A.2)

$$C_{sap} = \frac{A_{\text{样品}}}{A_{\text{标准}}} \times C_{\text{标准}} \dots\dots\dots(A.2)$$

Where:

$C_{\text{标准}}$ - aponin concentration in the standard, in terms of milligram/milliliter (mg/mL)(for example, $C_{\text{标准}}=13.5$ mg/mL means: the saponin content in 1.5g standard sample is 90%);

Saponin concentration in the standard, in terms of milligram/milliliter (mg/mL) (for example, $C_{\text{标准}}=13.5$ mg/mL means: the saponin content in 1.5g standard sample is 90%);

$A_{\text{样品}}$ - the sum of peak areas of 4 main saponin constituents, QS-7, QS-17, QS-18 and QS-21, is as shown in the chromatogram of Appendix B (the main peak of saponin will appear after the appearance of polyphenol's main peak, and see Fig. B.2 in Appendix B).

$A_{\text{标准}}$ - the sum of peak areas of 4 main saponins in the standard.

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 2.0%;

A.5.6.2 The mass fraction of saponin in test sample, W_1 , is calculated according to Formula (A.3)

$$w_1 = \frac{C_{sap} \times v_{\text{样品}}}{m_{\text{样品}}} \times 100\% \dots\dots\dots(A.3)$$

Where:

C_{sap}- saponin content in the test sample solution, in terms of milligram/milliliter (mg/mL);

Saponin content in the test sample solution, in terms of milligram/milliliter (mg/mL);

m_{样品}- the sample mass taken from the prepared samples, in terms of milligram (mg);

v_{样品}- the volume of prepared test sample solution, in terms of milliliter (mL).

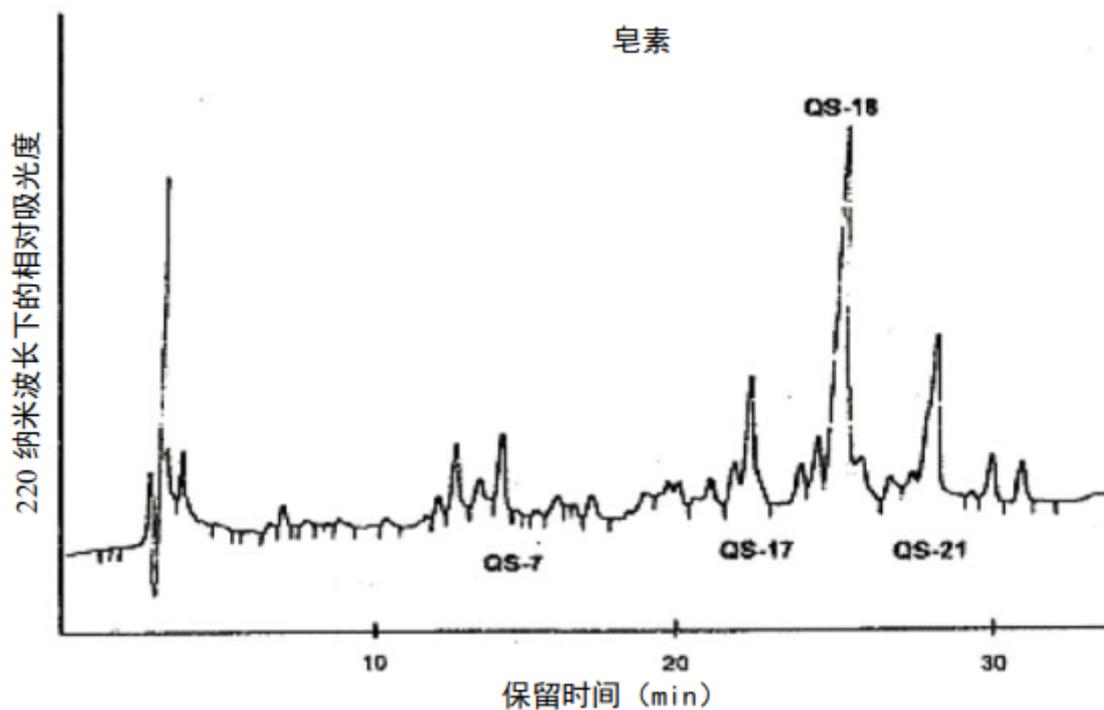
The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 2.0%.

Appendix B

High Performance Liquid Chromatogram of Quillaia Extract

B.1 Chromatogram Of Saponin Standard

For the chromatogram of saponin standard, see Fig. B.1.



皂素

saponin

220 纳米波长下的相对吸光度

relative absorbance in the case of 220nm wavelength

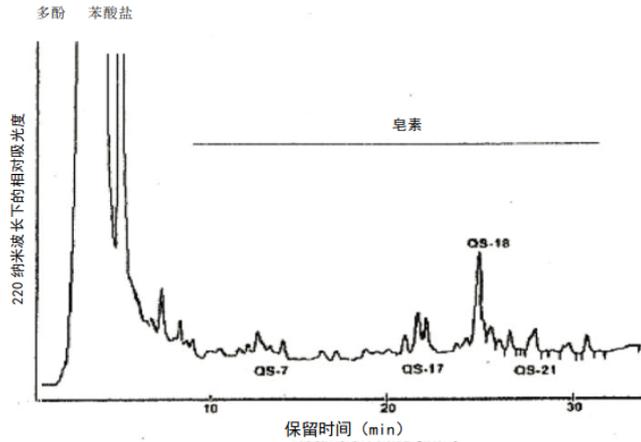
保留时间 (分钟)

retention time (min)

Fig. .1 Chromatogram Saponin Standard (15mg/mL dry matter content, is equivalent to 13.5mg/mlsaponin content)

B.2 Chromatogram of Quillaia Extract (Type 1)

For the chromatogram of quillaia extract (type 1), see Fig. B.2. For the chromatogram of quillaia extract (type 2), please refer to the chromatogram of quillaia extract (type 1).



多酚/polyphenol

苯酸盐/benzoates

Fig.B.2 Chromatogram of Quillaia Extract (Type 1) (about 55mg/mL dry matter content)

III. Phosphoric Acid (Wet Method)

English Name: Phosphoric acid (Wet process)

Functional classification: acidity regulator

(I) Amount and Range of Application

Food Category No.	Food Name	the largest application amount/(g/kg)	Note
14.04.01	Coke-type sodas	5.0	On the basis of PO_4^{3-}

(II) Quality Specification and Requirements

1 Scope

The quality specifications and requirements apply to the food additive phosphoric acid (wet method) produced through solvent extraction, impurity removal and refinement.

2. Molecular Formula and Relative Molecular Mass

2.1 Molecular Formula

H_3PO_4

2.2 Relative Molecular Weight

97.99

3 Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	colorless, transparent, or with a light color	Take appropriate amount of test samples, place them in clean, dry comparison tubes, and observe their color, luster and state.
State	thick liquid	

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item		Index	Test Method
phosphoric acid(H ₃ PO ₄) content, W/%		75.0 ~86.0	GB 1886.15
chroma, Hazen	≤	20	GB/T 605
total organic carbon (on the basis of C), W/%	≤	0.006	A.4 in Appendix A
easily oxidized substances (on the basis of H ₃ PO ₃),W/%	≤	0.008	GB 1886.15
sulfate (on the basis of SO ₄), W/%	≤	0.01	A.5 in Appendix A
chloride (on the basis of Cl),W/%	≤	0.0007	GB/T 2091
ferrum (on the basis of Fe),W/%	≤	0.001	A.6 in Appendix A
arsenic (on the basis of As)/ (mg/kg)	≤	0.5	GB 1886.15
fluoride (on the basis of F)/ (mg/kg)	≤	10	GB 1886.15
lead (Pb) (w) / (mg/kg)	≤	2.0	SN/T 2049
cadmium (on the basis of Cd) / (mg/kg)	≤	2.0	SN/T 2049
mercury (on the basis of Hg), w/%	≤	0.0001	A.7 in Appendix A
heavy metal (on the basis of Pb)/ (mg/kg)	≤	5.0	GB 1886.15

Appendix A

Test Method

A.1 Safety Instructions

A part of reagents used in the test methods in the quality specifications are corrosive, and operation personnel shall be cautious! If it is splashed onto skin, rinse it with water immediately, and seek medical help immediately in the case of severe injury. When an inflammable substance is used, heating with open fire is forbidden.

A.2 General Provisions

Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

All reagents and water used under the quality specifications are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard solutions, preparations and products for determination of impurity are prepared according to provisions of GB/T 601, GB/T602 and GB/T603, unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.3 Identification Test

A.3.1 Reagents and Materials

A.3.1.1 Sodium hydrate solution: 40 g/L

A.3.1.2 Silver nitrate solution: 10 g/L

A.3.1.3 Phenolphthalein indicator solution: 10 g/L

A.3.2 Identification Method

Weigh about 1g test sample, place it in a 100mL flask, add 10mL water and a drop of phenolphthalein into the flask, adjust the solution to be neutral with sodium hydrate solution, add drops of silver nitrate solution to generate yellow precipitate which is soluble in diluted nitric acid (5%) or ammonia water.

A.4 Determination of Total Organic Carbon

A.4.1 Method Abstract

The total organic carbon in the test sample is oxidized to carbon dioxide with the effect of sulfate and ultraviolet light, and determine the total carbon content with a TOC (total organic carbon) analyzer.

A.4.2 Reagents and Materials

A.4.2.1 distilled water or high purity water without carbon dioxide

A.4.2.2 Standard solution of potassium acid phthalate: 1mL solution contains 1.0mg carbon (C), weigh 2.1254g standard reagent (potassium acid phthalate) which is dried for 2h at 120°C, add water to dissolve it, transfer it into a 1000mL volumetric flask, distill it to the graduation with water, and e up.

A.4.2.3 high-purity nitrogen: purity 99.999%

A.4.3 Instrument and Equipment

TOC (total organic carbon) analyzer.

A.4.4 Analysis Steps

A.4.4.1 Drawing of Working Curve

Respectively take 0.00mL, 1.00mL, 2.00mL, 3.00mL and 4.00mL standard solutions of potassium acid phthalate, place it in a 100mL volumetric flask, titrate to the graduation with high-purity water, and shake up them. Draw a working curve with TOC (total organic carbon) analyzer.

A.4.4.2 Determination

Weigh about 3g sample, with a precision of 0.0001g. Place it in a 100mL volumetric flask, dilute it to the graduation, and shake up them. Determine it with a TOC (total organic carbon) analyzer.

A.4.5 Result Calculation

The mass fraction of total organic carbon (on the basis of C), w_1 is calculated according to Formula (A.1):

The mass fraction of total organic carbon (on the basis of C) is calculated according to Formula (A.1):

$$w_1 = \frac{m_1 \times 10^{-3}}{m} \times 100 \dots\dots\dots(A.1)$$

Where:

m_1 - the determined mass of total organic carbon in test solution, in terms of milligram (mg);

m - the mass of sample, with a unit of gram (g);

Take the arithmetic mean value of parallel determination results as the determination result. The absolute difference of parallel determination results shall not exceed 0.0002%.

A.5 Determination of Sulfate

A.5.1 Method Abstract

The energy needed during the process that atoms jump from a lower energy level to a higher energy level, is high-frequency electromagnetic energy generated by RF generator, which pass through coil coupling to the quarter bend with argon flow and thus produce plasma. Measure the light intensity of the characteristic spectrum-line emitted by the standard solution and then measure the intensity of the characteristic spectrum-line to be determined so as to determine the concentration of the solution to be determined.

A.5.2 Reagents and Materials

A.5.2.1 Nitric acid solution: 1+1

A.5.2.2 Standard solution: the content of SO_4 standard stock solution is 1mg/mL. Prepare the standard solution with a desired concentration with pure water temporarily.

A.5.3 Instrument and Equipment

Inductively coupled plasma optical emission spectrometry

A.5.4 Analysis Steps

Weigh about 3~4g test sample, with a precision of 0.0002g. place it in a 100mL volumetric flask, add 5mL nitric acid solution, dilute it to the graduation, and shake up them. Select SO_4 curve on the inductively coupled plasma optical emission spectrometry to conduct determination.

A.5.5 Result Calculation

The mass fraction of sulfate (on the basis of SO_4), w_2 , is calculated according to Formula (A.2)

$$w_2 = \frac{m_1 \times 10^{-4}}{m} \times 100 \dots\dots\dots(A.2)$$

Where:

m₁- the instrument's readout, in terms of milligram per liter (mg/L);

m- the mass of sample, with a unit of gram (g);

Take the arithmetic mean value of parallel determination results as the determination result. The absolute difference of parallel determination results shall not exceed 0.0005%.

A.6 Determination of Ferrum (on the basis of Fe)

A.6.1 Reagents and Materials

A.6.1.1 Nitric acid solution: 1+1

A.6.1.2 standard solution: the content of Fe standard stock solution is 1mg/mL. prepare the standard solution with a desired concentration with pure water temporarily.

A.6.2 Instrument and Equipment

Inductively Coupled Plasma Optical Emission Spectrometry. Inductively coupled plasma optical emission spectroscopy.

A.6.3 Analysis Steps

Weigh 3~4g test sample, with a precision of 0.0002g. Place it in a 100mL volumetric flask, add 5mL nitric acid solution, dilute it to the graduation with water, shake up them. Select Fe curve on the inductively coupled plasma optical emission spectrometry to conduct determination.

A.6.4 Result Calculation

The mass fraction of Fe (on the basis of Fe), w₃ is calculated according to Formula (A.3)

$$w_3 = \frac{m_1 \times 10^4}{m} \times 100 \dots\dots\dots(A.3)$$

Where:

m₁- the instrument's readout, in terms of milligram per liter (mg/L);

m- the mass of sample, with a unit of gram (g);

Take the arithmetic mean value of parallel determination results as the determination result. The absolute difference of parallel determination results shall not exceed 0.0002%.

A.7 Determination of Mercury (on the basis of Hg)

A.7.1 Method Abstract

In acid medium, mercury in the test sample is deoxidized into mercury in the atomic state with potassium borohydride (KBH₄), and is brought into an atomizer by the carrier gas (argon); with the illumination of the special mercury hollow cathode lamp, mercury of the ground state is excited to a higher energy state, and emit fluorescence light of characteristic wavelength when it is subject to deactivation and returns to the ground state; the fluorescence intensity is in proportion to the mercury content; compare it with the standard series and quantify it.

A.7.2 Reagents and Materials

A.7.2.1 hydrochloric acid solution: 1+1

A.7.2.2 Sodium hydrate solution: 5g/L.

A.7.2.3 potassium borohydride solution: weigh 5.0g potassium borohydride, dissolve it in 5g/L sodium hydrate solution, dilute it to 1000mL, mix them, and use it immediately after preparation.

A.7.2.4 mercury standard solution: 1mL solution contains 0.010mg mercury (Hg), and use it immediately after preparation. Suck 1mL mercury standard solution prepared according to HG/T 3696.2 with suction pipet, place it in a 100mL volumetric flask, dilute it to the graduation, and shake up them.

A.7.2.5 mercury standard solution: 1mL solution contains 0.1µg mercury (Hg), and use it immediately after preparation. A.7.2.6 argon: its purity shall be higher than 99.99%.

A.7.3 Instrument and Equipment

Dual-channel atomic fluorescence spectrometer

A.7.4 Analysis Steps

A.7.4.1 For the working parameters of the instrument, see Table A.1.

Table A.1 Working Parameters of the Instrument

element to be determined	negative high-voltage (V)	lamp current (mA)	column height (mm)	carrier gas (mL/min)	shrouding gas (mL/min)
Hg	230	15	10	300	900

A.7.4.2 Preparation of Standard Series of Solutions

Measure 0.00mL, 1.00mL, 2.00mL, 4.00mL, 8.00mL, 10.00mL Hg standard solution (100µg/mL), place them in six 100 volumetric flasks respectively, add 10mL (1+1) HCl in to each flask, dilute them to the graduation with water, and shake up them. Determine the light intensity of standard solution for test under the condition with the above working parameters, and draw the standard curve.

A.7.4.3 Treatment and Determination of Sample

Weigh 0.5g test sample (with a precision of 0.0001g), place it in a 100mL volumetric flask, add 10mL (1+1) HCl, dilute it to the graduation with water, and shake up them. Determine it on the drawn working curve.

A.7.5 Result Calculation

The mass fraction of mercury (on the basis of Hg), w_4 , is calculated according to Formula (A.4)

$$w_4 = \frac{c \times 10^{-4}}{m} \times 100 \dots\dots\dots(A.4)$$

Where:

C - the instrument's readout, in terms of milligram per liter (mg/L);

m- the mass of sample, with a unit of gram (g);

IV. Iron Tartrate

English Name: iron tartrate

Functional classification: antitackiness agent

(II) Amount and Range of Application

Food Category No.	Food Name	the largest application amount/ (g/kg)	Note
12.01	salt and its substitutes	0.106	The largest application amount is calculated on the basis of iron tartrate.

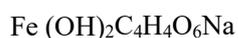
(II) Quality Specification and Requirements

1 Scope

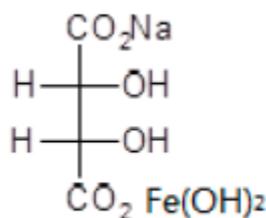
The quality specifications and requirements apply to the food additive iron tartrate derived from L-tartaric acid, sodium hydrate and ferric chloride subject to complexation.

2 Molecular Formula, Structural Formula and Relative Molecular Mass

2.1 Molecular Formula



2.2 Structural Formula



2.3 Relative Molecular Mass

261.93 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	dark green	place an appropriate amount of test sample in a 50mL flask, and observe it by eyes.
state	liquid	

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item		Index	Test Method
mesotartaric acid (disodium salt, on a dry basis), W%	\geq	37	A.2 in Appendix A
D- and L-tartaric acid (disodium salt, on a dry basis), W%	\geq	14	A.2 in Appendix A
oxalate (oxalic acid, on a dry basis), w/%	\leq	1.5	A.2 in Appendix A
ferrum (Fe)(on a dry basis), W%	\geq	8	GB/T 5009.90
water content, w/‰	\geq	65	GB 5009.3
chlorine (Cl)(on a dry basis), w/%	\leq	25	GB/T 12457
sodium(Na)(on a dry basis), w/%	\leq	23	GB/T 5009.91

arsenic (As) / (mg/kg)	\leq	3.0	GB 5009.76
lead (Pb) / (mg/kg)	\leq	5.0	GB 5009.12
mercury (Hg) / (mg/kg)	\leq	1.0	GB 5009.17

Appendix A

Test Method

A.1 General Provisions

All reagents and water used under the quality specifications are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard titration solutions in the test and standard solutions, preparations and products for determination of impurity are prepared according to provisions of GB/T 601, GB/T602 and GB/T603, unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A. Determination of mesotartaric acid content, D- and L-tartaric acid content and oxalic acid content

A.2.1 Method Abstract

Iron tartrate reacts with excessive hydroxide and decomposes to form $\text{Fe}(\text{OH})_3$ after filtration. Use organic acid chromatographic column as the solid phase, take 0.01 mol/L sulfuric acid as the mobile phase, and use liquid chromatograph to separate constituents. Use the differential refractive index detector to conduct detection, and calculate the results with the help of external standard.

A.2.2 Instrument and Equipment

A.2.2.1 high performance liquid chromatograph: differential refractive index detector

A.2.2.2 pump.

A.2.2.3 automatic Sample Injector: is equipped with 20 μL sample loop

A.2.2.4 separation column: stainless steel tube, 300 mm long, organic chromatographic column with an internal column of 7.8 mm.

A.2.2.5 column compartment

A.2.2.6 data collection and integration system.

A.2.2.7 syringe filter, with a diameter of 30 mm and precision of 0.45 μm , color number: green

A.2.3 Reagents and Materials

A.2.3.1 sulfuric acid, concentration 0.01 mol/L

A.2.3.2 mesotartaric acid monohydrate, concentration > 98 %

A.2.3.3 D-tartaric acid, concentration > 99 %

A.2.3.4 L-tartaric acid, concentration > 99 %

A.2.3.5 oxalic acid dihydrate, concentration > 99 %

A.2.3.6 sodium hydrate solution, concentration 5 mol/L

A.2.4 Sample

The test sample is stored in an air tight brown bottle and is isolated from oxygen. If it is impossible to fill the bottle up, it is necessary to fill the bottle with nitrogen to cover the sample. Keep it away from light (UV) and store it in refrigerator at a low temperature (4°C). The sample solution keep stable in 2 weeks.

A.2.5 Analysis Steps

A.2.5.1 Chromatographic Conditions

A.2.5.1.1 Separation column chromatographic column: organic acid chromatographic column, internal diameter 300 x 7.8 mm;

- A.2.5.1.2 Column temperature: 10°C;
- A.2.5.1.3 Mobile phase: sulfuric acid (A.2.3.1);
- A.2.5.1.4 Flow rate: 0.3 ml/min;
- A.2.5.1.5 Injection volume: 20 µL;
- A.2.5.1.6 Detector: differential refractive index detector.

A.2.5.2 Preparation of Standard Solution

A.2.5.2.1 Multi-Constituent Standard Solution (2)

Add 50~60mg mesotartaric acid monohydrate (A.2.3.2) and 20 ~ 30mg D-tartaric acid (A.2.3.3) D-tartaric acid (A.2.3.3) or L-tartaric acid (A.2.3.4) into a 50mL flask, with a precision of 0.01 mg. add 50mL sulfuric acid (A.2.3.1) to dissolve it. Determine the total mass, with a precision of 0.1mg.

A.2.5.2.2 standard solution b of oxalic acid

Weigh 250mg oxalic acid dihydrate (A.2.3.5), with a precision of 0.1mg, dissolve it with sulfuric acid (A.2.3.1), and produce 500mL. determine the total mass, with a precision of 1 mg.

A.2.5.3 Concentrations of Mesotartaric acid and D- And L-Tartaric Acid in Multi-Constituent Standard Solution A

Analyze two multi-constituent standard solutions A according to A.2.5.6.

Extract standard solution A with a suction pipet, inject it into a little glass bottle for analysis.

The concentrations of mesotartaric acid and D- and L-tartaric acid in multi-constituent standard solution A is calculated according to Formula (A.1),(A.2), (A.3),(A.4) respectively:

The mass of mesotartaric acid, M₁:

$$M_1 = M_{eq1} \times \left[\frac{150.1 \times X}{168.1 \times 100} \right] \dots\dots\dots(A.1)$$

Where:

M₁- the mass of mesotartaric acid in multi-constituent standard solution A, in terms of milligram (mg);

M_{eq1} - the mass of mesotartaric acid monohydrate in multi-constituent standard solution A, in terms of milligram (mg);

X – the mass percent of mesotartaric acid in standard substance;

150.1 - the molecular weigh of mesotartaric acid;

168.1- the molecular weigh of mesotartaric acid monohydrate;

100- conversion factor.

The mass of D- and L-tartaric acid, M₂:

$$M_2 = M_{eq2} + M_{eq1} \times \left[\frac{150.1 \times Y}{168.1 \times 100} \right] \dots\dots\dots(A.2)$$

Where:

M₂ – the mass of anhydrous D- and L-tartaric acid in multi-constituent standard solution, in terms of milligram (mg);

M_{eq1}- the mass of mesotartaric acid monohydrate in multi-constituent standard solution A, in terms of milligram (mg);

M_{cq2}- the mass of anhydrous D- or L-tartaric acid in multi-constituent standard solution A, in terms of milligram (mg);

Y - the mass percent of anhydrous D- and L-tartaric acid in standard substance;

150.1 - the molecular weight of mesotartaric acid;

168.1 – the molecular weight of mesotartaric acid monohydrat;

100- conversion factor.

In the multi-constituent standard solution A, the concentration of mesotartaric acid x_1 , and the concentration of D- and L-tartaric acid x_2 , are calculated according to Formula (A.3) and (A.4):

$$x_1 = \frac{M_1}{M_t} \dots\dots\dots(A.3)$$

$$x_2 = \frac{M_2}{M_t} \dots\dots\dots(A.4)$$

Where:

M_t – the mass of multi-constituent standard solution A, in terms of gram (g)

M₁ – the mass of mesotartaric acid in multi-constituent standard solution A, in terms of milligram (mg)

M₂ – the mass of D- and L-tartaric acid in multi-constituent standard solution A, in terms of milligram (mg)

A.2.5.4 Concentration of Oxalic Acid in the Standard Solution of Oxalic Acid

Prepare calibration solutions (I - VII) according to Table A.1: transfer the standard solutions of oxalic acid with following volumes with transfer liquid gun to 7 50mL flasks for analysis.

Table A.1 Calibration Solution

Solution (mL)	I	II	III	IV	V	VI	VII
Oxalic acid standard solution B(A.2.5.2.2)	0	0.2	1.0	2.5	5.0	7.5	10.0

Add 50mL sulfuric acid (A.2.3.1), determine the total mass, and calculate the result with a precision of 0.1 mg. draw a curve with 7 standard solutions in Table A.1 to calculate the Formula (A.2.6.1.2).

The concentration of oxalic acid in oxalic acid standard solution B of oxalic acid is calculated according to Formula (A.5) and (A.6):

the mass of oxalic acid, M₃:

$$M_3 = M_{cq3} \times \frac{90.0}{126.1} \dots\dots\dots(A.5)$$

Where:

M₃- oxalic acid content in the standard solution B of -oxalic acid, in terms of milligram (mg);

M_{cq3} - oxalic acid dihydrate content in the standard solution B of oxalic acid, in terms of milligram (mg);

90.0 - the molecular weight of oxalic acid

126.1- the molecular weight of oxalic acid dihydrate

The concentration of oxalic acid in the standard solution B of oxalic acid, x_3 :

$$x_3 = \frac{M_s V_c}{M_t M_{aq}} \dots\dots\dots(A.6)$$

Where:

M_t – the mass of the standard solution B of oxalic acid, in terms of gram (g)

M_s - the mass of oxalic acid in the standard solution B of oxalic acid, in terms of milligram (mg)

V_c - in the Table A.1, the mass of extracted standard solution B of oxalic acid, in terms of gram (g)

M_{aq} - in the Table A.1, the mass of prepared standard solution B of oxalic acid, in terms of milligram (mg)

A.2.5.5 Test Sample

Weigh 500mg sample, place it in a 50mL flask, dilute it with 25mL water, add 1mL NaOH solution (A.2.3.6), keep it still for at least 1h to make $Fe(OH)_3$ fully deposit. Filter the test samplesolution with a syringe filter, inject it into a little glass bottle for future analysis.

A.2.5.6 Determination

Inject 20 μ L multi-constituent standard solution A (A.2.5.2.1), standard solution of oxalic acid (A.2.5.4) and filtered test sample solution (A.2.5.5) into liquid chromatograph respectively. Record the results of liquid chromatography with refractive index detector, and determine the peak area of each constituent (=A).

A.2.6 Result Calculation

A.2.6.1 Drawing of Standard Curve

A.2.6.1.1 For the measurement range of standard curve, see Table A.2.

Table A.2 Measurement Rage Of Standard Curve

Constituent	concentration range of standard sample	measurement range of mTA concentrated solution
Mesotartaric acid	45 mg~55mg	9%~11%
D- and L-tartaric acid	20 mg~30mg	40%~6%
Oxalic acid	0.05 mg~2.5mg	0.01%~0.5%

According to A.2.5.6, test two multi-constituent standard solutions A (A.2.5.2.1), and conduct integration of the peak area. Take constituent the concentration of q as the horizontal ordinate and and the prea area of q as the vertical ordinate to draw standard curve and calculate the regression equation (A.7).

A.2.6.1.2 calibration function of constituent q (mesotartaric acid, D- and L-tartaric acid andoxalic acid)

The intercept (a_q) and slope (b_q) of constituent q standard curve are calculated according to Function (A.7):

$$y = a_q + b_q x \dots\dots\dots(A.7)$$

Where:

a_q - intercept of the constituent q standard curve

b_q - slope of the constituent q standard curve

Y- peak area(A_c) of constituent q in standard sample

X- concentration of constituent q in standard sample, in terms of milligram per gram (mg/g), is calculated according to Formula (A.3)(A.4)(A.6).

A.2.6.2 Concentration of Constituent q in Test Sample

The concentration of constituent q in the test sample is calculated according to Formula (A.8):

$$c(q) = \frac{(A_{sq} - a_q) \times M}{M_s \times b_q} \times 100\% \dots\dots\dots(A.8)$$

Where:

a_q - intercept of the constituent q standard curve

b_q - slope of the constituent q standard curve

A_{sq} - peak area of constituent q in test sample solution

M_s - mass of the test part, in terms of milligram (mg)

M- mass of constituent in the 50mL flask (A.2.5.5), in terms of gram (g)

A.2.7 Precision

Take the arithmetic mean value of two parallel determination results as the report result, and the absolute difference of two parallel determination results shall not exceed 0.2%.

V. Theaflavin

English Name: Theaflavin

Functional classification: antioxidant

(I) amount and range of application

Food Category No.	Food Name	The Largest Application Amount/(g/kg)	Note
02.0	fat, oil and emulsified fat products	0.4	
02.01	fat and oil basically containing no water	0.4	
04.05.02.01	cooked nuts and seeds (only limited to fried nuts and seeds)	0.2	
04.05.02.03	canned nuts and seeds	0.2	
05.02.01	gum based candy	0.4	
06.03.02.05	fried flour products	0.2	
06.06	instant cereals including rolled oat	0.2	
06.07	instant rice and wheat products	0.2	
07.0	baked food	0.4	
08.02	uncooked meat products	0.3	
08.03	cooked meat products	0.3	
09.0	aquatic products and their processed products (including such aquatic products and their processed products as fish, crustacea, seashells, mollush and echinodermata)	0.3	
09.03	uncooked aquatic products (semi-finished products)	0.3	
12.10	compound seasonings	0.1	
14.03.02	plant protein drinks	0.1	
14.04	sodas	0.2	
14.06	solid drinks	0.8	
14.07	drinks for special use	0.2	
14.08	flavored drinks	0.2	
14.09	other drinks	0.2	
16.01	jellies	0.2	If it is used for jelly powder, the application amount shall be increased according to the dissolving factor.
16.02.02	tea products (including flavored tea and substitute tea)	0.2	
16.06	puffed food	0.2	

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive theaflavin derived from fresh tea and tea polyphenols and is produced through biological fermentation, extraction with acetic ether, purification with polymeric adsorbent for the food industry, concentration and drying for polyphenol oxidases contained in fresh tea.

2. Technical Requirements

2.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	dark brown or light brown	Place an appropriate amount of test samples on a clean, dry, white porcelain plate in natural light, and observe their color, luster and state.
state	powder	

2.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item		Index	Test Method
theaflavin, W/%	≥	20.0	A.3 in Appendix A
caffeine, W/%	≤	5.0	GB/T 8312
water content, W/%	≤	6.0	GB/T 8304a
total ash, w/%	≤	2.0	GB/T 8306
Arsenic (on the basis of As)/ (mg/kg)	≤	2.0	GB 5009.11
Heavy metal (on the basis of Pb)/ (mg/kg)	≤	10	GB5009.74

a the drying temperature is 105°C±2°C, and the drying time is 4h.

2.3 Microbial Index: shall meet provisions of Table 3.

Table 3 Microbial Index

Item		Limit (it is expressed in terms of /25g, unless otherwise specified)	Test Method
total numbers of colony / (CFU/g)	≤	1000	GB 4789.2
mould and yeast / (CFU/g)	≤	100	GB 4789.15
coli group / (MPN/g)	≤	3.0	GB 4789.3
escherichia coli		not detected	GB 4789.38
salmonella		not detected	GB 4789.4

Appendix A

Test Method

A.1 General Provisions

All reagents and water used under the quality specifications are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard titration solutions in the test and standard solutions, preparations and products for determination of impurity are prepared according to provisions of GB/T 601, GB/T602 and GB/T603, unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.2 Identification Test

A.2.1 Color Reaction

Aluminum salts, together with theaflavin, produce red, and have the maximum absorption in the case of 525nm wavelength.

Take 1mL theaflavin-methanol solution with a concentration of 0.2mg/mL, place it in a 10mL volumetric flask, add 2mL aluminium muriate solution with a concentration of 0.1mol/L, produce a constant volume with methanol, keep full color rendering for 20 min, and there is the maximum absorption in the case of 525 nm.

A.2.2 Analysis of Finger-print

Weigh 10mg theaflavin standard (theaflavin content $\geq 80\%$), dissolve it with 95% ethanol solution to get 50mL solution, and filter it with 0.45 μ m filter membrane.

A.2.2.2 preparation of sample: weigh 0.1g sample, dissolve it with 15mL ethanol, remove it in a 100mL volumetric flask, produce 100mL with distilled water, mix them, and filter it with 0.45 μ m filter membrane.

A.2.2.3 Chromatographic Conditions:

a) chromatographic column: C18 reversed-phase chromatographic column 5.0 μ m x 4.6mm x 200mm;

b) mobile phase: A 0.1% phosphoric acid solution, is filtered with 0.45 μ m filter membrane.

Bacetonitrile (chromatographic pure)

c) column temperature: 35.0 $^{\circ}$ C

d)flow rate: 2.0mL/min

e)wavelength: 380nm

f)For the elution gradient, see Table A.1.A.1

Table A.1 Elution Gradient

time (min)	A%	B%
0	90	10
0.5	90	10
5	79	21
25	74	26
28	90	10

A.2.2.4 determination: take 10 μ L standard solution and sample solution, inject them into chromatograph, determine them, draw standard chromatogram, and compare it with that of the sample.

A.2.2.5 For the finger-print, see Fig. A.1

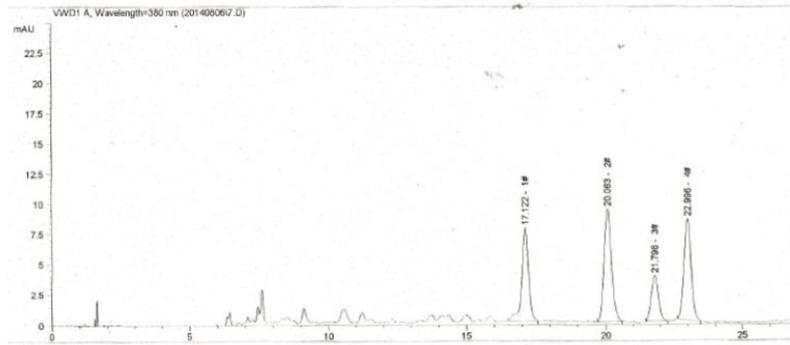


Fig. A.1 Finger-Print

A.3 Determination of Theaflavin Content

A.3.1 Reagents and Materials

- A.3.1.1 95% ethanol
- A.3.1.2 ethyl acetate (analytical reagent)
- A.3.1.3 sodium bicarbonate (analytical reagent)

A.3.2 Instrument and Equipment

Ultraviolet-visible spectrophotometer

A.3.3 Operation Steps

Accurately weigh 0.1g sample, and produce 100mL with water, shake up them, accurately remove 30mL uniform test solution in a 60mL cylindrical separatory funnel, add 30mL ethyl acetate in the funnel immediately, shake for 5 min, keep it still for layering, remove 15mL ethyl acetate phase to another 30mL cylindrical separatory funnel, add 15mL freshly prepared 2.5% sodium hydrogen carbonate solution, shake for 30s, at last remove 4mL ethyl acetate to a 25mL volumetric flask, and shake fully. Take ethanol solution as blank, and determine the absorbance A in the case of 380nm in 1cm cuvette.

A.3.4 Drawing of Standard Curve

Weigh 0.1g 80% standard substance, place it in a 100mL volumetric flask to obtain mother solution. Respectively remove 0mL,5mL,10mL,15mL, 20mL solutions to produce 100mL with ethanol, prepare them into standard solutions, determine the absorbance in the case of 380 nm, draw standard curve in which the slope is a and intercept is b.

A.3.5 Calculation

The mass fraction of theaflavin, W, is calculated according to Formula (A.1):

$$w = \frac{E \times 100 \times 25 / 4}{m \times (1 - w_1) \times 1000} \times 100\% \dots\dots\dots(A.1)$$

Where:

E - sample concentration calculated according to the standard curve, in terms of milligram/milliliter (mg/mL), E = aA+b(A is absorbance)

m- the mass of sample, with a unit of gram (g)

w₁- loss on drying for test sample, %

100 - produce 100mL

25/4 - 4mL is diluted to 25mL

1000 – conversion of unit value, 1g=1000mg

VI. 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane

English Name: 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane produced through chemical reaction among propionic aldehyde, hydrogen sulfide, acetaldehyde, ammonia and other materials.

2. Chemical Name, Molecular Formula, Structural Formula and Molecular Weight

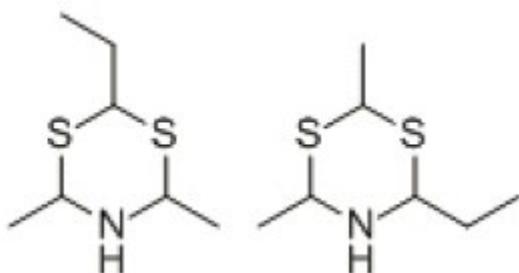
2.1 Chemical Name

2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane

2.2 Molecular Formula

C₇H₁₅NS₂

2.3 Structural Formula



2.4 Relative Molecular Mass

312.51 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 sensory requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	light yellow	place the test sample in comparison tubes, and observe them by eyes.
state	liquid	
aroma	onion and garlic-like odor	GB/T 14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
content, w/%	≥ 90.0 (the sum of two isomers, 2-ethyl-4,6-dimethyldihydro-1,3,5-dithiazinane and 4-	Appendix A

		ethyl-2,6-dimethyldihydro-1,3,5-dithiazinane) ^a	
refraction index (20 °C)		1.543 ~1.546	GB/T 14454.4
relative density (25°C/25°C)		1.072 ~1.075	GB/T 11540
a Minor constituents are 1,2,4-Trithiolane, 3,5-diethyl- and 2,4,6-trimethyldihydro--4H-1,3,5-dithiazinane			

Appendix A

Determination of 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane Content

A.1 Instrument and Equipment

A.1.1 Chromatograph: in accordance with provisions of Chapter 5 in GB/T 11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

Normalization method: determine the content according to Chapter 10.4 in GB/T 11538-2006.

A.3 Repeatability and Result Expression

It shall be conducted according Article 11.4 in GB/T 11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of food additive 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane, see Appendix B.

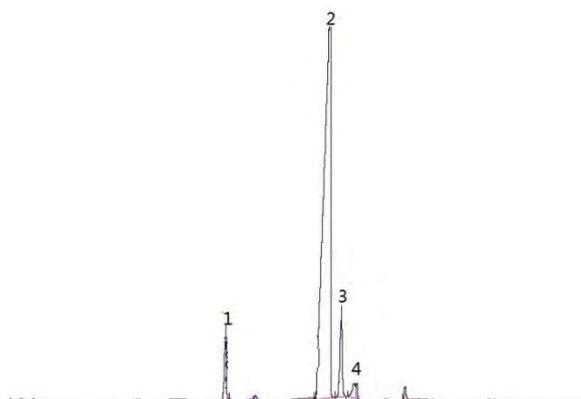
Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane

(normalization method)

B.1 Gas Chromatogram of the Food Additive 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane

For the gas chromatogram of the food additive 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-dithiazinane, see Table B.1.



Description:

- 1- 2,4,6-trimethyldihydro--4H-1,3,5-dithiazinane;
- 2- 2-Ethyl-4,6-dimethyldihydro-1,3,5-dithiazinane;
- 3- 4-Ethyl-2,6-dimethyldihydro-1,3,5-dithiazinane;
- 4- 1,2,4-Trithiolane, 3,5-diethyl-.

Fig.B.1 Gas Chromatogram of the Food Additive 2(4)-Ethyl-4(2),6-dimethyldihydro-1,3,5-Dithiazinane

B.2 Operation Conditions

B.2.1 Column: capillary column, 50 m long, with a diameter of 0.32 mm.

B.2.2 stationary phase: polyethylene glycol 20000.

B.2.3 membrane thickness: 0.50 μ m

B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75 °C for 4 min, then raise the temperature to 225°C from 75°C with a linear temperature program at a rate of 5°C/min, and at last keep the constant temperature of 225°C for 10 min.

B.2.5 Injection-port temperature: 250 C

B.2.6 Detector temperature: 250 C

B.2.7 Detector: hydrogen flame ionization detector

B.2.8 Carrier gas: nitrogen

B.2.9 Inlet pressure: 0.06 MPa

B.2.10 Sample size: 0.1 u L

B.2.11 Split ratio: 75: 1

VII. 3-Heptyldihydro-5-methyl-2(3H)-furanone

English Name: 3-Heptyldihydro-5-methyl-2(3H)-furanone

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive 3-Heptyldihydro-5-methyl-2(3H)-furanone produced through chemical reaction between 3-acetyl-5-methyl-2(3H)-furanone and heptanal.

2. Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

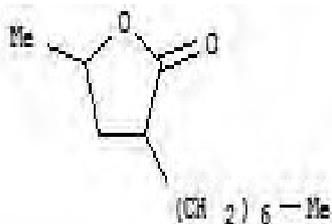
2.1 Chemical Name

3-Heptyldihydro-5-methyl-2(3H)-furanone

2.2 Molecular Formula

$C_{12}H_{22}O_2$

2.3 Structural Formula



2.4 Relative Molecular Weight

198.31 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	colorless	place the samples in comparison tubes, and observe them by eyes.
state	liquid	
aroma	fruit fragrance	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
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content, w/%	≥	95.0 (the sum of cis- and trans-isomers)	Appendix A
refraction index (20°C)		1.443~1.450	GB/T14454.4
relative density (25°C/25°C)		0.928~0.942	GB/T11540

Appendix A

Determination of 3-Heptyldihydro-5-methyl-2(3H)-furanone Content

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

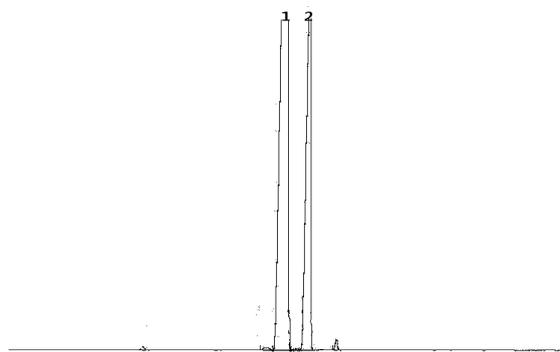
For the gas chromatogram and operation conditions of the food additive 3-Heptyldihydro-5-methyl-2(3H)-furanone, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 3-Heptyldihydro-5-methyl-2(3H)-furanone (normalization method)

B.1 Gas Chromatogram of the Food Additive 3-Heptyldihydro-5-methyl-2(3H)-furanone

For the gas chromatogram of food additive 3-Heptyldihydro-5-methyl-2(3H)-furanone, see Fig. B.1.



Description:

1-cis-3-Heptyldihydro-5-methyl-2(3H)-furanone;

2-trans-3-Heptyldihydro-5-methyl-2(3H)-furanone.

Fig. B.1 Gas Chromatogram of the Food Additive 3-Heptyldihydro-5-methyl-2(3H)-furanone

B.2 Operation Conditions

- B.2.1 Column: capillary column, with a length of 25 m and an internal diameter of 0.20 mm.
- B.2.2 stationary phase: polyethylene glycol20000
- B.2.3 Membrane thickness: 0.20 μ m
- B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75°C for 4 min, then raise the temperature to 225°C from 75°C with a linear temperature program at a rate of 8°C/min, and at last keep the constant temperature of 225°C for 8 min.
- B.2.5 Injection-port temperature: 250°C
- B.2.6 Detector temperature: 250°C
- B.2.7 Detector: hydrogen flame ionization detector
- B.2.8 Carrier gas: nitrogen
- B.2.9 Inlet pressure: 0.06MPa
- B.2.10 Sample size: 0.1 μ L
- B.2.11 Split ratio: 75: 1

VIII. Vanillic Alcohol

English Name: vanillyl alcohol

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive vanillic alcohol derived from vanillin.

2. Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

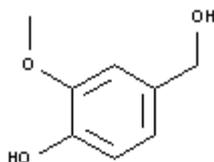
2.1 Chemical Name

4-Hydroxy-3-methoxybenzyl alcohol

2.2 Molecular Formula

$C_8H_{10}O_3$

2.3 Structural Formula



2.4 Relative Molecular Weight

154.17 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	white to light yellow, becoming light brown in the case of long-time placement.	Place test samples on a clean, white paper, and observe them by eyes.
State	crystalline powder	
Aroma	mild sweet fragrance, paste fragrance, vanillin-like fragrance	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
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content, w/%	≥	98.0	Appendix A
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Appendix A

Determination of Vanillic Alcohol Content

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

Normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

Preparation of test sample: weigh 2g test sample, dissolve it in 1mL absolute ethyl alcohol, and shake up for future use.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

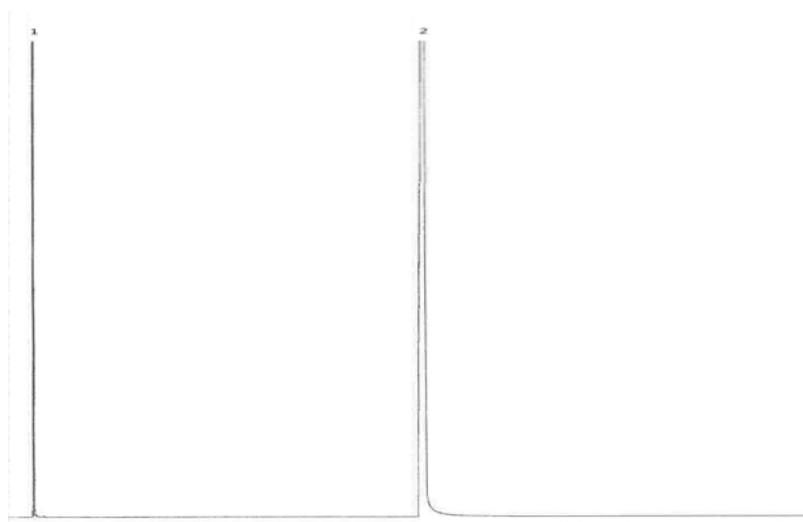
For the gas chromatogram and operation conditions of food additive vanillic alcohol, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive Vanillic Alcohol (normalization method)

B.1 Gas Chromatogram of the Food Additive Vanillic Alcohol

For the gas chromatogram of food additive vanillic alcohol, see Table B.1.



Description:

- 1- Ethanol (solvent)
- 2- Vanillic alcohol

Fig.B.1 Gas Chromatogram of the Food Additive Vanillic Alcohol

B.2 Operation Conditions

- B.2.1 Column: capillary column, with a length of 25 m and an internal diameter of 0.20 mm.
- B.2.2 Stationary phase: polyethylene glycol20000
- B.2.3 Membrane thickness: 0.33 μ m
- B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75°C for 4 min, then raise the temperature to 225°C from 75°C with a linear temperature program at a rate of 8°C/min, and at last keep the temperature of 225°C for 8 min.
- B.2.5 Injection-port temperature: 250°C
- B.2.6 Detector temperature: 250°C
- B.2.7 Detector: hydrogen flame ionization detector
- B.2.8 Carrier gas: nitrogen
- B.2.9 Inlet pressure: 0.06MPa
- B.2.10 Sample size: 0.1 μ L
- B.2.11 Split ratio: 75: 1

IX. 6-[5(6)-Decenoyloxy]decanoic Acid

English Name: 6-[5(6)-Decenoyloxy]decanoic acid

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive 6-[5(6)-Decenoyloxy]decanoic acid is derived from ϵ - Decalactone subject to hydrolyzation, dehydration and distillation.

2. Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

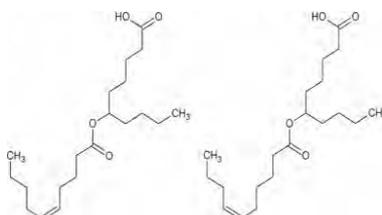
2.1 Chemical Name

6-[5(6)-Decenoyloxy]decanoic acid

2.2 Molecular Formula

$C_{20}H_{36}O_4$

2.3 Structural Formula



2.4 Relative Molecular Weight

340.5 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	colorless to yellowish	place the samples in comparison tubes, and observe them by eyes.
state	liquid	
odor	milky	GB/T14454.2

1.2 Technical Requirements: shall meet provisions of Table 2.

Table 2 Technical Requirements

Item	Index	Test Method
6-[5(6)-Decenoyloxy]decanoic acid content (GC,	96	GB/T11538

normalization method), w/%		
refraction index (20°C)	1.4550~1.4620	GB/T14454.4
relative density (20°C/20°C)	0.9520~0.9620	GB/T11540

X. Glucosyl Steviol Glycosides

English Name: Glucosyl Steviol Glycosides

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive glucosyl steviol glycosides derived from stevioside which is extracted from leaves of *Stevia Rebaudiana Bertoni*, then subject to glucosylation, evaporation for concentration and spray drying.

2. Technical Requirements

2.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Index	Test Method
color and luster	white or yellowish	Take an appropriate amount of sample, place it in a clean, dry glassware, and observe its color, luster and state in natural light.
properties	powdery	

2.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
glucosyl steviol glycosides(GSG), w/%	≥ 75.0	A.3 in Appendix A
rebaudioside A+stevioside, w/%	≤ 6.0	
rebaudioside A, w/%	≤ 4.0	
stevioside, w/%	≤ 4.0	
maltodextrin , w/%	≤ 20.0	
optical rotation	+65°~+75°	GB/T14454.5
relative density	0.2~0.6	GB/T11540
pH	4.5~7.0	GB/T9724

Appendix A

Test Method

A.1 General Provisions

Reagents and water used under the quality specifications are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.2 Identification Test

white or yellowish powder, soluble in water, slightly soluble in ethanol.

A.3 Determination Method for Glucosyl Steviol Glycosides, Stevioside and Maltodextrin

A.3.1 Principle

Adsorption chromatography and high performance liquid chromatography can be adopted to determine the total content (TSG), residual maltodextrin (RD), unreacted stevioside and proportion of glucosyl steviol glycosides.

A.3.2 Scope

It applies to final products containing mixtures of α -1, 4-glucosyl steviol glycosides (GSG), and solid samples whose total stevioside content (on the dry basis) is 60~102%.

A.3.3 Equipment and Reagents

- A.3.3.1 High Efficiency Liquid Chromatography(HPLC); the equipment need to be equipped with binary pump, automatic sample injector, column compartment, DAD detector, interface and data collection software;
- A.3.3.2 HPLC animo column, 4.6mm x 250mm, 5 μ m particles
- A.3.3.3 analytical balance with a precision of 0.0001 g
- A.3.3.4 Karl Fischer titration instrument
- A.3.3.5 vacuum rotary evaporator for lab
- A.3.3.6 vacuum oven
- A.3.3.7 moisture meter
- A.3.3.8 vacuum solvent filter system, with all glass materials
- A.3.3.9 vacuum system filter: with material of polypropylene, 0.2 μ m,47mm
- A.3.3.10 grade A volumetric flask and suction pipet
- A.3.3.11 glass column filled up with 200mL macroporous adsorbent resin (internal diameter is 25mm)
- A.3.3.12 acetonitrile, HPLC grade
- A.3.3.13 water, HPLC grade
- A.3.3.14 ethanol, reagent grade, system equipment, or other equivalent
- A.3.3.15 rebaudioside A standard
- A.3.3.16 stevioside standard
- A.3.3.17 rebaudioside C standard
- A.3.3.18 rebaudioside F standard
- A.3.3.19 dulcoside A standard
- A.3.3.20 rubusoside standard
- A.3.3.21 ammonium acetate , reagent grade
- A.3.3.22 glacial acetic acid. reagent grade

A.3.4 Safety Instructions

- A.3.4.1 during treatment of materials and cleaning for overflowing liquid and wastes, safety measures and emergency disposal principles for hazard chemicals shall be followed consistently.
- A.3.4.2 With regard to chemicals used in the above steps, all preventive measures and hazard warnings listed in Material Safety Data Sheet.
- A.3.4.3 Stevioside is always powdery, is easy to produce dust in air during shaking, feeding and stirring, and thus may be absorbed into human's mouth and nose, so cautious operation is needed to avoid generation of dust.

A.3.5 Steps

A.3.5.1 TSG

Test solution - accurately Weigh 5g GSG, pour it into 250mL to be dissolved. Add the solution to a glass column filled up with 200mL macroporous adsorbent resin (the internal diameter is 25mm) at a rate lower than 15 mL/min, and then rinse the resin with 1000mL water. Elute the absorbed stevioside with 1000mL 50% (volume) ethanol at a rate of 15mL/min or lower. Distill the ethanol eluate and cleaning water to make them dried, place them in a vacuum oven, and dry them for two hours at a temperature of 105°C. Weigh the dry weight of each group of constituents and records the data. Calculate the content of TSG and RD (%) according to formulas.

Test solution – accurately weigh 5g GSG, and pour it into 250mL water to dissolve it.

The mass fraction of TSG, w_1 , is calculated according to Formula (A.1), and mass fraction of RD content, w_2 , is calculated according to Formula (A.2):

$$w_1 = \frac{m_1}{m_2 \times (100 - w_h) \times 10^{-2}} \times 100\% \quad \dots\dots\dots (A.1)$$

Where:

m_1 - the total amount of ethanol constituents after being dried, in terms of gram (g);

m_2 - wet weight of the original sample, in terms of gram (g);

w_h - rate of water content (%);

$$w_2 = \frac{m_3}{m_2 \times (100 - w_h) \times 10^{-2}} \times 100\% \quad \dots\dots\dots (A.2)$$

Where:

m_3 - the total amount of water constituents after being dried, in terms of gram (g);

m_2 - wet weight of the original sample, in terms of gram (g);

w_h - rate of water content (%);

Acceptance Level:

The sample recovery rate, w_3 , must be between 98.0% and 102.0% and is calculated according to Formula (A.3).

$$w_3 = w_1 + w_2 \quad \dots\dots\dots (A.3)$$

Where:

w_1 - mass fraction of the total TSG content;

w_2 - mass fraction of the RD content;

Where the stevioside content in cleaning water is lower than 10mg/L, the cleaning water shall be tested with HPLC.

A.3.5.2 Content Of Unreacted Stevioside

Weigh 3g GSG, pour it into buffer solution (A.3.6.1.2) to be dissolved to prepare 100mL solution which is taken as test solution. Determine the content of unreacted stevioside (SG) according to the HPLC determination steps for stevioside. The chromatogram of the sample is consistent with the demonstrated chromatogram. The mass fraction of the content of α -glucosyl steviol glycosides is calculated according to the total content of stevioside(A.3.5.1), and the mass fraction of the content of α -glucosyl steviol glycosides, w_{α} , is calculated according to Formula (A.4):

$$w_{\alpha} = w_1 - w_4 \quad \dots\dots\dots (A.4)$$

Where:

w_1 - mass fraction of TSG (%);

w_4 - mass fraction of the content of unreacted stevioside (%);

A.3.5.3 Proportion of α -Glucosyl Steviol Glycosides

Weigh about 5g GSG, dissolve it in water to prepare 100mL solution, take it as test solution.

The area ratio of α -glucosyl steviol glycosides (%) is determined in HPLC analysis according to the HPLC steps for glucosyl steviol glycosides(A.3.6.2).

calculate the proportion of α -glucosyl steviol glycosides according to the content of α -glucosyl steviol glycosides (A.3.5.2), and the proportion of α -glucosyl steviol glycosides, w_5 , is calculated according to Formula (A.5):

$$w_5 = w_{\alpha} \times A_1 \times 10^{-2} \quad \dots\dots\dots (A.5)$$

Where:

w_{α} - mass fraction of the content of α -glucosyl steviol glycosides (%);

A_1 - area ratio of α -glucosyl steviol glycosides;

A.3.6 HPLC Analysis

A.3.6.1 HPLC Analysis of Stevioside

A.3.6.1.1 moisture balance between the standard and sample

Stevioside is a hydrophilic compound. Before analysis, moisture balance shall be reached for the standard and sample. The standard, sample and analytical balance shall be placed in the same room, and shall be exposed in the air for at least 24h, and the dry powder shall be stirred from time to time to ensure that the sample absorb moisture evenly. During weighing, Karl Fischer titration instrument shall be used to determine the moisture value of all standards. The moisture value in sample shall be determined at the temperature of 105°C with the method of loss on drying. Other moisture meters can be also used at a set temperature of 105°C.

A.3.6.1.2 Preparation of Mobile Phase Solution

Prepare mobile phase solution with an appropriate volume depending on the demands.

Mobile phase solution with appropriate volume can be prepared depending on demands.

Aqueous buffer solution (0.0125% acetic acid, 0.0125% ammonium acetate)- the buffer solution is prepared with 0.125g ammonium acetate (NH₄OAc) and 125 μ L glacial acetic acid (ethylic acid) dissolved in 1 L water.

Mobile phase (acetonitrile: buffer solution) - acetonitrile and buffer solution is mixed to prepare mobile phase solution whose ratio of acetonitrile to aqueous buffer solution is 80: 20 (volume). Mix acetonitrile and aqueous buffer solution with an appropriate amount, make the solution to reach room temperature, and degas the solution.

Diluent (100% buffer solution) – filter 1000mL aqueous buffer solution, and use it immediately.

A.3.6.1.3 Preparation of Standard Solution

Reb-A standard curve - Reb-A curve consists of five concentration points at 200mg/L~2000mg/L. Respectively weigh 5mg, 10mg, 25mg, 40mg and 50mg(± 2 mg) Reb-A (with moisture balance) samples, dissolve them with diluent in 25mL volumetric flasks respectively to produce the constant volume.

Stevioside standard curve - stevioside calibration curve consists of 7 concentration points distributed at 2.5mg/L, 5mg/L, 50mg/L, 100mg/L, 500mg/L, 1000mg/L and 2000mg/L. Prepare 2000mg/L stevioside stock solution similar to Reb-A standard reference. Dilute it to the needed concentration.

Stevioside – retention time labeling solution (M6), containing each of following 100mg/L stevioside (prepared with diluent): rubusoside, dulcoside A, stevioside, rebaudioside C, rebaudioside F and rebaudioside A.

Sample preparation- prepare sample solution according to the steps in A.3.5.1 and A.3.5.2.

A.3.6.1.4 For the conditions of instrument operation, see Table A.1.

Table A.1 Conditions of Instrument Operation

chromatographic column	animo column, 250 x 4.6mm, 5 μ m
temperature	30°C
isocratic mobile phase	20% buffer solution, 80% acetonitrile
flow rate	1.5mL/min
injection volume	12 μ L
detection wavelength	UV210nm(4nmbw), reference: 260nm(100nmbw)
runtime	60min
temperature of automatic sample injector	room temperature

A.3.6.1.5 Analysis Steps

A.3.6.1.5.1 system start-up/applicability

Check of detector sensitivity: inject 2.5mg/L stevioside standard solution, and confirm that the peak-to-noise ratio (signal-to-noise ratio) of stevioside ≥ 3 ; or need to check the instrument to ensure signal-to-noise ratio ≥ 3 for further operation.

Tailing factor: inject Reb-A2000mg/L standard solution, and use the peak to calculate the tailing factor -T. Tailing factor: $0.8 \leq T \leq 2$.

Signal-to-noise ratio: calculate the signal-to-noise ratio of stevioside standard solution. The limit of detection(LOD) is 5mg/L stevioside standard solution: signal-to-noise ratio of the

standard solution must be ≥ 10 . With regard to the stevioside standard solution whose limit of detection is 2.5mg/L: the signal-to-noise ratio must be ≥ 3 .

Separation of stevioside: Inject M6 standard solution, and the two peaks of stevioside and rebaudioside C shall be obviously separated. Record the retention time of each stevioside (A.3.8.1).

Inject M6 standard solution, and the two peaks of stevioside and rebaudioside C shall be separated obviously. Record the retention time of each stevioside (A.3.8.1).

A.3.6.1.5.2 Analytic Sequence

After checking the system adaptability, inject the remained standard solutions in a order from high concentration to low concentration, then inject the sample; after at most 12 times of sample injection and completion of analytic sequence, inject 2000mg/L stevioside and Reba standard solution respective for backup calibration.

A.3.6.1.5.3 Integral Parameter

Use the software of the liquid chromatograph to complete integration.

A.3.6.1.6 Calculation

A.3.6.1.6.1 relative standard deviation of peak area

The relative standard deviation of peak area r_1 is calculated according to (A.6):

$$r_1 = \frac{s_1}{x} \times 100\% \quad \dots\dots\dots (A.6)$$

Where:

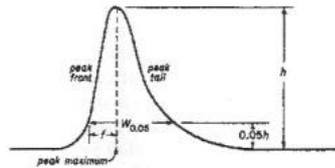
s_1 - standard deviation= $(\frac{\sum(x-x)^2}{(N-1)})^{1/2}$;

x - average value $= (x_1+x_2+x_3+x_n)/N$

x_n - peak area

N - total quantity of samples

A.3.6.1.6.2 tailing factor (T)



The tailing factor T is calculated according to Formula (A.7):

$$T = \frac{W_{0.05}}{2f} \quad \dots\dots\dots (A.7)$$

Where:

$W_{0.05}$ - the peak width at 5% height;

f - the distance between the maximum peak value and the peaks value along the x axis, and measure at the place of 5% above the peak value baseline.

A.3.6.1.6.3 Standard Recovery Rate

The standard recovery rate p is calculated according to Formula (A.8):

$$p = \frac{c_1}{c_2} \times 100\% \quad \dots\dots\dots (A.8)$$

Where:

c_1 - the calculated concentration value in the curve

c_2 - theoretical concentration

A.3.6.1.6.4 Analysis and Calculation

Determine the target analyte through matching M6 standard solution to the retention time.

Determine the peak response area of target analyst in standard solution and sample.

Determine the system drift of RebA standard. Determine the response area of RebA at 2000mg/L, and calculate relative standard deviation, with the requirement for relative standard deviation: $\leq 2.0\%$.

Take RebA or stevioside concentration (in terms of mg/L) as the vertical axis and the corresponding response area as the horizontal axis to draw fully fitted linear regression curve. Or data collection software can also be used to draw the calibration curve.

Calculate the concentration of analyte in the sample from the linear regression equation of standard curve (in terms of mg/L) (RebA curve is adopted for RebA, and stevioside curve is adopted for all other analytes). Or data collection software can also be used to (calibration curve drawn with software) calculate the concentration of analytes. The concentration of an analyte, Y , is calculated according to Formula (A.9):

$$Y = AX + B \quad \dots\dots\dots (A.9)$$

Where:

X - peak response area:

A - slope;

B - Y-intercept.

Correction of the concentration of all analytes in the sample is as follows:

Multiple the concentration of each glucoside and its calibration factor to correct the difference of molecular weight between it and stevioside (see Table A.2).

The structural formula of stevioside is as follows:

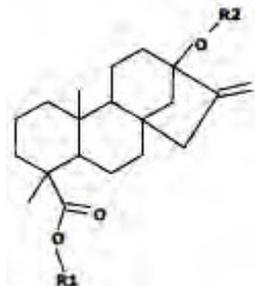


Table A.2 Stevioside R1 and R2 Groups, Molecular Formula and Corresponding Molecular Weight

Name	Abbreviation	R1	R2	Molal Weight(g/mol)	Correction Factor
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Dulcoside A	DulA	β glc-	α rha- β glc-	788.88	0.98
Rebaudioside A	RebA	β glc-	(β glc) ₂ - β glc-	967.03	-
Rebaudioside C	RebC	β glc-	(β glc, α rha)- β glc-	951.02	1.18
Rebaudioside F	RebF	β glc-	(β glc, β xyl)- β glc-	936.99	1.16
rubusoside	Rub	β glc- β glc-	β glc- β glc-	642.73	0.80
stevioside	Stev	β glc-	β glc- β glc-	804.88	-

The weight percentage of RebA and other glucosides in sample, w , is calculated according to Formula (A.10):

$$w = c_3 / c_4 \times 100 \quad \dots\dots\dots (A.10)$$

Where:

c_3 - analyte concentration, mg/L;

c_4 - sample concentration, mg/L.

The weight percentage of RebA and all other glucosides (water is deducted) can be corrected according to W (weight percentage) multiplied by the following factor (F). Correction Factor F is calculated according to Formula (A.11):

$$F = 100 / (100 - M) \quad \dots\dots\dots (A.11)$$

Where:

M - water content in the sample.

The weight percentage of stevioside(SG) in the sample, w_{SG} , is calculated according to Formula (A.12):

$$w_{SG} = w_{Rub} + w_{DulA} + w_{RebC} + w_{RebF} + w_{Stev} + w_{RebA} \quad \dots\dots\dots (A.12)$$

Where:

w_{DulA} - The weight percentage of DulA in the sample,(%)

w_{RebC} - The weight percentage of RebC in the sample,(%)

w_{RebF} - The weight percentage of RebF in the sample,(%)

w_{Stev} - The weight percentage of Stev in the sample,(%)

w_{RebA} - The weight percentage of RebA in the sample,(%)

A.3.6.1.7 Acceptance Level

A.3.6.1.7.1 Acceptance Level of the Standard Curve

The standard curve of RebA- for all different RebA concentration levels used in the calibration curve, the recovery rate of the standard substance must be within 100±3%, and the acceptable standard of the correlation coefficient of the standard curve is ≥ 0.9900 .

for all different stevioside concentration levels used in the calibration curve, the recovery rate of the standard substance must be within 100.0±10% except the range of 100.0±20% in the

case of the minimum concentration level (2.5mg/L). The acceptable standard of the correlation coefficient of the standard curve is ≥ 0.9900 .

A.3.6.1.7.2 Serial standard (standard examination) – the recovery rate of serial standards of stevioside and RebA (see A.3.6.1.6.3) must be within $100.0 \pm 2\%$.

A.3.6.1.7.3 Sample- the % relative standard deviation RSD of SG and Reb-A test results of parallel samples shall not exceed 2.0%. the % relative standard deviation other glucosides shall not exceed 50% when the content is lower than 5mg/L (the content in the sample is 0.1% correspondingly); shall not exceed 20% when the content is higher than 5mg/L. When the % relative standard deviation is not in the above scope, fresh sample shall be prepared again until the fresh sample pass the quality control examination.

A.3.6.2 HPLC Determination Steps of Glucosyl Steviol Glycosides Gradient

A.3.6.2.1 mobile phase (A - acetonitrile, B - water)

Filter and degas acetonitrile and water.

A.3.6.2.2 diluent (100% water)

Filter 1000mL water, and use it immediately

A.3.6.2.3 (M6) preparation of the standard substance (M6)

Weigh 100mg/L of each kind of rubusoside, Dulcoside A, stevioside, Rebaudioside C, Rebaudioside F and Rebaudioside A standard and prepare it into mixed standard sample solution with diluent.

A.3.6.2.4 Sample Preparation

Prepare sample solution according to the method described in A.3.5.3 (about 5%).

A.3.6.2.5 For the conditions of instrument operation, see Table A.3

Table A.3 Conditions of Instrument Operation

chromatographic column	amino column, 250 x 4.6mm, 5 μ m
temperature	30°C
gradient mobile phase	A- acetonitrile, B - wate 0 min A: B-80: 20 0~2minA: B-80: 20 2~70minA: B-50: 50
flow rate	1.0mL/min
injection volume	10 μ L
detection wavelength	UV210nm(4nmbw), reference: 260nm(100nmbw)
runtime	70min
temperature of automatic sample injector	room temperature

A.3.6.2.6 Analysis Steps

Separation of stevioside: inject M6 standard solution, and the two peaks of stevioside and rebaudioside C shall be separated obviously. Record the retention time of each stevioside (A.3.8.2).

A.3.6.2.7 Analytic Sequence

Inject sample first, then after injecting at most 12 samples and completing sample sequence test, inject the standard substances for quantitative test.

A.3.6.2.8 Integral Parameter

Use the software of the liquid chromatograph to complete integration. The demonstrated chromatogram is attached to the appendix part (Fig. A.3).

A.3.6.2.9 Calculation

Identify each α -glucosyl steviol glycoside through comparison between the elution profile and demonstrated chromatogram (Fig. A.2, Fig. A.3)

Integrate all peaks (except unreacted glucoside). Determine the proportion of α -glucosyl steviol glycosides (% area) with the data collection software of chromatograph.

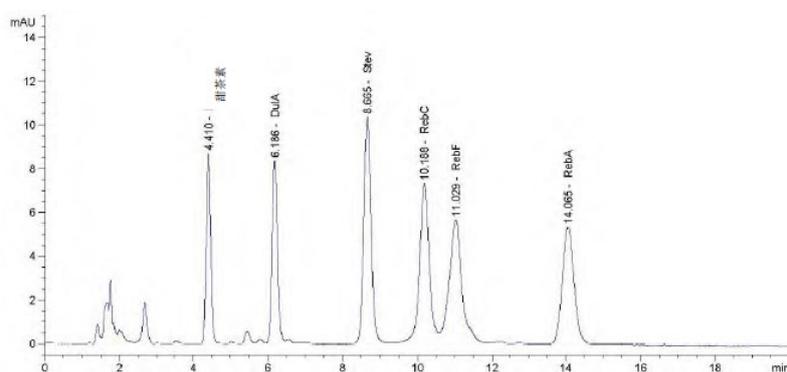
Record the proportion of each α -glucosyl steviol glycoside.

A.3.7 Result Report

The concentration of unreacted stevioside and the concentration of TSG shall be reported on the basis of dry weight %. The proportion of α -glucosyl steviol glycoside is reported on the basis of area %. The average value of two samples' repeated test results is taken as the reported value.

A.3.8 Attachment

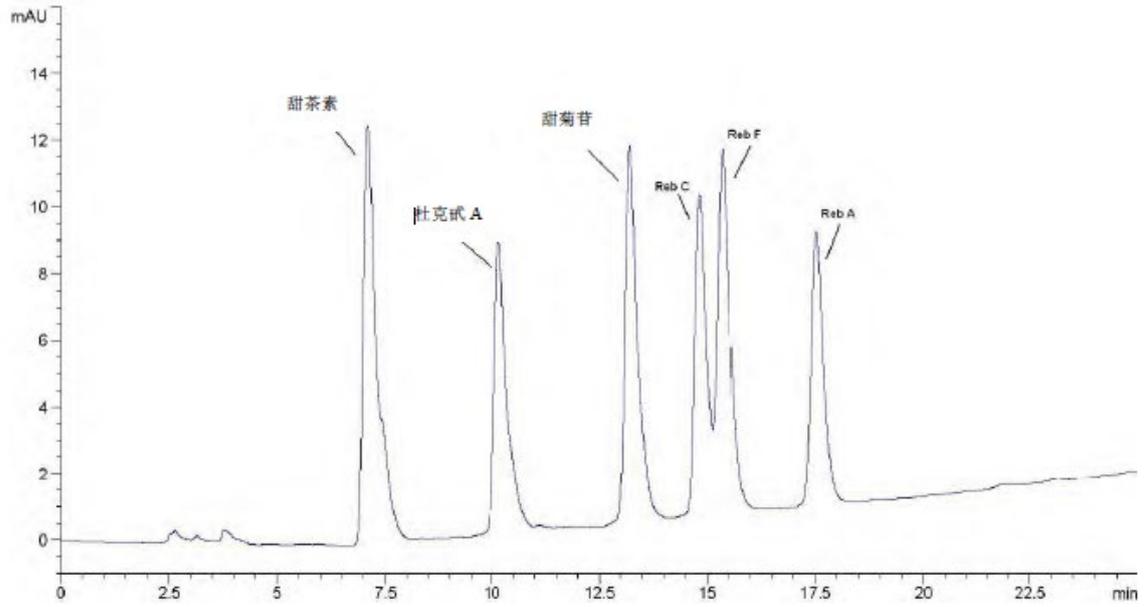
A.3.8.1 HPLC Chromatogram of M6 Sample



甜茶素/Rubusoside

Fig.A.1 HPLC Chromatogram of M6 Sample

A.3.8.2 HPLC Chromatogram of M6 Sample (Gradient)



甜茶素/Rubusoside

杜克忒A/Dulcoside A

甜菊苷/Stevioside A

Fig.A.2HPLC Chromatogram of M6 Sample (Gradient)

A.3.8.3 Demonstrated Chromatogram Set of Sample Gradient Analysis

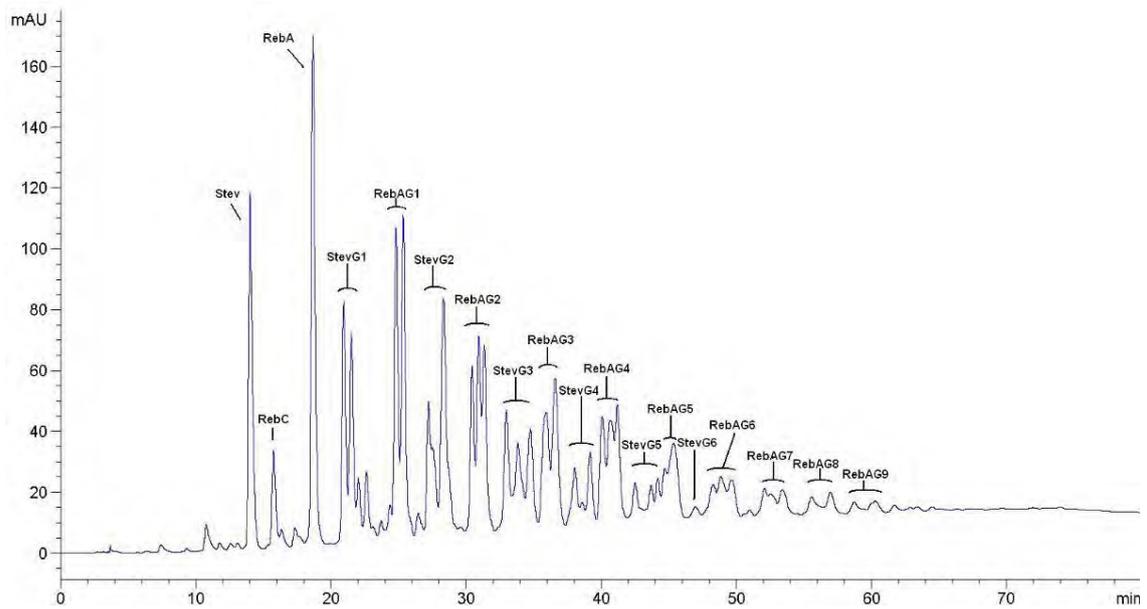


Fig. A.3Demonstrated Chromatogram Set of Sample Gradient Analysis

Attachment 2

Extension of the Range or Amount of Application for 19 Food Additives Including L (+)- Tartaric Acid

No	Name	Function	Food Category No.	Food Name	The largest application amount (g/kg)	Note
1.	L (+)-TARTARIC ACID,	acidity regulator	05.02	candies	30	on the basis of tartaric acid
2.	Dimethyl dicarbonate	preservative	14.08	flavored drinks	0.25	The application amount of solid drinks is increased according to the dilution factor.
3.	titanium dioxide	colorant	16.03	collagen casings	use with an appropriate amount depending on production demands	
4.	monascus red	colorant	10.03	egg products (change their physical characteristics)	use with an appropriate amount depending on production demands	
			10.04	other egg products	use with an appropriate amount depending on production demands	
5.	caramel color class I- plain	colorant	04.04.01.03	dried bean curd products	use with an appropriate amount depending	

					on production demands	
6.	potassium metabisulphite	antioxidant, preservative	15.02	compound wine	0.25g/L	The largest application amount is calculated on the basis of the residual quantity of sulfur dioxide.
7.	sodium metabisulphite	color fixative, antioxidant	04.02.02.04	canned vegetable	0.05	The largest application amount is calculated on the basis of the residual quantity of sulfur dioxide.
		processing agent for the food industry (viscosity regulator)	-	Processing for soybean protein (only limited to soybean protein isolate and protein concentrate)	0.03	on the basis of the residual quantity of sulfur dioxide
8.	ascorbyl palmitate	antioxidant	14.05.01	tea drinks	0.2	The application amount of solid drinks is increased according to the dilution factor.
9.	curdlan	stabilizer and coagulant, thickener	01.02.02	flavored fermented milk	use with an appropriate amount depending on production demands	
			03.01	ice cream, popsicles	use with an	

					appropriate amount depending on production demands	
			05.02.01	gum based candy	use with an appropriate amount depending on production demands	
			12.10.02.01	mayonnaise, salad dressing	use with an appropriate amount depending on production demands	

No.	Name	Function	Food Category No.	Food Name	The largest application amount (g/kg)	Note
			14.03.02	plant protein drinks	use with an appropriate amount depending on production demands	The application amount of solid drinks is increased according to the dilution factor.
			14.06.04	other solid drinks	use with an appropriate amount depending on production demands	
10.	paprika red	colorant	04.03.02.03	pickled edible	use with an	

				fungi and algae	appropriate amount depending on production demands	
11.	paprika oleoresin	flavoring agent, colorant	04.04.01.03	dried bean curd products	use with an appropriate amount depending on production demands	
			04.04.01.05	new bean products (including soybean protein and its puffed food and soybean meat, etc.)	use with an appropriate amount depending on production demands	
12.	brilliant blue, brilliant blue aluminum lake	colorant	07.02.04	colorful decoration on cakes	0.025	on the basis of brilliant blue
13.	glycerol ester of wood rosin	emulsifier	05.03	coatings for candies and chocolate products coatings of candies and chocolate products	0.32	
14.	potassium sorbate	preservative	02.02.02	emulsified products with a fat content of less	1.0	on the basis of sorbic acid

				than 80%		
15.	sorbitol and sorbitol syrup	humectant	09.02.03	frozen surimi products (including fish balls)	20	
16.	tertiary butylhydroquinone (TBHQ)	antioxidant	07.02	cakes	0.2	on the basis of content in fat and oil
17.	vegetable carbon, carbon black	colorant	16.03	collagen casings	use depending on production demands	
18.	insoluble polyvinylpyrrolidone	processing agent for the food industry (adsorbent)	-	processing for tea drinks	use with an appropriate amount depending on production demands	
19.	calcium silicate	processing agent for the food industry (filter aid)	-	processing for frying oil	40	

**Announcement Concerning New Varieties of Food Additives Including Ascorbyl
Palmitate (Enzymic Method)**

2016 No. 9

According to Food Safety Law, the review body has organized experts to review the safety evaluation materials for and approved 3 new varieties of food additive including ascorbyl palmitate (enzymic method), extension of the range of application of 8 varieties of food additives including paprika oleoresin, and extension of the food nutrient enhancer selenium enriched yeast.

It is hereby announced.

Attachment:

1. 3 New Varieties of Food Additive Including Ascorbyl Palmitate (Enzymic Method)
2. Extension of The Range of Application of 8 Varieties of Food Additives Including Paprika Oleoresin
3. Extension of The Food Nutrient Enhancer Selenium Enriched Yeast

National Health and Family Planning Commission of PRC

July 22, 2016

Attachment 1

3 New Varieties of Food Additives Including Ascorbyl Palmitate (Enzymic Method)

I. Ascorbyl Palmitate (Enzymic Method)

English Name: ascorbyl palmitate(enzymatic)

Functional classification: antioxidant

(I) Amount and Range of Application

Food Category No.	Food Name	the largest application amount/(g/kg)	Note
02.0	fat, oil and emulsified fat products	0.2	
02.01	fat and oil basically containing no water		

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive ascorbyl palmitate derived from palmitic acid (ethyl palmitate) and ascorbic acid subject to catalytic reaction with lipase. Other technical requirement is implemented according to Food Additive L-ascorbyl Palmitate (GB16314-1996).

II. 3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione

English Name:

3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive 3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione produced through chemical reaction among N,N-dimethylformamide, Ethyl 4-pyrazolecarboxylate, tertbutyldimethylsilyl chloride, N,N-diisopropylethylamine, sodium triacetoxymethylborohydride and tetrahydrofuran.

2. Chemical Name, Molecular Formula, Structural Formula, Molecular Weight

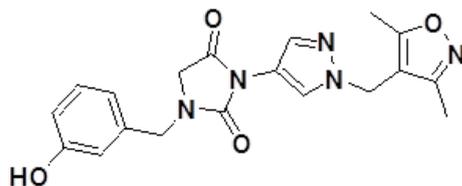
2.1 Chemical Name

3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione

2.2 Molecular Formula

C₁₉H₁₉N₅O₄

2.3 Structural Formula



2.4 Relative Molecular Weight

354.42 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	white	Place test samples on a clean, white paper, and observe them by eyes.
state	powder	
aroma	mild aroma	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item		Index	Test Method
content, w/%	≥	99.0	Appendix A
melting point/°C		145~150	GB/T14457.3

Appendix A

Determination of the Content of the Food Additive 3-{1-[(3,5-Dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Article 5 in GB/T27579-2011

A.1.2 Column: reversed-phase liquid chromatography column

A.1.3 Detector: diode array detector

A.2 Determination Method

Internal standard method: determine the content according to Chapter 9 in GB/T27579—2011.

A.3 Repeatability and Result Expression

It is conducted according to provisions of Article 9.2 in GB/T27579-2011.

For the high performance liquid chromatogram of food additive 3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione, see Appendix B.

For the high performance liquid chromatogram of 3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione, see Appendix B.

Appendix B

High Performance Liquid Chromatogram of Food Additive 3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione (internal standard method)

B.1 High Performance Liquid Chromatogram of Food Additive 3-{1-[(3,5-Dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione

For the high performance liquid chromatogram of food additive 3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione, see Fig. B.1

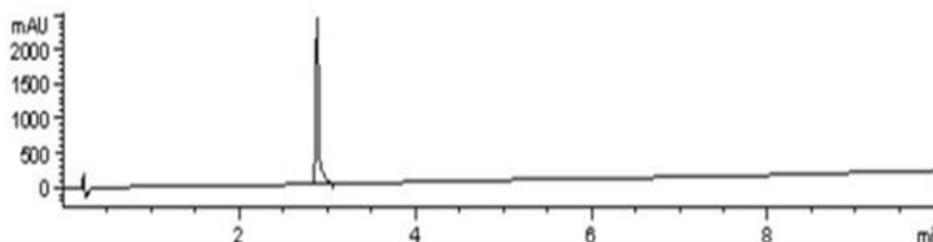


Fig.B.1 High Performance Liquid Chromatogram of Food Additive 3-{1-[(3,5-dimethyl-1,2-oxazol-4-yl)methyl]-1H-pyrazol-4-yl}-1-(3-hydroxybenzyl)imidazolidine-2,4-dione

B.2 Operation Conditions

B.2.1 Column: reversed-phase liquid chromatography column(Φ 4.6mm \times 150mm, particle diameter 4 μ m).

B.2.2 Mobile Phase A: 0.1% formic acid solution.

B.2.3 Mobile Phase B: 0.1% formic acid – acetonitrile solution.

B.2.4 Flow rate: 1mL/min.

B.2.5 Detection wavelength: 230nm.

B.2.6 Sample size: 1 μ L.

B.2.7 Column temperature: 25°C.

B.2.8 Gradient elution condition: see Table B.1.

Table B.1 Gradient Elution Condition

time (min)	mobile phase A (%)	mobile phase B (%)
0	95	5
20	5	95
25	5	95
27	95	5
30	95	5

III. 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic Acid Sulfate

English Name:

4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid sulfate

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1. Scope

The quality specifications and requirements apply to the food additive 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid sulfate produce through chemical reaction among isopropylamine, isopropylmagnesium chloride, methyl hydroxyl trimethyl acetate and ethyl acetoacetate.

2. Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

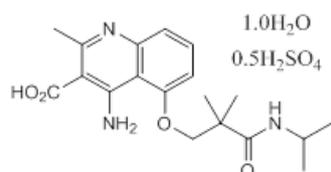
2.1 Chemical Name

4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid sulfate

2.2 Molecular Formula

$C_{19}H_{28}N_3O_7S_{0.5}$

2.3 Structural Formula



2.4 Relative Molecular Weight

426.47 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	white to light yellow	Place test samples on a clean, white paper, and observe them by eyes.
State	powder	
Aroma	mild aroma	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
content, w/% \geq	98.0	Appendix A

Appendix A

Determination of The Food Additive 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic Acid Sulfate 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic Acid Sulfate

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Article 5 in GB/T27579-2011

A.1.2 Column: reversed-phase liquid chromatography column

A.1.3 Detector: diode array detector

A.2 Determination Method

Internal standard method: determine the content according to Chapter 9 in GB/T27579-2011.

A.3 Repeatability and Result Expression

It is conducted according to provisions of Article 9.2 in GB/T27579-2011.

For the high performance liquid chromatogram of 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid sulfate, see Appendix B.

Appendix B

High Performance Liquid Chromatogram of the Food Additive 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic Acid Sulfate

(internal standard method)

B.1 High Performance Liquid Chromatogram of the Food Additive 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic Acid Sulfate

For the high performance liquid chromatogram of 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid sulfate, see Fig. B.1.

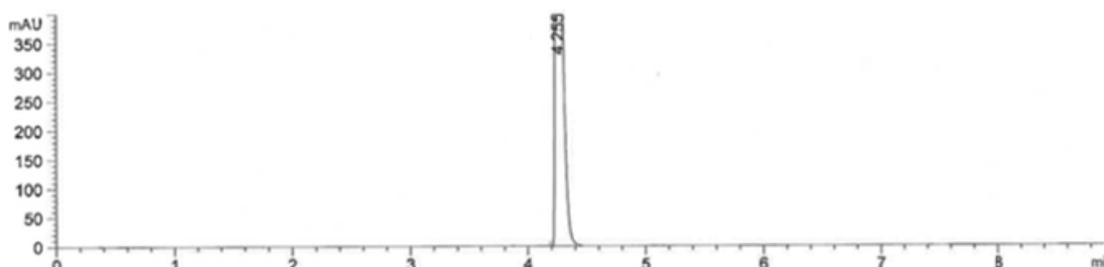


Fig.B.1 High Performance Liquid Chromatogram of the Food Additive 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic Acid Sulfate

B.2 Operation Conditions

- B.2.1 column: reversed-phase liquid chromatography column(Φ 4.6mm \times 150mm, particle diameter 4 μ m).
- B.2.2 Mobile phase A: 0.1% formic acid solution.
- B.2.3 Mobile phase B: 0.1% formic acid - acetonitrile solution.
- B.2.4 Flow rate: 1mL/min.
- B.2.5 Detection wavelength: 230nm.
- B.2.6 Sample size: 1 μ L.
- B.2.7 Column temperature: 25°C.
- B.2.8 Gradient elution condition: See table B.1.

TableB.1Gradient Elution Condition

time (min)	mobile phase A (%)	mobile phase B (%)
0	95	5
20	5	95
25	5	95
27	95	5
30	95	5

Attachment 2

Extension of the Range of Application for 8 Varieties of Food Additives Including Paprika Oleoresin

No.	Name	Function	Food Category No.	Food Name	Largest Application amount (g/kg)	Note
1.	paprika oleoresin	flavoring agent, colorant	04.04.01.02	dried beancurd	use with an appropriate amount depending on production demands	-
			09.04.02	cooked or fried aquatic products		
2.	paprika red	colorant	04.04.01.02	dried beancurd	use with an appropriate amount depending on production demands	-
			09.04.02	cooked or fried aquatic products		
3.	isomaltulose	edulcorant	05.01.02	chocolate and chocolate products, and cocoa products except 05.01.01	use with an appropriate amount depending on production demands	-
			05.01.03	CBR (cocoa butter replacer) chocolate and chocolate-like products using cocoa butter substitute		
			05.03	coatings for candies and chocolate products		
			06.10	fillings for cereal products		
			07.04	fillings and syrup for the surface of baked food		
4.	potassium sorbate	preservative	09.03.02	Pickled aquatic products (only limited to instant)	1.0	on the basis of sorbic acid
5.	sodium metabisulphite	Preservative, antioxidant	09.01	fresh aquatic products (only limited to seawater shrimps and crabs)	0.1	The largest application amount is calculated on the basis

			09.02	frozen aquatic products and their processed products (only limited to seawater shrimps and crabs and their products)		of the residual quantity of sulfur dioxide.
6.	shellac	colorant	16.03	collagen casings	use with an appropriate amount depending on production demands	-
7.	polydimethylsiloxane and its emulsion	processing agent for the food industry (defoamer)	-	processing for the potato	use with an appropriate amount depending on production demands	-
8.	octyl and decyl glycerate	processing agent for the food industry (antisticking agent)	-	processing for chocolate and chocolate products	0.08	-

Attachment 3**Extension of the Range of Application for the Food Nutrient Enhancer Selenium Enriched Yeast**

No.	Name	Function	Food Category No.	Food Name	Application Amount	Note
1.	selenium enriched yeast	food nutrient enhancer	01.03.02	modified milk powder (except milk powder for children)	140µg/kg~280µg/kg	on the basis of selenium
				modified milk powder (limited to milk powder for children)	60µg/kg~130µg/kg	
			06.02	rice and its products	140µg/kg~280µg/kg	
			06.03	wheat flour and its products	140µg/kg~280µg/kg	
			06.04	multi-grain and its products	140µg/kg~280µg/kg	
			07.01	bread	140µg/kg~280µg/kg	
			07.03	cookies	30µg/kg~110µg/kg	

**Announcement Concerning A New Variety of Food Flavor Ingredient (9-Decen-2-one),
Extension of the Range of Application for 7 Varieties of Food Additives Including Tea
Polyphenols and Extension of the Range of Application for the Food Nutrient Enhancer
Calcium**

2016 No. 14

According to Food Safety Law, the review body has organized experts to review the safety evaluation materials for and approved a new variety of food flavor ingredient (9-decen-2-one), extension of the range of application for 7 varieties of food additives including tea polyphenols and extension of the range of application for the food nutrient enhancer calcium.

It is hereby announced.

Attachment:

1. A New Variety of Food Flavor Ingredient, 9-Decen-2-one
2. Extension of the Range of Application for 7 Varieties of Food Additives Including Tea Polyphenols
3. Extension of the Range of Application for the Food Nutrient Enhancer calcium

National Health and Family Planning Commission of PRC

November 1, 2016

Attachment 1

A New Variety of Food Flavor Ingredient, 9-Decen-2-one

English Name: 9-Decen-2-one

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive 9-Decen-2-one derived from 10- hendecenoic acid

2.Chemical Name, Molecular Formula, Structural Formula, Molecular Weight

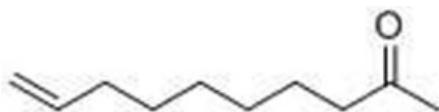
2.1 Chemical Name

9-Decen-2-one

2.2 Molecular Formula

C₁₀H₁₈O

2.3 Structural Formula



2.4 Relative Molecular Weight

154.25 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless to yellow	place the test samples in comparison tubes, and observe them by eyes
State	transparent liquid	
Aroma	with aroma of pears, pineapples and apples	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2Physical and Chemical Indexes

Item	Index	Test Method
content, w/% ≥	99	Appendix A
refraction index (20°C)	1.431~1.441	GB/T14454.4

relative density (25°C/25°C)	0.840~0.850	GB/T11540
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Appendix A

Determination of the Content of the Food Additive 9-Decen-2-one

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Article 5 in GB/T11538-2006

A.1.2 Column: capillary column

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: determine the content according to Chapter 10.4 in GB/T11538-2006

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive 9-Decen-2-one, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 9-Decen-2-one (normalization method)

B.1 Gas Chromatogram of the Food Additive 9-Decen-2-one

For the gas chromatogram of the food additive 9-Decen-2-one, see Fig. B.1.

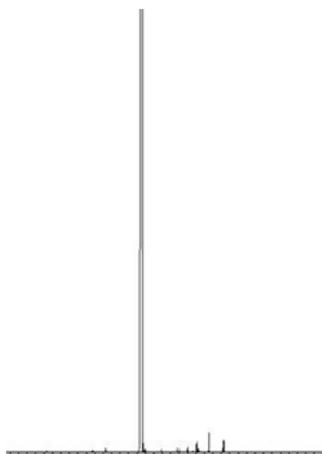


Fig.B.1 Gas Chromatogram of the Food Additive 9-Decen-2-one

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 60 m and an internal diameter of 0.25 mm.

B.2.2 Stationary phase: 100% polydimethylsiloxane

B.2.3 Membrane thickness: 0.25um

B.2.4 Temperature of chromatographic stove: keep the temperature of 70°C for 0 min, and raise the temperature to 220°C at a rate of 5°C per minute.

B.2.5 Injection-port temperature: 250°C

B.2.6 Detector temperature: 300°C

B.2.7 Detector: hydrogen flame ionization detector

B.2.8 Carrier gas: helium

B.2.9 Inlet pressure: 0.13MPa

B.2.10 Sample size: 1.0uL

B.2.11 Split ratio: 350: 1

Attachment 2

Extension of The Range of Application for 7 Food Additives Including Tea Polyphenols

No.	Name	Function	Food Category No.	Food Name	Largest Application amount (g/kg)	Note
1.	Tea polyphenols	antioxidant	04.01.02.05	jams	0.5	on the basis of catechuic acid
			11.05.01	fruit flavoring syrup		
2.	Carbon dioxide	others	14.01.01	natural mineral water for drinking	use with an appropriate amount depending on production demands	-
3.	Caramel color class I-plain	colorant	14.03.04	other protein drinks	use with an appropriate amount depending on production demands	-
4.	Lactic acid	acidity regulator	01.05.01	single cream	use with an appropriate amount depending on production demands	-
5.	Cellulose	antitackiness agent, stabilizer and coagulant, thickener	01.06	cheese, processed cheese and its similar products	use with an appropriate amount depending on production demands	-
			06.03.02.04	flour paste (such as topping paste for fish and poultry meat), coating flour, and flour for frying		
			07.0	baked food		

			08.03.04	spice sauce (mustard and green mustard)		
			08.03.05	sausages		
			12.05	sauce and sauce products		
			12.09.03	spice sauce (for example, mustard and green mustard)		
			16.03	collagen casings		
6.	Sodium sulfite	color fixative, antioxidant	04.01.02.05	jams	0.1	on the basis of the residual quantity of sulfur dioxide
7.	Polydimethylsiloxane and its emulsion	processing agent for the food industry (defoamer)	-	processing for livestock and poultry blood products	0.2	-

Attachment 3

Extension of The Range of Application for the Food Nutrient Enhancer Calcium

No.	Name	Function	Food Category No.	Food Name	Application amount	Note
1.	calcium	food nutrient enhancer	01.02.02	flavored fermented milk	250mg/kg~ 1000mg/kg	The chemical compound source of calcium shall meet provisions of Appendix B in GB 14880.

Interpretation on Announcement Concerning A New Variety of Food Flavor Ingredient (9-Decen-2-one) and Extension of the Range of Application for 7 Varieties of Food Additives Including Tea Polyphenols

I. 9-Decen-2-one

(I) Background information. The molecular formula of 9-Decen-2-one is C₁₀H₁₈O, and European Commission and Flavor and Extract Manufacturers Association of the United States have approved it as a food flavor ingredient for various kinds of food with an appropriate application amount depending on production demands.

(II) Technological Necessity. The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760) to improve the taste of food. The quality specifications shall be implemented according to relevant content of the Announcement.

II. Tea Polyphenols

(I) Background information. Tea polyphenols, as a food additive, has been listed in National Food Safety Standard Standard for Use of Food Additives(GB2760-2014) is permitted to be used for fat and oil basically containing no water, fried flour products, instant cereals, instant rice and wheat products, cakes, pot stewed meat products, fermented meat products, uncooked aquatic products, compound seasonings and plant protein drinks. This time it applies for extension of the range of application to jams (food category 04.01.02.05) and fruit flavoring syrup (food category 11.05.01).

(II) Technological Necessity. The substance, as an antioxidant, is used for jams (food category 04.01.02.05) and fruit flavoring syrup (food category 11.05.01) to retard browning of products. Its quality specifications shall be implemented according to Food Additive Tea Polyphenols (GB1886.211-2016).

III. Carbon Dioxide

(I) Background information. Carbon dioxide, as a food additive, has been listed in GB2760, and is permitted to be used for food categories including other candies except gum based candies, beverages, compound wine, other brewed wine (sparkling). Product categories in Natural Mineral Water for Drinking (GB8537) includes sparkling natural mineral water. This time it applies for extension of the range of application to natural mineral water for drinking (food category 14.01.01). Codex Alimentarius Commission, European Commission and U.S. Food and Drug Administration have approved it to be used for natural mineral water. According to the review results of FAO/WHO Expert Committee on Food Additive, the acceptable daily intake for the substance “needs no limit” .

(II) Technological Necessity. The substance is used for natural mineral water for drinking (food category 14.01.01) to adjust the taste of natural mineral water for drinking. Its quality specifications shall be implemented according to Food Additive Carbon Dioxide (GB1886.228-2016).

IV. Caramel Color ClassI- Plain

(I) Background information. caramel color classI- plain, as a food additive, has been listed in GB 2760, and is permitted to be used for food categories including formulated condensed milk, frozen drinks, jellies and puffed food. This time it applies for extension of the range of application to other protein drinks (food category 14.03.04). Codex Alimentarius Commission, European Commission and Food Standards Australia New Zealand have approved it as a colorant for food. According to the review results of FAO/WHO Expert Committee on Food Additive, the acceptable daily intake for the substance “needs no limit” .

(II) Technological Necessity. The substance, as a colorant, is used for other protein drinks (food category 14.03.04) to improve the sensory quality of products. Its quality specifications shall be implemented according to Food Additive Caramel Color (GB1886.64-2015).

V. Lactic Acid

(I) Background information. Lactic acid, as a food additive, has been listed in GB2760, and is permitted to be used for infant formula. This time it applies for extension of the range of application to single cream (food category 01.05.01). Codex Alimentarius Commission, European Commission and Food Standards Australia New Zealand have approved it as an acidity regulator for food. According to the review results of FAO/WHO Expert Committee on Food Additive, the acceptable daily intake for the substance “needs no limit” .

(II) Technological Necessity. The substance, as an acidity regulator, is used for single cream (food category 01.05.01) to adjust the acidity of single cream. Its quality specifications shall be implemented according to Food Additive lactic acid (GB1886.173-2016).

VI. Cellulose

(I) Background information. Cellulose, as a food additive, has been listed in GB2760, and is permitted to be used for processing of various food categories. This time it applies for extension of the range of application as an antitackiness agent , stabilizer, coagulant and thickener to cheese, processed cheese and its similar products (food category 01.06), flour paste (such as topping paste for fish and poultry meat), coating flour, flour for frying (food category 06.03.02.04), baked food (food category 07.0), Western hams (cured, smoked, steamed and stewed hams) (food category 08.03.04), sausages (food category 08.03.05), sauce and sauce products (food category 12.05), spice sauce (for example, mustard and green mustard) (food category 12.09.03) and collagen casings (food category 16.03). Codex Alimentarius Commission, European Commission and Food Standards Australia New Zealand have approved it as an antitackiness agent, stabilizer, coagulant and thickener for food. According to the review results of FAO/WHO Expert Committee on Food Additive, the acceptable daily intake for the substance “needs no limit” .

(II) Technological Necessity. The substance, as an antitackiness agent , stabilizer, coagulant and thickener, is used for various food categories to improve the quality of products. Its quality specifications shall be implemented according to Food Additive Cellulose (GB29946-2013).

VII. Sodium sulfite

(I) Background information. Sodium sulfite, as a food additive, has been listed in GB2760, and is permitted to be used for food categories including fresh fruit subject surface treatment , dried fruit and candied succades. This time it applies for extension of the range of application to jams (food category 04.01.02.05). Codex Alimentarius Commission, European Commission and Food Standards Australia New Zealand have approved it as a color fixative, antioxidant for food. According to the review results of FAO/WHO Expert Committee on Food Additive, the acceptable daily intake for the substance shall not exceed 0.7mg/kgbw (on the basis of sulfur dioxide).

(II) Technological Necessity. The substance, as a color fixative and antioxidant, is used for jams (food category 04.01.02.05) to improve the quality of products and restrain browning of products in the shelf life. Its quality specifications shall be implemented according to Food Additive Sodium sulfite (GB1886.8-2015).

VIII. Polydimethylsiloxane and Its Emulsion

(I) Background information. Polydimethylsiloxane and its emulsion, as a processing agent for the food industry, has been listed in GB2760, and is permitted to be used for processing for foods including bean products, meat products, beer, baked food, oil, fat and chips. This time it applies for extension of the range of application to processing for livestock and poultry blood products. European Commission, Food Standards Australia New Zealand, U.S. Food and Drug Administration and Ministry of Health, Labor and Welfare of Japan have approved it as a processing agent for the food industry. According to the review results of FAO/WHO

Expert Committee on Food Additive, the acceptable daily intake for the substance shall not exceed 1.5mg/kgbw.

(II) Technological Necessity. The substance, as a processing agent for the food industry, is used for processing of livestock and poultry blood products to eliminate air bubbles produced in processing of livestock and poultry blood. Its quality specifications shall be implemented according to Food Additive polydimethylsiloxane and its emulsion (GB30612-2014).

IX. Calcium

(I) Background information. Calcium, as a food nutrient enhancer, has been listed in National Food Safety Standard Standard for the Use of Food Nutrient Enhancers, and is permitted to be used for food categories including modified milk, modified milk powder, cheese and processed cheese. This time it applies for extension of the range of application to flavored fermented milk (food category 01.02.02). U.S. Food and Drug Administration have approved it to be used for food. According to the review results of FAO/WHO Expert Committee on Food Additive, the acceptable daily intake for the substance “needs no limit” .

(II) Technological Necessity. The substance, as a food nutrient enhancer, is used for flavored fermented milk (food category 01.02.02) to enhance the calcium element in food.

Relative link: Interpretation on Announcement Concerning A New Variety of Food Flavor Ingredient 9-Decen-2-one and Extension of the Range of Application for 7 Varieties of Food Additives Including Tea Polyphenols

Announcement Concerning A New Variety of Food Additive (Ammonium Carbonate), 9 New Varieties of Food Flavor Ingredients Including 6-Methylheptanal and Extension of The Range of Application for 2 Food Additives Including Sodium Metabisulphite

2017 No. 1

According to Food Safety Law, the review body has organized experts to review the safety evaluation materials for and approved a new variety of food additive, ammonium carbonate, 9 new varieties of food flavor ingredients including 6-Methylheptanal and extension of the range of application for 2 food additives including sodium metabisulphite.

It is hereby announced.

Attachment:

1. A New Variety of Food Additive, Ammonium Carbonate
2. 9 New Varieties of Food Flavor Ingredients Including 6-Methylheptanal
3. Extension of the Range of Application for 2 Food Additives Including Sodium Metabisulphite

National Health and Family Planning Commission of PRC

February 6, 2017

Attachment1

A New Variety of Food Additive, Ammonium Carbonate

English Name: Ammonium Carbonate

Functional classification: swelling agent

(I) Amount and Range of Application

Food Category No.	Food Name	the largest application amount/(g/kg)	Note
07.03	cookies	use with an appropriate amount depending on production demands	

(II) Quality Specification and Requirements

1. Scope

The quality specifications apply to the food additive ammonium carbonate derived from ammonia, carbon dioxide and water vapor which are subject to absorption, crystallization, separation and drying.

2. Molecular Formula



3. Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	white	Take an appropriate amount of sample, place it in a 50mL beaker, and observe its color, luster and state in natural light. Flap slightly with a hand to make a small amount of the gas go into the nose, and smell it.
Odor	strong ammonia odor	
State	crystalline powder	

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table2Physical and Chemical Indexes

Item	Index	Test Method
Content (on the basis of NH ₃), w/%	30.5~34.0	A.4 in Appendix A
Ignition residue, w/% ≤	0.1	GB/T9741
Chloride (on the basis of Cl)/(mg/kg) ≤	30	A.5 in Appendix A
Sulfate (on the basis of SO ₄)/(mg/kg) ≤	30	A.6 in Appendix A
Nonvolatile matter /(mg/kg) ≤	100	A.7 in Appendix A

Heavy metal (on the basis of Pb)/(mg/kg)	≤	10	GB5009.74
Total arsenic (on the basis of As)/(mg/kg)	≤	1.0	GB5009.11
Lead (Pb)/(mg/kg)	≤	1.0	GB5009.12

Appendix A

Test Method

A.1 Safety Instructions

A part of reagents used in the test methods in the quality specifications are corrosive, and operation personnel shall be cautious! If it is splashed onto skin, rinse it with water immediately, and seek medical help immediately in the case of severe injury. Operation with volatile . When an inflammable substance is used, heating with open fire is forbidden.

A.2 General Provisions

Reagents and water used under the quality specifications are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard titration solutions in the test and standard solutions, preparations and products for determination of impurity are prepared according to provisions of GB/T 601, GB/T602 and GB/T603, unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.3 Identification Test

A.3.1 Reagents and Materials

A.3.1.1 Hydrochloric acid solution: 1+1.

A.3.1.2 Red litmus paper.

A.3.2 Identification

A.3.2.1 Identification of carbonate

When hydrochloric acid solution is added to the test sample, air bubbles are produced.

A.3.2.2 Hot test

The test sample decomposes when heated, and the produced vapor can make wet red litmus paper become blue.

A.4 Determination of the Content (on the basis of NH₃)

A.4.1 Method Abstract

The test sample is soluble in water, take methyl orange as the indicator, titrate with the standard titration solution of hydrochloric acid, and determine the ammonia content.

A.4.2 Reagents and Materials

A.4.2.1 standard titration solution of hydrochloric acid: c(HCl)=1mol/L.

A.4.2.2 methyl orange indication solution

A.4.3 Analysis Steps

Weigh and take 1.5 ~ 2.0g test sample, with a precision of 0.0001g, place it in a 250mL conical flask, add 100mL water to make it fully dissolved. Add 3 drops of methyl orange indication solution, titrate it with the standard titration solution with hydrochloric acid until the yellow test solution turns into orange.

A.4.4 Result Calculation

The mass fraction of the content (on the basis of NH₃), w₁, is calculated according to Formula (A.1):

$$w_1 = \frac{c \times V \times M}{m \times 1000} \times 100\% \dots\dots\dots (A.1)$$

Where:

V – the volume of standard titration solution of hydrochloric acid consumed by titration of test solution, in terms of milliliter (mL);

C – the concentration of the standard titration solution of hydrochloric acid, in terms of liter/mol (mol/L);

m- the mass of sample, with a unit of gram (g);

M- the molar mass of ammonia, in terms of gram/mol (g/mol)[M(NH₃)=17];

1000 - conversion factor.

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 0.2%.

A.5 Determination of Chloride (on the basis of Cl)

A.5.1 Method Abstract

Add silver nitrate solution in acid medium. When white silver chloride suspension is produced from chloridion, compare it with standard turbid solution.

A.5.2 Reagents and Materials

A.5.2.1 Nitric acid solution: the mass fraction is 10%.

A.5.2.2 Silver nitrate solution: 17g/L.

A.5.2.3 Sodium carbonate.

A.5.2.4chloride standard solution: 1mL solution contains 10µg chlorine (Cl). Standard solution of chloride: 1mL solution contains 10µg chlorine (Cl).

Weigh 165mg sodium chloride, place it in a 100mL volumetric flask, add distilled water until it reaches the tick mark, prepare it into standard stock solution of sodium chloride. Each milliliter of the solution contains 0.01mg chlorine.

A.5.3 Analysis Steps

Weigh 0.5g test sample, place it in 50mL flask, add 10mL distilled water to make it dissolved. Add 5mg sodium carbonate, place it on vapor bath for slow evaporation until it is dried. Then dissolve the residue with 30mL distilled water, acidize it with hydrogen nitrate, add 1mL silver nitrate solution, dilute it to the graduation, shake up, keep it still for 5 min and then

standard turbid solution : take 1.5mL chloride standard solution, place it in a 50mL comparison tube, add 40mL distilled water into the tube, acidize it with nitric acid, add 1mL silver nitrate solution, dilute it to the graduation with water, and shake up.

Protect the test solution from light.

A.6 Determination of Sulfate (on the basis of SO₄)

A.6.1 Method Abstract

Add hydrogen peroxide into the test sample to make various ions containing sulfur transferred to sulfate ions which react with barium ions in acid mediums to produce white barium sulfate aerosols, and compare it with standard turbid solution.

A.6.2 Reagents and Materials

A.6.2.1 Hydrogen peroxide: the mass fraction is 30%.

A.6.2.2 hydrochloric acid: the mass fraction is 10%.

A.6.2.3 Sodium carbonate.

A.6.2.4 Barium chloride solution: the mass fraction is 10%.

A.6.2.5 Sulfate standard solution: 1mL solution contains 10 μ g sulfate radical (SO₄).

Weigh 48mg anhydrous sodium sulfate, place it in a 100mL volumetric flask, dissolve it with distilled water until it reaches the tick mark, prepare it into sulfate standard stock solution. Suck 10mL sulfate standard stock solution, place it in a 1000mL volumetric flask, and add distilled water until it reaches the tick mark. Each milliliter of the solution contains 10 μ g sulfate ions.

A.6.3 Analysis Steps

Weigh 4g test sample, place it in a 50mL flask, and add 40mL distilled water to dissolve it. Add 10mg sodium carbonate and 1mL 30% hydrogen peroxide, and place it on the vapor bath for slow evaporation until it is dried. Then use 40mL distilled water to dissolve the residue, acidize it with hydrochloric acid, add 3mL barium chloride solution, dilute it with water to the graduation, shake up, keep it still for 10 min, and then carry out turbidimetry. Its turbidity shall not exceed that produced by standard turbid solution.

standard turbid solution: take 20mL sulfate standard solution, place it in a 50mL flask, add 20mL distilled water, and acidize it with hydrochloric acid. Add 3mL barium chloride solution, dilute it to the graduation with water, and shake up.

A.7 Determination of Nonvolatile Matter

A.7.1 Method Abstract

Place the sample on a evaporating dish, place the dish on a vapor bath for evaporation until it is dried, dry it in an electrothermal constant-temperature dry box until the mass is constant, and weigh the mass of nonvolatile matter.

A.7.2 Instrument and Equipment

A.7.2.1 porcelain evaporating dish: 50mL.

A.7.2.2 electrothermal Constant-temperature Dry Box: can control the temperature the range of 105 $^{\circ}$ C~110 $^{\circ}$ C.

A.7.3 Analysis Steps

Weigh about 4g test sample, with a precision of 0.0002g, place it on a porcelain evaporating dish which is preheated at 105 $^{\circ}$ C~110 $^{\circ}$ C, and add 10mL water into it. Place it in an electrothermal constant-temperature dry box, dry it for 1h at 105 $^{\circ}$ C~110 $^{\circ}$ C, then put it in a drier to cool it, and weigh it.

A.7.4 Result Calculation

The mass fraction of the nonvolatile matter content is calculated according to Formula (A.2).

$$w_2 = \frac{m_1 - m_2}{m_3} \times 100\% \dots\dots\dots (A.2)$$

Where:

m_1 - mass of nonvolatile matter and evaporating dish after drying, in terms of gram (g);

m_2 - mass of evaporating dish, in terms of gram (g);

m_3 - mass of the sample, with a unit of gram (g);

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 0.005%.

Attachment 2

9 New Varieties of Food Flavor Ingredients Including 6-Methylheptanal

I. 6-Methylheptanal

English Name: 6-Methylheptanal

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive 6-methylheptanal produced through chemical reaction between hexane,1-chloro-5-methyl- and N,N-dimethylformamide.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

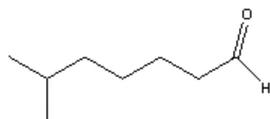
2.1 Chemical Name

6-Methylheptanal

2.2 Molecular Formula

C₈H₁₆O

2.3 Structural Formula



2.4 Relative Molecular Weight

128.21 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: shall meet provisions of Table 1.

Table 1Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless, transparent	place the test samples in comparison tubes, and observe them by eyes
State	liquid	
Aroma	delicate orange-like fragrance	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
relative density (25°C/25°C)	0.806~0.816	GB/T11540
refraction index (20°C)	1.411~1.416	GB/T14454.4
acid value (on the basis of KOH)/(mg/g) <	2	GB/T14455.5
6-methylheptanal content, w/% ≥	98.0	Appendix A

Appendix A

Determination of the Content of the Food Additive 6-Methylheptanal

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive 6-methylheptanal, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 6-Methylheptanal (normalization method)

B.1 Gas Chromatogram of the Food Additive 6-Methylheptanal

For the gas chromatogram of food additive 6-methylheptanal, see Table B.1.

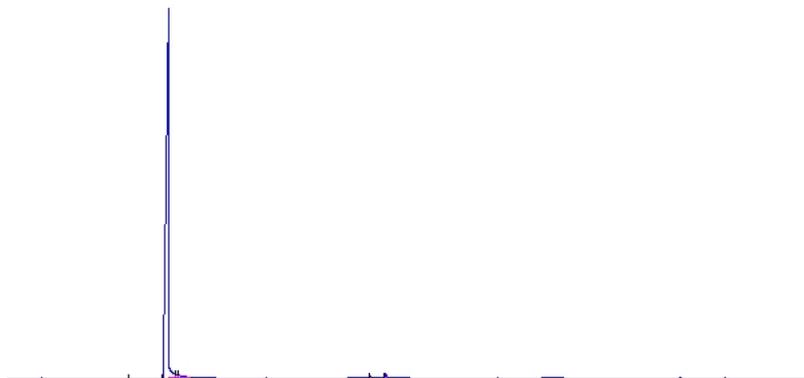


Fig.B.1 Gas Chromatogram of the Food Additive 6-Methylheptanal

B.2 Operation Conditions

- B.2.1 Column: capillary column, with a length of 30 m and an internal diameter of 0.32 mm.
- B.2.2 stationary phase: polyethylene glycol 20000
- B.2.3 Membrane thickness: 0.25 μ m
- B.2.4 Temperature of chromatographic stove: raise the temperature to 240°C from 35°C with a linear temperature program at a rate of 10°C/min, and at last keep the constant temperature of 240°C for 10 min.
- B.2.5 Injection-port temperature: 250°C
- B.2.6 Detector temperature: 250°C
- B.2.7 Detector: hydrogen flame ionization detector
- B.2.8 Carrier gas: helium
- B.2.9 Flow rate of carrier gas: inlet pressure 15kPa
- B.2.10 Sample size: about 1 μ L
- B.2.11 Split ratio: 50: 1

II. Cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

English Name: Cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide produce through chemical reaction among 2- isopropyl-5- methylcyclohexanone, ammonium formate and cyclopropanecarbonyl chloride.

2.Chemical Name, Molecular Formula, Structural Formula, Molecular Weight

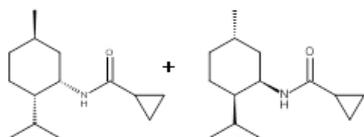
2.1 Chemical Name

Cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

2.2 Molecular Formula

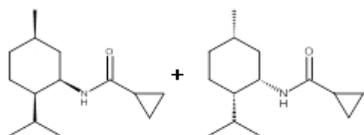
C₁₄H₂₅NO

2.3 Structural Formula



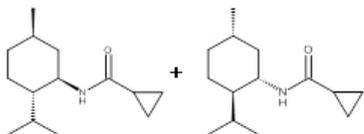
(1S,2S,5R)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

and (1R,2R,5S)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide



(1R,2R,5R) -cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

and (1S,2S,5S)- cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide;



(1R,2S,5R)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

and (1S,2R,5S)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

2.4 Relative Molecular Weight

223.4 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	pearl white	Place test samples on a clean, white paper, and observe them by eyes.
State	powder	
Aroma	aroma of salted meat gravy	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item		Index	Test Method
Cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide,w/%	≥	96.0 (the sum of three pairs of optical isomers)	Appendix A
Solubility (25°C)		0.83g sample is fully dissolved in 10mL 95% (volume fraction) ethanol solution, and the solution is clear and transparent.	GB/T14455.3
Melting point/°C	≥	166	GB/T14457.3

Appendix A

Determination of Content of the Food Additive Cyclopropanecarboxylic Acid (2-isopropyl-5-methyl-cyclohexyl)-amide

A.1 Instrument and Equipment

- A.1.1 Chromatograph: comply with provisions of Article 5 in GB/T11538-2006
- A.1.2 Column: capillary column
- A.1.3 Detector: hydrogen flame ionization detector

A.2 Determination Method

normalization method: determine the content according to Chapter 10.4 in GB/T11538-2006

Preparation of test sample: weigh 1g of the product, dissolve it in 10mL absolute ethyl alcohol, and shake up for future use.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

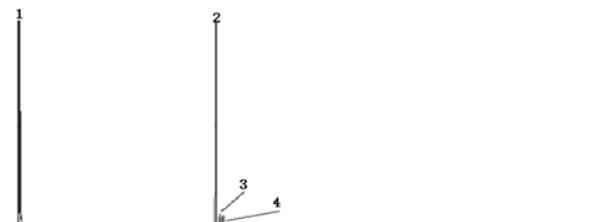
For the gas chromatogram and operation conditions of the food additive cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide, see Appendix B.

Appendix B

**Gas Chromatogram and Operation Conditions of the Food Additive
Cyclopropanecarboxylic Acid(2-isopropyl-5-methyl-cyclohexyl)-amide
(normalization method)**

B.1 Gas Chromatogram of the Food Additive Cyclopropanecarboxylic Acid(2-isopropyl-5-methyl-cyclohexyl)-amide

For the gas chromatogram of the food additive cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide, see Fig. B.1.



Description:

1 - solvent (absolute ethyl alcohol)

(1S,2S,5R)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide and
(1R,2R,5S)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide

3 - (1R,2R,5R) -cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide and
(1S,2R,5S)- cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide;

4 - (1R,2S,5R)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide and
(1S,2R,5S)-cyclopropanecarboxylic acid(2-isopropyl-5-methyl-cyclohexyl)-amide;

Fig.B.1 Gas Chromatogram of the Food Additive Cyclopropanecarboxylic Acid(2-isopropyl-5-methyl-cyclohexyl)-amide

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 50 m and an internal diameter of 0.25 mm.

B.2.2 Stationary phase: polyethylene glycol

B.2.3 Membrane thickness: 0.25 μ m

B.2.4 Temperature of chromatographic stove: 5min keep a constant temperature of 60°C for 5 min, and then raise the temperature to 250°C from 60°C with a linear temperature program at a rate of 5°C/min.

B.2.5 Injection-port temperature: 250°C

B.2.6 Detector temperature: 280°C

B.2.7 Detector: hydrogen flame ionization detector

B.2.8 Carrier gas: nitrogen

B.2.9 Flow rate of carrier gas: 2.0mL/min

B.2.10 Sample size: 1 μ L

B.2.11 Split ratio: 30: 1

III. 4-Hydroxy-4-methyl-5-hexenoic Acid Gamma Lactone

English Name: 4-Hydroxy-4-methyl-5-hexenoic acid gamma lactone

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive 4-hydroxy-4-methyl-5-hexenoic acid gamma lactone derived from epoxydihydrolinalool, linalool oxide subject to chemical reaction.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

2.1 Chemical Name

4-Hydroxy-4-methyl-5-hexenoic acid gamma lactone

2.2 Molecular Formula

$C_7H_{10}O_2$

2.3 Structural Formula



2.4 Relative Molecular Weight

126.15 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1Sensory Requirements

Item	Requirement	Test Method
color and luster	colorless	place the test samples in comparison tubes, and observe them by eyes
state	liquid	
aroma	flower fragrance	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table2Physical and Chemical Indexes

Item	Index	Test Method
4-Hydroxy-4-methyl-5-hexenoic acid gamma lactone content, w/% \geq	97.0	Appendix A
acid value (on the basis of KOH)/(mg/g) \leq	1.0	GB/T14455.5

refraction index (20°C)	1.440~1.462	GB/T14454.4
relative density (20°C/20°C)	1.015~1.025	GB/T11540

Appendix A

Determination of the Content of the Food Additive 4-Hydroxy-4-methyl-5-hexenoic Acid Gamma Lactone

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Article 5 in GB/T11538-2006

A.1.2 Column: capillary column

A.1.3 Detector: hydrogen flame ionization detector

A.2 Determination Method

normalization method: determine the content according to Chapter 10.4 in GB/T11538-2006

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive 4-hydroxy-4-methyl-5-hexenoic acid gamma lactone, see Appendix B

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 4-Hydroxy-4-methyl-5-hexenoic Acid Gamma Lactone

(normalization method)

B.1 Gas Chromatogram of the Food Additive 4-Hydroxy-4-methyl-5-hexenoic Acid Gamma Lactone

For the gas chromatogram of the food additive 4-hydroxy-4-methyl-5-hexenoic acid gamma lactone, see Fig. B.1.

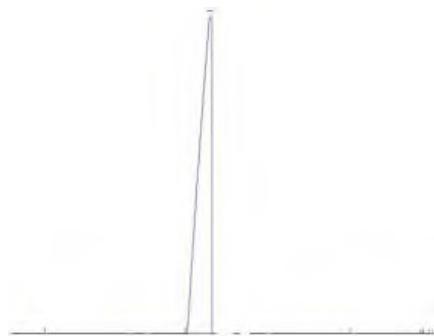


Fig.B.1 Gas Chromatogram of the Food Additive 4-Hydroxy-4-methyl-5-hexenoic Acid Gamma Lactone

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 50 m and an internal diameter of 0.32 mm.

B.2.2 Stationary phase: methylsilane

- B.2.3 Membrane thickness: 0.50 μ m
- B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75°C for 4 min, then raise the temperature to 225°C from 75°C with a linear temperature program at a rate of 2°C/min, and at last keep the temperature of 225°C for 8 min.
- B.2.5 Injection-port temperature: 250°C
- B.2.6 Detector temperature: 250°C
- B.2.7 Detector: hydrogen flame ionization detector
- B.2.8 Carrier gas: nitrogen
- B.2.9 Inlet pressure: 0.06MPa
- B.2.10 Sample size: 0.1 μ L
- B.2.11 Split ratio: 75: 1

IV. Furfuryl 2-methyl-3-furyl Disulfide

English Name: Furfuryl 2-methyl-3-furyl disulfide

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760-2014), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive furfuryl 2-methyl-3-furyl disulfide produced through chemical reaction between furfuryl mercaptan and 2-methyl-3-furanthiol.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

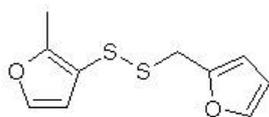
2.1 Chemical Name

Furfuryl 2-methyl-3-furyl disulfide

2.2 Molecular Formula

C₁₀H₁₀O₂S₂

2.3 Structural Formula



2.4 Relative Molecular Weight

226.32 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 **Sensory Requirements:** Sensory requirements shall meet provisions of Table 1.

Table1Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless, becoming yellow to brown in the case of long-time placement.	place the test samples in comparison tubes, and observe them by eyes
State	liquid	
Aroma	odor of sulfur, odor of stewed meat	GB/T14454.2

3.2 **Physical and chemical indexes shall meet provisions of Table 2.**

Table2Physical and Chemical Indexes

Item	Index	Test Method
furfuryl 2-methyl-3-furyl disulfide content, w/% ≥	90.0	Appendix A

relative density (25°C/25°C)	1.227~1.283	GB/T11540
refraction index (20°C)	1.581~1.587	GB/T14454.4
acid value (on the basis of KOH)/(mg/g)≤	3	GB/T14455.5

Appendix A

Determination of Content of the Food Additive Furfuryl 2-Methyl-3-furyl Disulfide

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Article 5 in GB/T11538-2006

A.1.2 Column: capillary column

A.1.3 Detector: hydrogen flame ionization detector

A.2 Determination Method

normalization method: determine the content according to Chapter 10.4 in GB/T11538-2006

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive furfuryl 2-methyl-3-furyl disulfide, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive Furfuryl 2-Methyl-3-furyl Disulfide

(normalization method)

B.1 Gas Chromatogram of the Food Additive Furfuryl 2-Methyl-3-furyl Disulfide

For the gas chromatogram of food additive Furfuryl 2-methyl-3-furyl disulfide, see Fig. B.1.



Description:

- 1- Bis(2-methyl-3-furyl)disulfide
- 2- Furfuryl 2-methyl-3-furyl disulfide
- 3- Furan, 2,2'-[dithiobis(methylene)]bis-

Fig.B.1 Gas Chromatogram of the Food Additive Furfuryl 2-methyl-3-furyl Disulfide

B.2 Operation Conditions

- B.2.1 Column: capillary column, with a length of 50 m and an internal diameter of 0.32 mm.
- B.2.2 Stationary phase: methylsilane.
- B.2.3 Membrane thickness: 0.5 μ m.
- B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75°C for 4 min, then raise the temperature to 220°C from 75°C with a linear temperature program at a rate of 2°C/min, and at last keep the temperature of 225°C for 30 min.
- B.2.5 Injection-port temperature: 250°C.
- B.2.6 Detector temperature: 250°C.
- B.2.7 Detector: hydrogen flame ionization detector.
- B.2.8 Carrier gas: nitrogen.
- B.2.9 Inlet pressure: 0.06MPa.
- B.2.10 Sample size: 0.1 μ L.
- B.2.11 Split ratio: 75: 1.

V. 4-Decenoic acid

English Name: 4-Decenoic acid

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760-2014), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive 4-decenoic acid produced from 1-Octen-3-ol and triethyl orthoacetate.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

2.1 Chemical Name

4-Decenoic acid

2.2 Molecular Formula

C₁₀H₁₈O₂

2.3 Structural Formula



2.4 Relative Molecular Weight

170.25 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table1Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless	place the test samples in comparison tubes, and observe them by eyes
State	transparent liquid	
Aroma	fruit aroma	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table2 Physical and Chemical Indexes

Item	Index	Test Method
Solubility (25°C)	1g test sample is fully dissolved in 1mL 95% ethanol (volume fraction).	GB/T14455.3
4-Decenoic acid content, w/% ≥	97	Appendix A
refraction index (20°C)	1.140~1.160	GB/T14454.4
relative density (20°C/20°C)	0.915~0.925	GB/T11540

Appendix A

Determination of 4-Decenoic Acid Content

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of food additive 4-Decenoic acid, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 4-Decenoic Acid (normalization method)

B.1 Gas Chromatogram of the Food Additive 4-Decenoic acid

For the gas chromatogram of food additive 4-Decenoic acid, see Fig. B.1.

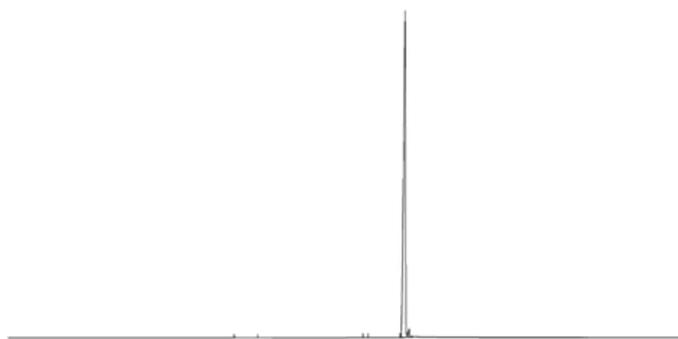


Fig.B.1 Gas Chromatogram of the Food Additive 4-Decenoic acid

B.2 Operation Conditions

- B.2.1 Column: capillary column, with a length of 30 m and an internal diameter of 0.25 mm.
- B.2.2 stationary phase: polyethylene glycol 20000.
- B.2.3 Membrane thickness: 0.25 μ m.
- B.2.4 Temperature of chromatographic stove: raise the temperature to 230°C from 150°C with a linear temperature program at a rate of 5°C/min, and at last keep the constant temperature of 230°C for 10 min.
- B.2.5 Injection-port temperature: 250°C.
- B.2.6 Detector temperature: 300°C.
- B.2.7 Detector: hydrogen flame ionization detector.
- B.2.8 Carrier gas: nitrogen.
- B.2.9 Flow rate of carrier gas: 1mL/min.
- B.2.10 Sample size: 0.2 μ L.
- B.2.11 Split ratio: 100: 1.

VI. 2-(4-Methyl-5-thiazolyl)ethyl Propionate

English Name: 2-(4-methyl-5-thiazolyl)ethyl propionate

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760-2014), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive 2-(4-methyl-5-thiazolyl)ethyl propionate produced from 2-(4-methyl-5-thiazolyl)ethanol 2-(4-Methyl-5-thiazolyl) ethanol and propionic acid.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

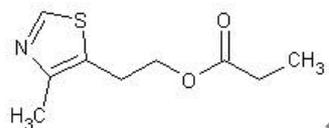
2.1 Chemical Name

2-(4-methyl-5-thiazolyl)ethyl propionate

2.2 Molecular Formula

C₉H₁₃O₂NS

2.3 Structural Formula



2.4 Relative Molecular Weight

199.27 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table1Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless to yellow	place the test samples in comparison tubes, and observe them by eyes
State	transparent liquid	
Aroma	with baking and nut-like aroma	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table2Physical and Chemical Indexes

Item	Index	Test Method
Solubility (25°C)	1g test sample is fully dissolved in 1mL 95% ethanol (volume fraction).	GB/T14455.3

2-(4-methyl-5-thiazolyl)ethyl propionate content, w/% \geq		Appendix A
acid value (on the basis of KOH)/(mg/g) \leq	1	GB/T14455.5
refraction index (20°C)	1.502~1.506	GB/T14454.4
relative density (25°C/25°C)	1.136~1.140	GB/T11540

Appendix A

Determination of the Food Additive 2-(4-Methyl-5-thiazolyl)ethyl Propionate Content

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

Normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive 2-(4-methyl-5-thiazolyl)ethyl propionate, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 2-(4-Methyl-5-thiazolyl)ethyl Propionate (normalization method)

B.1 Gas Chromatogram of the Food Additive 2-(4-Methyl-5-thiazolyl)ethyl Propionate

For the gas chromatogram of the food additive 2-(4-methyl-5-thiazolyl)ethyl propionate, see Fig. B.1.

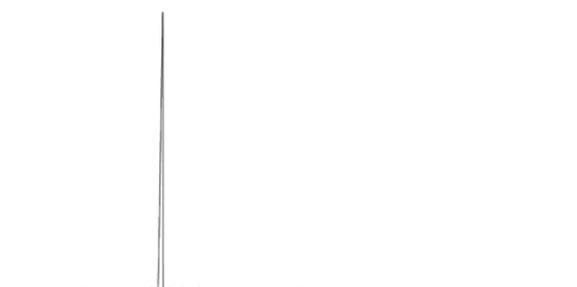


Fig. B.1 Gas Chromatogram of the Food Additive 2-(4-Methyl-5-thiazolyl)ethyl Propionate

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 30 m and an internal diameter of 0.25 mm.

B.2.2 stationary phase: polyethylene glycol 20000.

B.2.3 Membrane thickness: 0.25 μ m.

B.2.4 Temperature of chromatographic stove: constant temperature of 220°C.

B.2.5 Injection-port temperature: 250°C.

B.2.6 Detector temperature: 250°C.

B.2.7 Detector: hydrogen flame ionization detector.

B.2.8 Carrier gas: nitrogen.

B.2.9 Flow rate of carrier gas: 1mL/min.

B.2.10 Sample size: 0.2 μ L.

B.2.11 Split ratio: 100: 1.

VII. 4,5-Octanedione

English Name: 4,5-Octanedione

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760-2014), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive 4,5-Octanedione produced from 5-hydroxy-4-octanone.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

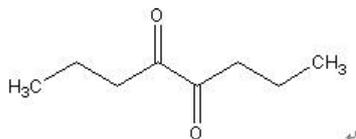
2.1 Chemical Name

4,5-Octanedione

2.2 Molecular Formula

C₈H₁₄O₂

2.3 Structural Formula



2.4 Relative Molecular Weight

142.2 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table1Sensory Requirements

Item	Requirement	Test Method
color and luster	yellow	place the test samples in comparison tubes, and observe them by eyes
state	transparent liquid	
aroma	There is strong fat and butter odor in the case of high concentration and pleasant cream-like aroma after dilution.	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table2Physical and Chemical Indexes

Item	Index	Test Method
solubility (25°C)	1g test sample is fully dissolved in 1mL 95% ethanol (volume fraction).	GB/T14455.3
4,5-octanedione content, w/% \geq	95	Appendix A
refraction index (20°C)	1.414~1.424	GB/T14454.4
relative density (20°C/20°C)	0.908~0.918	GB/T11540

Appendix A

Determination of Content of the Food Additive 4,5-Octanedione

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive 4,5-Octanedione, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 4,5-Octanedione (normalization method)

B.1 Gas Chromatogram of the Food Additive 4,5-Octanedione

For the gas chromatogram of the food additive 4,5-Octanedione, see Table B.1.



Fig. B.1 Gas Chromatogram of the Food Additive 4,5-Octanedione

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 60 m and an internal diameter of 0.32 mm.

B.2.2 Stationary phase: polyethylene glycol 20000.

B.2.3 Membrane thickness: 0.25 μ m.

B.2.4 Temperature of chromatographic stove: constant temperature of 200°C.

B.2.5 Injection-port temperature: 250°C.

B.2.6 Detector temperature: 250°C.

B.2.7 Detector: hydrogen flame ionization detector.

B.2.8 Carrier gas: nitrogen.

B.2.9 Flow rate of carrier gas: 1mL/min.

B.2.10 Sample size: 0.2 μ L.

B.2.11 Split ratio: 100: 1.

VIII. Ethyl 5-hydroxydecanoate

English Name: Ethyl 5-hydroxydecanoate

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760-2014), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to ethyl 5-hydroxydecanoate produced from δ -decanolactone.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

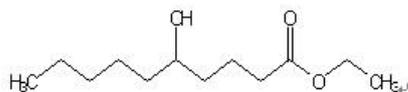
2.1 Chemical Name

Ethyl 5-hydroxydecanoate

2.2 Molecular Formula

$C_{12}H_{24}O_3$

2.3 Structural Formula



2.4 Relative Molecular Weight

216.32 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table1Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless	place the test samples in comparison tubes, and observe them by eyes
State	transparent liquid	
Aroma	sweet fat and peach-like aroma	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
Solubility (25°C)	1g test sample is fully dissolved in 1mL 95% ethanol (volume fraction).	GB/T14455.3
Ethyl 5-hydroxydecanoate,	56	Appendix A

w/% ≥		
acid value (on the basis of KOH)/(mg/g) ≤	10	GB/T14455.5
refraction index (20°C)	1.442~1.452	GB/T14454.4
relative density (20°C/20°C)	0.945~0.956	GB/T11540

Appendix A

Determination of Content of the Food Additive Ethyl 5-hydroxydecanoate

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of food additive ethyl 5-hydroxydecanoate, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive Ethyl 5-hydroxydecanoate

(normalization method)

B.1 Gas Chromatogram of the Food Additive Ethyl 5-hydroxydecanoate

For the gas chromatogram of the food additive ethyl 5-hydroxydecanoate, see Table B.1.

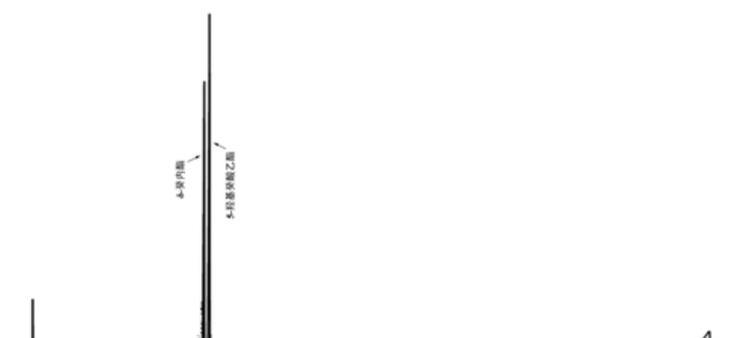


Fig. B.1 Gas Chromatogram of the Food Additive Ethyl 5-hydroxydecanoate

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 30 m and an internal diameter of 0.32 mm.

B.2.2 Stationary phase: (5% phenyl) Polymethylsiloxane

B.2.3 Membrane thickness: 0.25 μ m.

B.2.4 Temperature of chromatographic stove: raise the temperature to 280°C from 160°C with a linear temperature program at a rate of 20°C/min, and keep the constant temperature of 280°C for 5 min; then raise the temperature to 300°C from 280°C with a linear temperature program at a rate of 30°C/min, and at last keep the constant temperature of 230°C for 15 min.

B.2.5 Injection-port temperature: 250°C.

B.2.6 Detector temperature: 250°C.

B.2.7 Detector: hydrogen flame ionization detector.

B.2.8 Carrier gas: nitrogen.

B.2.9 Flow rate of carrier gas: 1 mL/min.

B.2.10 Sample size: 0.2 μ L.

B.2.11 Split ratio: 100: 1.

IX. Dioctyl Adipate

English Name: Dioctyl adipate

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive of dioctyl adipate derived from adipic acid and 1-octyl alcohol.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

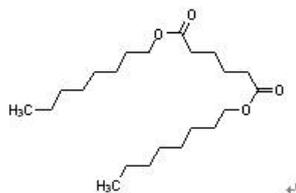
2.1 Chemical Name

Dioctyl adipate

2.2 Molecular Formula

C₂₂H₄₂O₄

2.3 Structural Formula



2.4 Relative Molecular Weight

370.57 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless	place the test samples in comparison tubes, and observe them by eyes
Sate	transparent liquid	
Aroma	slight fat aroma	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2Physical and Chemical Indexes

Item	Index	Test Method
Solubility (25°C)	1g test sample is fully dissolved in 1mL 95% ethanol (volume fraction).	GB/T14455.3

Diethyl adipate content, w/% ≥	98	Appendix A
acid value (on the basis of KOH)/(mg/g) ≤	1	GB/T14455.5
refraction index (20°C)	1.444~1.450	GB/T14454.4
relative density (20°C/20°C)	0.924~0.930	GB/T11540

Appendix A

Determination of Content of the Food Additive Diethyl Adipate

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

Normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall be conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of food additive diethyl adipate, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive Diethyl Adipate (normalization method)

B.1 Gas Chromatogram of the Food Additive Diethyl Adipate

For the gas chromatogram of the food additive diethyl adipate, see Fig. B.1.

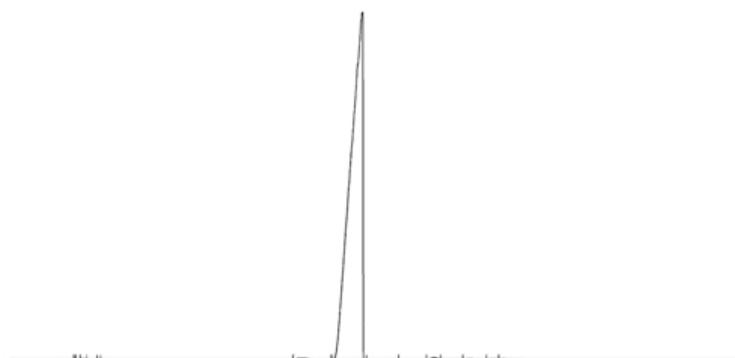


Fig. B.1 Gas Chromatogram of the Food Additive Diethyl Adipate

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 30 m and an internal diameter of 0.25 mm.

- B.2.2 stationary phase: polyethylene glycol 20000.
- B.2.3 Membrane thickness: 0.25 μ m.
- B.2.4 Temperature of chromatographic stove: constant temperature of 230°C.
- B.2.5 Injection-port temperature: 250°C.
- B.2.6 Detector temperature: 250°C.
- B.2.7 Detector: hydrogen flame ionization detector.
- B.2.8 Carrier gas: nitrogen.
- B.2.9 Flow rate of carrier gas: 1mL/min.
- B.2.10 Sample size: 0.2 μ L.
- B.2.11 Split ratio: 100: 1.

Attachment3

Extension of the Range of Application of 2 Varieties of Food Additives Including Sodium Metabisulphite

No.	Name	Function	Food Category No.	Food Name	Largest Application amount (g/kg)	Note
1	sodium metabisulphite	antioxidant	04.02.02.04	canned vegetable(only limited to silver dishes)	0.2	on the basis of the residual quantity of sulfur dioxide
2	glucono- δ -lactone	acidity regulator	01.05.01	single cream	use with an appropriate amount depending on production demands	

Announcement on A New Variety of Food Additive (Glycine (Glycolonitrile Method))

2017 No.3

According to Food Safety Law, the review body has organized experts to review the safety evaluation materials for and approved a new variety of food additive, glycine (glycolonitrile method), a new variety of food flavor ingredient ethyl linalyl ether and extension of the range of application for food additive β -carotene.

It is hereby announced.

Attachment:

1. A New Variety of Food Additive, Glycine (Glycolonitrile Method)
2. A New Variety of Food Flavor Ingredient, Ethyl Linalyl Ether
3. Extension of the Range of Application for Food Additive β -Carotene

National Health and Family Planning Commission of PRC

March 8, 2017

Relate links:

1. A New Variety of Food Additive, Glycine(Glycolonitrile Method)
2. A New Variety of Food Flavor Ingredient Ethyl Linalyl Ether
3. Extension of the Range of Application for Food Additive β -Carotene

Attachment 1

The New Variety of Food Additive Glycine(Glycolonitrile Method)

English Name: Glycine(Glycolonitrile method)

Functional classification: flavoring agent, food flavor ingredient

(I) Amount and Range of Application

The amount and range of application shall meet provisions on glycine in GB2760.

(II) Quality Specification and Requirements

1.Scope

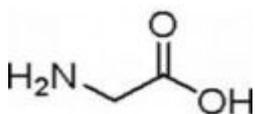
The quality specifications apply to the food additive glycine(glycolonitrile method)produced with ion exchange and membrane decolorization for sodium glycinate produced with ammoniation and alkaline hydrolysis of glycolonitrile.

2.Molecular Formula, Structural Formula and Relative Molecular Mass

2.1 Molecular Formula

C₂H₅NO₂

2.2 Structural Formula



2.3 Relative Molecular Weight

75.07 (on the basis of 2013 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1Sensory Requirements

Item	Requirement	Test Method
Color and luster	White	Place an appropriate amount of test samples on a clean, dry, white porcelain plate in natural light, and observe their color, luster and state.
State	crystalline granule or crystalline powder	

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
glycocoll (on a dry basis), w/%	98.5~101.5	A.4 in Appendix A
chloride (on the basis of Cl), w/% ≤	0.007	A.5 in Appendix A
heavy metal (on the basis of Pb)/(mg/kg) ≤	10	A.6 in Appendix A
dry reduction ,w/% ≤	0.20	A.7 in Appendix A
ignition residue, w/% ≤	010	A.8 in Appendix A
clarity test	pass the test	A.9 in Appendix A

pH value (50g/L aqueous solution)	55~70	A.10 in Appendix A
nitrilotriacetic acid, w/% ≤	0.05	A.11 in Appendix A
iminodiacetic acid, w/% ≤	0.05	A.12 in Appendix A
sulfate (on the basis of SO ₄), w/% ≤	0.01	A.13 in Appendix A
arsenic (As)/(mg/kg) ≤	1	GB5009.76

Appendix A

Test Method

A.1 Safety Instructions

A part of reagents used in the test methods in the quality specifications are poisonous or toxic, so there shall be proper safety and protection measures during operation.

A.2 General Provisions

Reagents and water used under the quality specifications are analytical reagents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard titration solutions in the test and standard solutions, preparations and products for determination of impurity for which no specific solvent is specified for preparation mean aqueous solutions, and are prepared according to provisions of GB/T 601, GB/T602 and GB/T603 unless otherwise specified.

A.3 Identification Test

A.3.1 Reagents and Materials

A.3.1.1 Ninhydrine solution : 1g/L.

Weigh 1.0 g ninhydrine, dissolve it in water, and dilute it to 1000 mL.

A.3.1.2 Hydrochloric acid solution: 1+3.

A.3.1.3 Sodium nitrite solution: 100g/L.

Weigh 100g sodium nitrite solution, dissolve it in water, and dilute it to 1000 mL.

A.3.1.4 Chromotropic Acid Solution

Weigh 0.5 g chromotropic acid, add and dissolve 50mL sulfuric acid (2+1), shake it, conduct centrifugal separation, and use the upper layer of clear liquid. The solution needs to be prepared before use.

A.3.2 Analysis Steps

A.3.2.1 Ninhydrine Test

Weigh about 0.1 g lab sample (with a precision of 0.01 g), dissolve it in 100mL water, and take 5mL of the solution, add 1mL ninhydrine solution in it, heat it until it boils, and after boiling it will become purple within about 3 minutes.

A.3.2.2 Nitroso Test

Weigh 1g lab test sample (with a precision of 0.01g), dissolve it in 10mL water, take 5mL of the solution, add 5 drops of hydrochloric acid solution and 1mL newly prepared sodium nitrite solution into it, and colorless gas will be produced. Take 5 drops of the solution remained after the reaction, add them into the tube,

A.4 Determination of Glycocoll Content

A.4.1 Method Abstract

take formic acid as the cosolvent, glacial acetic acid as the solvent and crystal violet as the indicator, titrate it with standard solution of perchloric acid, and calculate the glycocoll content according to the volume of consumed standard titration solution of perchloric acid.

A.4.2 Reagents and Materials

A.4.2.1 glacial acetic acid : analytical reagent.

A.4.2.2 anhydrous formic acid : analytical reagent.

A.4.2.3 standard solution of perchloric acid : $c(\text{HClO}_4)=0.1\text{mol/L}$.

A.4.2.4 crystal violet indicator: 2g/L glacial acetic acid solution. Weigh 0.2 g crystal violet , dissolve it in glacial acetic acid, and dilute it to 100mL.

A.4.3 Analysis Steps

Weigh about 0.15g test sample of dry matter (with a precision of 0.0001g) in A.7.1, place it in a 250mL dry conical flask, add about 2mL anhydrous formic acid to dissolve it, add 30mL glacial acetic acid , add two drops of crystal violet indication solution, and use

0.1mol/L standard titration solution of perchloric acid to titrate it until the purple solution turns into green.

When conducting determination, conduct blank test for the same quantity of reagent solution without addition of sample according to the same determination steps.

A.4.4 Result Calculation

The mass fraction of glycocoll content, w_1 , in terms of %, is calculated according to Formula (A.1):

$$w_1 = \frac{(V_1 - V_2)cM}{m \times 1000} \times 100\% \quad \dots\dots\dots (A. 1)$$

Where:

V_1 - the numerical value of the volume of standard titration solution of perchloric acid (A.4.2.3) consumed by test materials, in terms of milliliter (mL);

V_2 - the numerical value of the volume of standard titration solution of perchloric acid consumed by blank test, in terms of milliliter (mL);

C - actual concentration of standard titration solution of perchloric acid, in terms of liter/mol (mol/L);

M - mass of the mass, in terms of gram (g);

M - molar mass of glycocoll, in terms of gram/mol (g/mol)($M=75.07$).

The report result is subject to the arithmetic mean value of parallel determination results (keep 1 decimal place). The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 0.3%.

A.5 Determination of Chloride (on the Basis of Cl)

A.5.1 Reagents and Materials

A.5.1.1 Nitric acid solution: 1+9.

A.5.1.2 Silver nitrate solution: 17g/L.

A.5.1.3 chloride (Cl) standard solution: 0.1mg/mL.

Chloride (Cl) standard solution: Weigh sodium chloride 0.165g, place it in a 1000mL volumetric flask, add an appropriate amount of water to dissolve it and dilute it to the graduation, shake up them, take it as the stock solution, and concentration of the stock solution is 0.1mg/mL.

Before use, precisely Measure 10mL stock solution, place it in a 100mL volumetric flask, add water to dilute it to the graduation and shake up them to obtain the chloride standard solution (every 1mL is equivalent to 0.010mg Cl).

A.5.2 Analysis Steps

Weigh about 1.0g lab sample, with a precision of 0.01g, place it in a 50mL comparison tube, add 30mL to dissolve it, take it as the test solution, add 6mL acidification test solution of nitric acid solution, then add 1mL silver nitrate solution, add water to 50mL, shake up them, and keep it still for 10min, compare the shown turbidity with that of the standard turbid solution.

Preparation of standard turbid solution: precisely suck 7mL chloride standard solution, place it in a 50mL comparison tube, dilute it to a volume identical to the volume of test solution, and treat it in the same way as the test solution with the same volume at the same time.

A.5.3 Result Judgement

Place the comparison tube of test solution and the comparison tube of standard turbid solution in the same black background, observe it from top to bottom in the natural light, and the turbidity of test solution shall not exceed that of standard turbid solution.

A.6 Determination of Heavy metal (on the Basis of Pb)

A.6.1 Reagents and Materials

- A.6.1.1 Sodium hydrate solution: 43g/L.
- A.6.1.2 sodium sulfide solution: 100g/L, the solution shall be prepared before use.
- A.6.1.3 Lead (Pb) standard solution: 0.01mg/mL.

A.6.2 Analysis Steps

Weigh about 1.0g lab sample, with a precision of 0.01g, place it in a 25mL comparison tube, add 5mL sodium hydrate solution, add water to dissolve it and dilute it to 25mL, add five drops of sodium sulfide solution, shake up them, keep it still for 2min, and the show color shall not deeper than the standard. The standard is to precisely suck 1mL lead (Pb) standard solution (containing 0.01mg lead), and treat it in the same way with the test sample at the same time.

A.7 Determination of Dry Reduction

A.7.1 Analysis Steps

Weigh about 1.0g lab sample, with a precision of 0.0001 g, place it in a weighing bottle which has been dried to constant weight at 105°C±2°C, lay it with a thickness of less than 5mm, dry it in a constant-temperature loft drier at 105°C±2°C for 3h, place it in a drier to cool it for 30 min to weigh it. Keep the dry matter (it is dry matter A) to determine the content of glycocoll.

A.7.2 Result Calculation

The mass fraction of dry reduction, w_2 , in terms of %, is calculated according to Formula (A.2):

The mass fraction of dry reduction, w_2 , is expressed in terms of % and calculated according to Formula (A.2).

$$w_2 = \frac{m - m_1}{m} \times 100\% \dots\dots\dots (A. 2)$$

Where:

m - mass of the sample before drying, in terms of gram (g);

m_1 - mass of the sample after drying, in terms of gram (g).

Take the arithmetic mean value of two parallel determination results as the determination result. The absolute difference of two parallel determination results shall not exceed 0.02%.

A.8 Determination of Ignition Residue

A.8.1 Reagents and Materials

A.8.1.1 sulfuric acid: analytical reagent.

A.8.1.2 sulfuric acid solution: 1+8.

A.8.2 Analysis Steps

Weigh 2 ~ 3 g lab sample with a precision of 0.0001 g, place it in a porcelain crucible which has been burnt to a constant mass at 800°C±25°C, add an appropriate amount of sulfuric acid solution to make the sample totally soaked. Slowly heat it with small fire until the sample is totally carbonization, and cool it. And about 0.5mL sulfuric acid to soak the residue, heat it at a low temperature until the sulfuric acid vapor totally escape. Burn it for 45 min at 800°C±25°C. Place it in a drier to cool it to the room temperature, and weigh it.

A.8.3 Result Calculation

The mass fraction of ignition residue, w_3 , in terms of %, is calculated according to Formula (A.3):

$$w_3 = \frac{m_1}{m} \times 100\% \dots\dots\dots (A. 3)$$

Where:

m - mass of the mass, in terms of gram (g);

m_1 - mass of the residue, in terms of gram (g).

Take the arithmetic mean value of two parallel determination results as the determination result. The absolute difference of two parallel determination results shall not exceed 0.01%.

A.9 Clarity Test

A.9.1 Method Abstract

Dissolve the test sample in water, and compare it with the standard turbid solution.

A.9.2 Reagents and Materials

A.9.2.1 Nitric acid solution: 1+2.

A.9.2.2 Dextrin solution: 20g/L.

A.9.2.3 Silver nitrate solution: 20g/L.

A.9.2.4 Turbidity standard solution: contain Chlorine (Cl) 0.01 mg/mL.

Measure 14.10mL \pm 0.02mL hydrochloric acid standard solution ($c(\text{HCl})=0.1000\text{mol/L}$), place it in a 50mL volumetric flask, and dilute it to the graduation. Measure 10.0mL \pm 0.02mL of the solution in a 1000mL volumetric flask, dilute it to the graduation, and shake up them.

A.9.3 Analysis Steps

Weigh 1.0 g lab sample with an accuracy of 0.01 g, place it in a comparison tube, add water to dissolve it and dilute it to 25mL, and take the solution as the test solution; take another comparison tube, accurately add 0.2mL standard turbid solution, add water to it until it is 20mL, add 1mL nitric acid solution, 0.2mL dextrin solution and 1mL silver nitrate solution, add water to it until it is 25mL, shake it and place it in a dark place for 15 min to obtain the standard turbid solution.

In the natural light, observe it from top to bottom, and the turbidity of test solution shall not exceed that of standard turbid solution.

A.10 Determination of pH

It shall be conducted according to GB/T9724. During determination, weigh 1.0 g lab sample with an accuracy of 0.01 g, add 20mL of water without carbon dioxide, dissolve and mix it, then conduct determination.

A.11 Determination of Nitrilotriacetic Acid

A.11.1 Method Abstract

When $\text{pH}=1.6\sim 2.0$, nitrilotriacetic acid and Fe^{3+} ions are subject to quantitative complexation, take 5-sulfosalicylic acid dehydrate as the indicator, and titrate it with the standard titration solution of ammonium iron(III) sulfate until the solution shows slight purplish red.

A.11.2 Reagents and Materials

A.11.2.1 sulfuric acid: analytical reagent.

A.11.2.2 phosphoric acid: analytical reagent.

A.11.2.3 hydrochloric acid solution: 6mol/L.

A.11.2.4 stannous chloride solution: 400g/L.

A.11.2.5 mixture of sulfuric acid and phosphoric acid: take 150mL sulfuric acid, slowly add it into 700mL water, add 150 phosphoric acid after it is cooled, and mix them.

A.11.2.6 indication solution of diphenylaminesulfonic acid sodium salt: 5g/L.

A.11.2.7 indication solution of 5-sulfosalicylic acid dehydrate: 10g/L.

A.11.2.8 standard titration solution of Ammonium iron(III) sulfate: 0.05mol/L

A.11.2.8.1 Preparation

Weigh 24.1g ammonium iron(III) sulfate [$\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$], add 500mL water, slowly add 40mL sulfuric acid, heat it to make it dissolved, cool it, dilute it to 1000mL with water and mix them.

A.11.2.8.2 Calibration

Precisely such 25mL of the above ammonium iron(III) sulfate solution in a conical flask, add 10mL hydrochloric acid solution, heat it until it is near boiling, add stannous chloride solution in a dropwise way until the solution is colorless, add 1~2 drops more, cool it in flowing water, add 10mL saturated solution of mercuric chloride, shake up them, keep it still for 2~3min, add 10mL mixture of sulfuric acid and phosphoric acid, add water to 100mL, add 4 drops of indication solution of diphenylaminesulfonic acid sodium salt, and titrate it with potassium bichromate solution ($\frac{1}{6}K_2Cr_2O_7 = 0.05mol/L$) until the solution show purple steadily.

Concentration of the standard titration solution of ammonium iron(III) sulfate (in terms of mol/L):

$$c[FeNH_4(SO_4)_2] = \frac{c_1V_1}{V}$$

Where:

c_1 - concentration of potassium bichromate solution, in terms of mole per liter (mol/L);

V_1 - the value of volume of potassium bichromate solution, in terms of milliliter (mL);

V - volume of 5-sulfosalicylic acid dihydrate, in terms of milliliter (mL).

A.11.3 Analysis Steps

Weigh 10g lab sample, with a precision of 0.001g, place it in a 250mL flask, add 100mL water at a temperature of 50°C~55°C to dissolve it, add 17mL hydrochloric acid solution and 1mL 5-sulfosalicylic acid dihydrate indication solution, at 40°C~50°C, titrate it with the standard titration solution of Ammonium iron(III) sulfate(A.11.2.8) until the solution start to show slight purplish red, and the application amount of the standard titration solution of ammonium iron(III) sulfate doesn't exceed 0.5 mL.

A.12 Determination of Iminodiacetic Acid

A.12.1 Method Abstract

Dissolve the lab sample with mobile phase, take aqueous monopotassium phosphate solution with pH=2.2 and mobile phase, take aqueous monopotassium phosphate solution with pH=2.2 and acetonitrile as the mobile phase, use strong anion exchange column and UV detector (195nm), conduct separation of determination of high performance liquid chromatography for iminodiacetic acid in the lab sample, and quantify it with the external standard method.

A.12.2 Reagents and Materials

A.12.2.1 Acetonitrile: chromatographic pure.

A.12.2.2 Monopotassium phosphate: analytical reagent.

A.12.2.3 Water: Grade I water.

A.12.2.4 Phosphoric acid: analytical reagent.

A.12.2.5 Phosphoric acid solution: 50%.

A.12.2.6 Iminodiacetic acid standard: the know mass fraction of iminodiacetic acid \geq 98.0%.

A.12.3 Instrument

A.12.3.1 High performance liquid chromatograph: with variable-wavelength UV detector.

A.12.3.2 Chromatographic data processor.

A.12.3.3 chromatographic column: 250mm \times 4.6mm, SAX 5 μ m stainless steel column. (or strong anion exchange column with equivalent effect).

A.12.3.4 Filter: the pore diameter of filter membrane is about 0.45 μ m.

A.12.3.5 Quantitative tube: 20 μ L.

A.12.3.6 Sample injector: 50 μ L or 100 μ L.

A.12.3.7 Ultrasonic cleaner.

A.12.4 Operation Conditions of High Performance Chromatography

A.12.4.1 Mobile phase: Weigh 2.72g monopotassium phosphate, dissolve it with 800mL water, use phosphoric acid solution to adjust pH=2.2, add 200mL acetonitrile, vibrate it with ultrasound for 10 min after filtering it with filter membrane.

A.12.4.2 Flow rate: 1.0mL/min

A.12.4.3 Column temperature: room temperature(variation of temperature difference shall not be larger than 2°C)(the temperature difference variation shall not exceed 2 °C)

A.12.4.4 Detection wavelength: 195 nm

A.12.4.5 Injection volume: 20 μL

Retention time of iminodiacetic acid: about 4~5 min.

The above operation parameters is typical, which can be adjusted appropriately depending on the characteristics of specific instrument and chromatographic column to obtain the optimum effect. For the liquid chromatogram of iminodiacetic acid in the glycocoll sample, see Fig.

A.1.

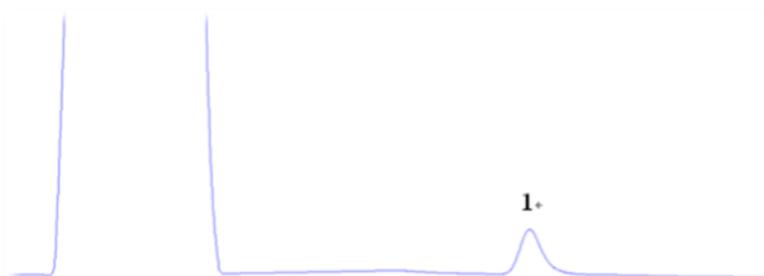


Fig. A.1 High Performance Liquid Chromatogram of The Glycocoll Sample Solution

Note: in Fig. A.1, 1- iminodiacetic acid

A.12.5 Analysis Steps

A.12.5.1 Preparation of Standard Sample Solution

Weigh 0.05g standard iminodiacetic acid, with a precision of 0.0001g, place it in a 100mL volumetric flask, use mobile phase to solve it and dilute it to the graduation, and shake up them. Accurately suck 20mL, place it in a 50mL volumetric flask, use mobile phase to dilute it to the graduation, and shake up them.

A.12.5.2 Preparation of Lab Sample

Weigh lab sample 2.5g, with a precision of 0.0001g, place it in a 100mL volumetric flask, add mobile phase to solve it and dilute it to the graduation, shake up them.

A.12.5.3 Determination

After the instrument is stable, inject standard sample solution and lab sample solution successively according to the operation conditions specified by the method.

A.12.6 Result Calculation

the mass fraction of iminodiacetic acid, w_4 , whose numerical value is expressed in terms of %, is calculated according to Formula (A.4):

$$w_4 = \frac{A \times \frac{m_1}{100} \times \frac{2}{50} \times P_1}{A_1 \times \frac{m}{100}} \dots\dots\dots(A.4)$$

Where:

A_1 - peak area of iminodiacetic acid in standard sample solution;

A - peak area of iminodiacetic acid in the lab sample solution;

m_1 – the mass of standard iminodiacetic acid, in terms of gram (g);

m - in terms of gram (g); the numerical value of the mass of test materials, in terms of gram (g);

P_1 - the mass fraction of iminodiacetic acid in standard substance.

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 10% of the mean value of the two results.

A.13 Determination of Sulfate (on the basis of SO₄)

A.13.1 Reagents and Materials

A.13.1.1 Hydrochloric acid solution: 1+2.

A.13.1.2 potassium sulfate –ethanol solution: 0.2g/L(dissolve 0.02g potassium sulfate into 100mL ethanol solution).

A.13.1.3 Barium chloride solution: 250g/L.

A.13.1.4 sulfate (SO₄) standard solution: Weigh 0.181g potassium sulfate, place it in a 1000mL volumetric flask, add an appropriate amount of water to dissolve it and dilute it to the graduation, and shake up them to obtain the sulfate standard solution(each milliliter is equivalent to 100μg SO₄).

A.13.2 Analysis Steps

Weigh lab sample 2.0g, with a precision of 0.01g, place it in a 50mL comparison tube, add 40mL water to dissolve it, take it as the test solution. Use 0.5mL hydrochloric acid solution to acidize test solution. Mix 0.25mL potassium sulfate- ethanol solution and 1mL barium chloride solution, after keeping it still for 1min, add it into the above acidized test solution, and dilute it to 50mL, shake up them, keep it still for 5min, compare the shown turbidity with that of the standard turbid solution.

preparation of standard turbid solution: precisely suck sulfate standard solution 2.0mL place it in a 50mL comparison tube, dilute it to a volume identical to the volume of test solution, treat it in the same way as the test solution with the same volume at the same time.

A.13.3 Result Judgement

Place the comparison tube of test solution and the comparison tube of standard turbid solution in the same black background, observe it from top to bottom in the natural light, and the turbidity of test solution shall not exceed that of standard turbid solution.

Attachment 2

New Variety of Food Flavor Ingredient Ethyl Linalyl Ether

English Name: ethyl linalyl ether

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760-2014), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive ethyl linalyl ether derived from linalool subject to chemical reaction.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

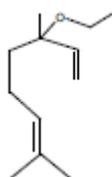
2.1 Chemical Name

3- Oxethyl-3,7- dimethyl-1,6-octadien

2.2 Molecular Formula

C₈H₁₆O

2.3 Structural Formula



2.4 Relative Molecular Weight

182.3 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	colorless	place the test samples in comparison tubes, and observe them by eyes
State	liquid	
Aroma	pleasant flower fragrance	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
ethyl linalyl ether content, w/% ≥	98.0	Appendix A
refraction index (20°C)	1.444~1.447	GB/T14454.4
relative density (20°C/20°C)	0.829~0.832	GB/T11540

Appendix A

Determination of the Content of Food Additive Ethyl Linalyl Ether

A.1 Instrument and Equipment

- A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.
- A.1.2 Column: capillary column.
- A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive ethyl linalyl ether, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive ethyl linalyl ether (normalization method)

B.1 Gas Chromatogram of the Food Additive Ethyl Linalyl Ether

For the gas chromatogram of the food additive ethyl linalyl ether , see Fig. B.1.

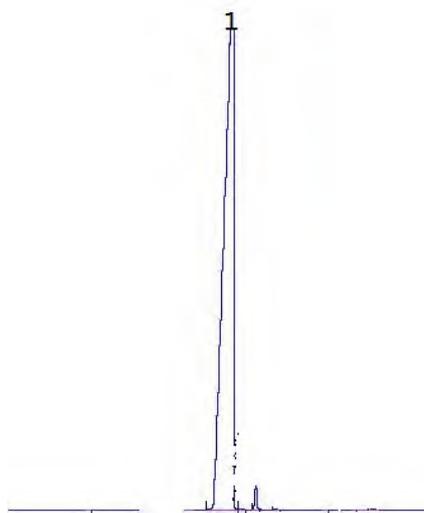


Fig. B.1 Gas Chromatogram of the Food Additive Ethyl Linalyl Ether

B.2 Operation Conditions

- B.2.1 Column: capillary column, with a length of 25 m and an internal diameter of 0.20 mm.
- B.2.2 Stationary phase: methylsilane.
- B.2.3 Membrane thickness: 0.33 μ m.
- B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75 $^{\circ}$ C for 4 min, then raise the temperature to 225 $^{\circ}$ C from 75 $^{\circ}$ C with a linear temperature program at a rate of 2 $^{\circ}$ C/min, and at last keep the constant temperature of 220 $^{\circ}$ C for 8 min.
- B.2.5 Injection-port temperature: 250 $^{\circ}$ C.
- B.2.6 Detector temperature: 250 $^{\circ}$ C.
- B.2.7 Detector: hydrogen flame ionization detector.
- B.2.8 Carrier gas: nitrogen.
- B.2.9 Inlet pressure: 0.06MPa.

B.2.10 Sample size: 0.1 μ L.

B.2.11 Split ratio: 75: 1.

Attachment3

Extension of the Range of Application for the Food Additive β -Carotene

Name	Function	Food Category No.	Food Name	The largest application amount (g/kg)	Note
β -Carotene	colorant	08.02.01	flavored meat products (flavoring materials for raw meat)	0.02	—

Announcement Concerning A New Variety of Food Additive (Glycine (Glycolonitrile Method)) and Others

2017 No. 3

According to Food Safety Law, the review body has organized experts to review the safety evaluation materials for and approved a new variety of food additive, glycine(glycolonitrile method), a new variety of food flavor ingredient ethyl linalyl ether and extension of the range of application for food additive β -carotene.

It is hereby announced.

Attachment:

1. A New Variety of Food Additive, Glycine(Glycolonitrile Method)
2. A New Variety of Food Flavor Ingredient, Ethyl Linalyl Ether
3. Extension of the Range of Application for Food Additive β -Carotene

National Health and Family Planning Commission of PRC

March 8, 2017

Relate links:

1. A New Variety of Food Additive, Glycine(Glycolonitrile Method)
2. A New Variety of Food Flavor Ingredient Ethyl Linalyl Ether
3. Extension of the Range of Application for Food Additive β -Carotene

Attachment1

The New Variety of Food Additive Glycine(Glycolonitrile Method)

English Name: Glycine(Glycolonitrile method)

Functional classification: flavoring agent, food flavor ingredient

(I) Amount and Range of Application

The amount and range of application shall meet provisions on glycine in GB2760.

(II) Quality Specification and Requirements

1.Scope

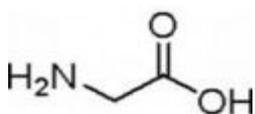
The quality specifications apply to the food additive glycine(glycolonitrile method)produced with ion exchange and membrane decolorization for sodium glycinate produced with ammoniation and alkaline hydrolysis of glycolonitrile.

2.Molecular Formula, Structural Formula and Relative Molecular Mass

2.1 Molecular Formula

$C_2H_5NO_2$

2.2 Structural Formula



2.3 Relative Molecular Weight

75.07 (on the basis of 2013 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1Sensory Requirements

Item	Requirement	Test Method
color and luster	white	place an appropriate amount of test samples on a clean, dry, white
state	crystalline granule or crystalline	

	powder	porcelain plate in natural light, and observe their color, luster and state.
--	--------	--

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
glycocoll (on a dry basis),w/%	98.5~101.5	A.4 in Appendix A
Chloride (on the basis of Cl),w/% ≤	0.007	A.5 in Appendix A
heavy metal (on the basis of Pb)/(mg/kg) ≤	10	A.6 in Appendix A
dry reduction ,w/% ≤	0.20	A.7 in Appendix A
ignition residue w/% ≤	010	A.8 in Appendix A
clarity test	pass the test	A.9 in Appendix A
pH value (50g/L aqueous solution)	55~70	A.10 in Appendix A
nitrilotriacetic acidw/% ≤	005	A.11 in Appendix A
iminodiacetic acid w/% ≤	005	A.12 in Appendix A
sulfate (on the basis of SO) w/% ≤	0.01	A.13 in Appendix A
arsenic (As)/(mg/kg) ≤	1	GB5009.76

Appendix A

Test Method

A.1 Safety Instructions

A part of reagents used in the test methods in the quality specifications are poisonous or poisonous, so there shall be proper safety and protection measures during operation.

A.2 General Provisions

Reagents and water used under the quality specifications are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard titration solutions in the test and standard solutions, preparations and products for determination of impurity for which no specific solvent is specified for preparation mean aqueous solutions, and are prepared according to provisions of GB/T 601,GB/T602 and GB/T603 unless otherwise specified.

A.3 Identification Test

A.3.1 Reagents and Materials

A.3.1.1 Ninhydrine solution : 1g/L.

Weigh 1.0 g ninhydrine, dissolve it in water, and dilute it to 1000 mL.

A.3.1.2 Hydrochloric acid solution: 1+3.

A.3.1.3 Sodium nitrite solution: 100g/L.

Weigh 100g sodium nitrite solution, dissolve it in water, and dilute it to 1000 mL.

A.3.1.4 Chromotropic Acid Solution

Weigh 0.5 g chromotropic acid, add and dissolve 50mL sulfuric acid (2+1), shake it, conduct centrifugal separation, and use the upper layer of clear liquid. The solution needs to be prepared before use.

A.3.2 Analysis Steps

A.3.2.1 Ninhydrine Test

Weigh about 0.1 g lab sample (with a precision of 0.01 g), dissolve it in 100mL water, and take 5mL of the solution, add 1mL ninhydrine solution in it, heat it until it boils, and after boiling it will become purple within about 3 minutes.

A.3.2.2 Nitroso Test

Weigh 1g lab test sample (with a precision of 0.01g), dissolve it in 10mL water, take 5mL of the solution, add 5 drops of hydrochloric acid solution and 1mL newly prepared sodium nitrite solution into it, and colorless gas will be produced. Take 5 drops of the solution remained after the reaction, add them into the tube,

A.4 Determination of Glycocoll Content

A.4.1 Method Abstract

Take forlic acid as the cosolvent, glacial acetic acid as the solvent and crystal violet as the indicator, titrate it with standard solution of perchloric acid, and calculate the glycocoll content according to the volume of consumed standard titration solution of perchloric acid.

A.4.2 Reagents and Materials

A.4.2.1 glacial acetic acid : analytical reagent.

A.4.2.2 anhydrous formic acid : analytical reagent.

A.4.2.3 standard solution of perchloric acid : $c(\text{HClO}_4)=0.1\text{mol/L}$.

A.4.2.4 crystal violet indicator: 2g/L glacial acetic acid solution. Weigh 0.2gcrystal violet, dissolve it in glacial acetic acid , and dilute it to 100mL.

A.4.3 Analysis Steps

Weigh about 0.15g test sample of dry matter (with a precision of 0.0001g) in A.7.1, place it in a 250mL dry conical flask, add about 2mL anhydrous formic acid to dissolve it, add 30mL glacial acetic acid , add two drops of crystal violet indication solution, and use 0.1mol/Lstandard titration solution of perchloric acid to titrate it until the purple solution turns into green.

In the meantime for determination, conduct blank test for the same quantity of reagent solution without addition of sample according to the same determination steps.

A.4.4 Result Calculation

The mass fraction of glycocoll content, w_1 , in terms of %, is calculated according to Formula (A.1):

$$w_1 = \frac{(V_1 - V_2)cM}{m \times 1000} \times 100 \% \quad \dots\dots\dots (A. 1)$$

Where:

V_1 - the numerical value of the volume of standard titration solution of perchloric acid (A.4.2.3) consumed by test materials, in terms of milliliter (mL);

V_2 - the numerical value of the volume of standard titration solution of perchloric acid consumed by blank test, in terms of milliliter (mL);

C - actual concentration of standard titration solution of perchloric acid, in terms of liter/mol (mol/L);

M - mass of the mass, in terms of gram (g);

M - molar mass of glycocoll, in terms of gram/mol (g/mol)($M=75.07$).

The report result is subject to the arithmetic mean value of parallel determination results (keep 1 decimal place). The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 0.3%.

A.5 Determination of Chloride (on the Basis of Cl)

A.5.1 Reagents and Materials

A.5.1.1 Nitric acid solution: 1+9.

A.5.1.2 Silver nitrate solution: 17g/L.

A.5.1.3 Chloride (Cl) standard solution: 0.1mg/mL.

Chloride (Cl) standard solution: Weigh sodium chloride 0.165g, place it in a 1000mL volumetric flask, add an appropriate amount of water to dissolve it and dilute it to the graduation, shake up them, take it as the stock solution, and concentration of the stock solution is 0.1 mg/mL.

Before use, precisely Measure 10mL stock solution, place it in a 100mL volumetric flask, add water to dilute it to the graduation, and shake up them to obtain chloride standard solution (every 1mL is equivalent to 0.010mg Cl).

A.5.2 Analysis Steps

Preparation of standard turbid solution: precisely suck chloride standard solution 7mL, place it in a 50mL comparison tube, dilute it to a volume identical to the volume of test solution, treat it in the same way as the test solution with the same volume at the same time.

A.5.3 Result Judgement

Place the comparison tube of test solution and the comparison tube of standard turbid solution in the same black background, observe it from top to bottom in the natural light, and the turbidity of test solution shall not exceed that of standard turbid solution.

A.6 Determination of Heavy metal (on the Basis of Pb)

A.6.1 Reagents and Materials

A.6.1.1 Sodium hydrate solution: 43g/L.

A.6.1.2 Sodium sulfide solution: 100g/L, the solution shall be prepared before use.

A.6.1.3 Lead (Pb) standard solution: 0.01mg/mL.

A.6.2 Analysis Steps

Weigh about 1.0g lab sample, with a precision of 0.01g, place it in a 25mL comparison tube, add 5mL sodium hydrate solution, add water to dissolve it and dilute it to 25mL, add five drops of sodium sulfide solution, shake up them, keep it still for 2min, and the shown color shall not be darker than the standard. The standard is to precisely suck 1mL Lead (Pb) standard solution (containing 0.01mg lead), and treat it in the same way with the test sample at the same time.

A.7 Determination of Dry Reduction

A.7.1 Analysis Steps

Weigh about 1.0g lab sample, with a precision of 0.0001g, place it in a weighing bottle which has been dried to constant weight at $105^{\circ}\text{C}\pm 2^{\circ}\text{C}$, lay it with a thickness of less than 5mm, dry it in a constant-temperature loft drier at $105^{\circ}\text{C}\pm 2^{\circ}\text{C}$ for 3h, place it in a drier to cool it for 30 min to weigh it. Keep the dry matter (it is dry matter A) to determine the content of glycocoll.

A.7.2 Result Calculation

The mass fraction of dry reduction, w_2 , is expressed in terms of % and calculated according to Formula (A.2):

$$w_2 = \frac{m - m_1}{m} \times 100\% \dots\dots\dots (A. 2)$$

Where:

m - mass of the sample before drying, in terms of gram (g);

m_1 - mass of the sample after drying, in terms of gram (g).

Take the arithmetic mean value of two parallel determination results as the determination result. The absolute difference of two parallel determination results shall not exceed 0.02%.

A.8 Determination of Ignition Residue

A.8.1 Reagents and Materials

A.8.1.1 sulfuric acid: analytical reagent.

A.8.1.2 sulfuric acid solution: 1+8.

A.8.2 Analysis Steps

Weigh 2 ~ 3 g lab sample with a precision of 0.0001 g, place it in a porcelain crucible which has been burnt to a constant mass at $800^{\circ}\text{C} \pm 25^{\circ}\text{C}$, add an appropriate amount of sulfuric acid solution to make the sample totally soaked. Slowly heat it with small fire until the sample is totally carbonization, and cool it. And about 0.5mL sulfuric acid to soak the residue, heat it at a low temperature until the sulfuric acid vapor totally escape. Burn it for 45 min at $800^{\circ}\text{C} \pm 25^{\circ}\text{C}$. Place it in a drier to cool it to the room temperature, and weigh it.

A.8.3 Result Calculation

The mass fraction of ignition residue, w_3 , in terms of %, is calculated according to Formula (A.3):

$$w_3 = \frac{m_1}{m} \times 100\% \dots\dots\dots (A. 3)$$

Where:

m - mass of the mass, in terms of gram (g);

m_1 - mass of the residue, in terms of gram (g).

Take the arithmetic mean value of two parallel determination results as the determination result. The absolute difference of two parallel determination results shall not exceed 0.01%.

A.9 Clarity Test

A.9.1 Method Abstract

Dissolve the test sample in water, and compare it with the standard turbid solution.

A.9.2 Reagents and Materials

A.9.2.1 Nitric acid solution: 1+2.

A.9.2.2 Dextrin solution: 20g/L.

A.9.2.3 Silver nitrate solution: 20g/L.

A.9.2.4 Turbidity standard solution: contain Chlorine (Cl) 0.01 mg/mL.

Measure $14.10\text{mL} \pm 0.02\text{mL}$ hydrochloric acid standard solution ($c(\text{HCl})=0.1000\text{mol/L}$), place it in a 50mL volumetric flask, and dilute it to the graduation. Measure $10.0\text{mL} \pm 0.02\text{mL}$ of the solution in a 1000mL volumetric flask, dilute it to the graduation, and shake up them.

A.9.3 Analysis Steps

Weigh 1.0 g lab sample with an accuracy of 0.01 g, place it in a comparison tube, add water to dissolve it and dilute it to 25mL, and take the solution as the test solution; take another comparison tube, accurately add 0.2mL standard turbid solution, add water to it until it is 20mL, add 1mL nitric acid solution, 0.2mL dextrin solution and 1mL silver nitrate solution, add water to it until it is 25mL, shake it and place it in a dark place for 15 min to obtain the standard turbid solution.

In the natural light, observe it from top to bottom, and the turbidity of test solution shall not exceed that of standard turbid solution.

A.10 Determination of pH

It shall be conducted according to GB/T9724. During determination, weigh 1.0 g lab sample with an accuracy of 0.01 g, add 20mL of water without carbon dioxide, dissolve and mix it, then conduct determination.

A.11 Determination of Nitrilotriacetic Acid

A.11.1 Method Abstract

When pH=1.6~2.0, nitrilotriacetic acid and Fe^{3+} ions are subject to quantitative complexation, take 5-sulfosalicylic acid dehydrate as the indicator, and titrate it with the standard titration solution of ammonium iron(III) sulfate until the solution shows slight purplish red.

A.11.2 Reagents and Materials

- A.11.2.1 Sulfuric acid: analytical reagent.
- A.11.2.2 Phosphoric acid: analytical reagent.
- A.11.2.3 Hydrochloric acid solution: 6mol/L.
- A.11.2.4 Stannous chloride solution: 400g/L.
- A.11.2.5 mixture of sulfuric acid and phosphoric acid: take 150mL sulfuric acid, slowly add it into 700mL water, add 150 phosphoric acid after it is cooled, and mix them.
- A.11.2.6 indication solution of diphenylaminesulfonic acid sodium salt: 5g/L.
- A.11.2.7 5-sulfosalicylic acid dehydrate indication solution: 10g/L.
- A.11.2.8 standard titration solution of ammonium iron(III) sulfate: 0.05mol/L

A.11.2.8.1 Preparation

Weigh 24.1g ammonium iron(III) sulfate [$FeNH_4(SO_4)_2 \cdot 12H_2O$], add 500mL water, slowly add 40mL sulfuric acid, heat it to dissolve it, cool it, dilute it to 1000mL with water, and mix them.

A.11.2.8.2 Calibration

Precisely such 25mL of the above ammonium iron(III) sulfate solution in a conical flask, add 10mL hydrochloric acid solution, heat it until it is near boiling, add stannous chloride solution in a dropwise way until the solution is colorless, add 1~2 drops more, cool it in flowing water, add 10mL saturated solution of mercuric chloride, shake up them, keep it still for 2~3min, add 10mL mixture of sulfuric acid and phosphoric acid, add water to 100mL, add 4 drops of indication solution of diphenylaminesulfonic acid sodium salt, and titrate it with potassium bichromate solution ($\frac{1}{6} K_2Cr_2O_7$) = 0.05mol/L until the solution show purple steadily.

Concentration of the standard titration solution of Ammonium iron(III) sulfate (in terms of mol/L):

$$c[FeNH_4(SO_4)_2] = \frac{c_1 V_1}{V}$$

Where:

- c_1 - concentration of potassium bichromate solution, in terms of mole per liter (mol/L);
- V_1 - the value of volume of potassium bichromate solution, in terms of milliliter (mL);
- V - volume of 5-sulfosalicylic acid dihydrate, in terms of milliliter (mL).

A.11.3 Analysis Steps

Weigh 10g lab sample, with a precision of 0.001g, place it in a 250mL flask, add 100mL water at a temperature of 50°C~55°C to dissolve it, add 17mL hydrochloric acid solution and 1mL 5-sulfosalicylic acid dihydrate indication solution, at 40°C~50°C, titrate it with the standard titration solution of Ammonium iron(III) sulfate(A.11.2.8) until the solution start to show slight purplish red, and the application amount of the standard titration solution of ammonium iron(III) sulfate doesn't exceed 0.5 mL.

A.12 Determination of Iminodiacetic Acid

A.12.1 Method Abstract

Dissolve the lab sample with mobile phase, take aqueous monopotassium phosphate solution with pH=2.2 and mobile phase, take aqueous monopotassium phosphate solution with pH=2.2 and acetonitrile as the mobile phase, use strong anion exchange column and UV detector (195nm), conduct separation of determination of high performance liquid chromatography for iminodiacetic acid in the lab sample, and quantify it with the external standard method.

A.12.2 Reagents and Materials

- A.12.2.1 Acetonitrile: chromatographic pure.
- A.12.2.2 Monopotassium phosphate: analytical reagent.
- A.12.2.3 Water: Grade I water.
- A.12.2.4 Phosphoric acid: analytical reagent.
- A.12.2.5 Phosphoric acid solution: 50%.
- A.12.2.6 Iminodiacetic acid standard: the know mass fraction of iminodiacetic acid \geq 98.0%.

A.12.3 Instrument

- A.12.3.1 High performance liquid chromatograph: with variable-wavelength UV detector.
- A.12.3.2 Chromatographic data processor.
- A.12.3.3 Chromatographic column: 250mm \times 4.6mm, SAX 5 μ m stainless steel column. (or strong anion exchange column with equivalent effect).
- A.12.3.4 Filter: the pore diameter of filter membrane is about 0.45 μ m.
- A.12.3.5 Quantitative tube: 20 μ L.
- A.12.3.6 Sample injector: 50 μ L or 100 μ L.
- A.12.3.7 Ultrasonic cleaner.

A.12.4 Operation Conditions of High Performance Liquid Chromatography

- A.12.4.1 Mobile phase: Weigh 2.72gmonopotassium phosphate, dissolve it with 800mL water, use phosphoric acid solution to adjust pH=2.2, add 200mL acetonitrile, vibrate it with ultrasound for 10 min after filtering it with filter membrane.
- A.12.4.2 Flow rate: 1.0mL/min
- A.12.4.3 Column temperature: room temperature(variation of temperature difference shall not be larger than 2°C)(the temperature difference variation shall not exceed 2 °C)
- A.12.4.4 Detection wavelength: 195nm
- A.12.4.5 Injection volume: 20 μ L

Retention time of iminodiacetic acid: about 4~5min.

The above operation parameters is typical, which can be adjusted appropriately depending on the characteristics of specific instrument and chromatographic column to obtain the optimum effect. For the liquid chromatogram of iminodiacetic acid in the glycocoll sample, see Fig.

A.1.

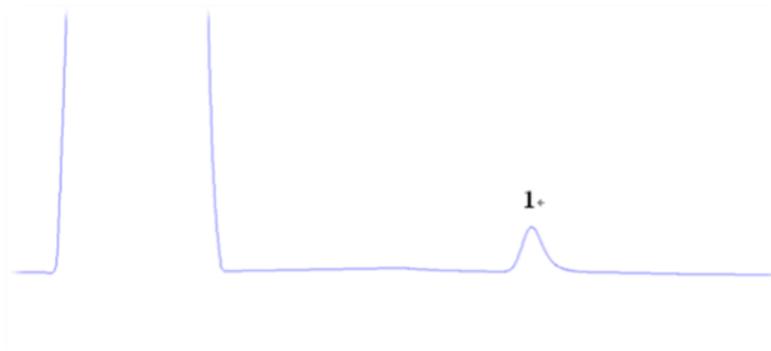


Fig. A.1 High Performance Liquid Chromatogram of The Glycocoll Sample Solution

Note: in Fig. A.1, 1- iminodiacetic acid

A.12.5 Analysis Steps

A.12.5.1 Preparation of Standard Sample Solution

Weigh 0.05g iminodiacetic acid standard, with a precision of 0.0001g, place it in a 100mL volumetric flask, use mobile phase to solve it and dilute it to the graduation, and shake up them. Accurately suck 20mL, place it in a 50mL volumetric flask, use mobile phase to dilute it to the graduation, and shake up them.

A.12.5.2 Preparation of Lab Sample

Weigh lab sample 2.5g, with a precision of 0.0001g, place it in a 100mL volumetric flask, add mobile phase to solve it and dilute it to the graduation, and shake up them.

A.12.5.3 Determination

After the instrument is stable, inject standard sample solution and lab sample solution successively according to the operation conditions specified by the method.

A.12.6 Result Calculation

the mass fraction of iminodiacetic acid, w_4 , whose numerical value is expressed in terms of %, is calculated according to Formula (A.4):

$$w_4 = \frac{A \times \frac{m_1}{100} \times \frac{2}{50} \times P_1}{A_1 \times \frac{m}{100}} \dots\dots\dots(A.4)$$

Where:

A_1 - peak area of iminodiacetic acid in standard sample solution;

B - peak area of iminodiacetic acid in the lab sample solution;

m_1 - the mass of standard iminodiacetic acid, in terms of gram (g);

m - in terms of gram (g); the numerical value of the mass of test materials, in terms of gram (g);

P_1 - the mass fraction of iminodiacetic acid in standard substance.

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 10% of the mean value of the two results.

A.13 Determination of Sulfate (on the basis of SO4)

A.13.1 Reagents and Materials

A.13.1.1 Hydrochloric acid solution: 1+2.

A.13.1.2 potassium sulfate – ethanol solution: 0.2g/L(dissolve 0.02g potassium sulfate into 100mL ethanol solution).

A.13.1.3 Barium chloride solution: 250g/L.

A.13.1.4 sulfate (SO_4) standard solution: Weigh 0.181g potassium sulfate, place it in a 1000mL volumetric flask, add an appropriate amount of water to dissolve it and dilute it to the graduation, and shake up them to obtain the sulfate standard solution (each milliliter is equivalent to $100\mu\text{g SO}_4$).

A.13.2 Analysis Steps

Weigh 2.0g lab sample, with a precision of 0.01g, place it in a 50mL comparison tube, add 40mL water to dissolve it, take it as the test solution. Use 0.5mL hydrochloric acid solution to acidize the test solution. Mix 0.25mL potassium sulfate-ethanol solution and 1mL barium chloride solution, after keeping it still for 1min, add it into the above acidized test solution, dilute it to 50mL, shake up them, keep it still for 5min, and compare the shown turbidity with that of the standard turbid solution.

Preparation of standard turbid solution: precisely suck 2.0mL sulfate standard solution, place it in a 50mL comparison tube, dilute it to a volume identical to the volume of test solution, and treat it in the same way as the test solution with the same volume at the same time.

A.13.3 Result Judgement

Place the comparison tube of test solution and the comparison tube of standard turbid solution in the same black background, observe it from top to bottom in the natural light, and the turbidity of test solution shall not exceed that of standard turbid solution.

Attachment 2

New Variety of Food Flavor Ingredient Ethyl Linalyl Ether

English Name: ethyl linalyl ether

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760-2014), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The quality specifications and requirements apply to the food additive ethyl linalyl ether derived from linalool subject to chemical reaction.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

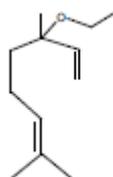
2.1 Chemical Name

3-oxethyl-3,7-dimethyl-1,6-octadien

2.2 Molecular Formula

C₈H₁₆O

2.3 Structural Formula



2.4 Relative Molecular Weight

182.3 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements: Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
color and luster	colorless	place the test samples in comparison tubes, and observe them by eyes
state	liquid	
aroma	pleasant flower fragrance	GB/T14454.2

3.2 Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
ethyl linalyl ether content, w/% ≥	98.0	Appendix A
refraction index (20°C)	1.444~1.447	GB/T14454.4
relative density (20°C/20°C)	0.829~0.832	GB/T11540

Appendix A

Determination of Content of the Food Additive Ethyl Linalyl Ether

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive ethyl linalyl ether, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive Ethyl Linalyl Ether

(normalization method)

B.1 Gas Chromatogram of the Food Additive Ethyl Linalyl Ether

For gas chromatogram of the food additive ethyl linalyl ether , see Fig. B.1.

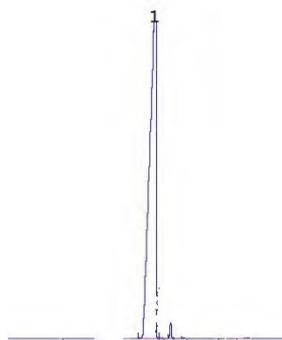


Fig. B.1 Gas Chromatogram of the Food Additive Ethyl Linalyl Ether

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 25 m and an internal diameter of 0.20 mm.

B.2.2 Stationary phase: methylsilane.

B.2.3 Membrane thickness: 0.33 μ m.

B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75 $^{\circ}$ C for 4 min, then raise the temperature to 225 $^{\circ}$ C from 75 $^{\circ}$ C with a linear temperature program at a rate of 2 $^{\circ}$ C/min, and at last keep the constant temperature of 220 $^{\circ}$ C for 8 min.

B.2.5 Injection-port temperature: 250 $^{\circ}$ C.

B.2.6 Detector temperature: 250 $^{\circ}$ C.

B.2.7 Detector: hydrogen flame ionization detector.

B.2.8 Carrier gas: nitrogen.

B.2.9 Inlet pressure: 0.06MPa.

B.2.10 Sample size: 0.1 μ L.

B.2.11 Split ratio: 75: 1.

Attachment 3**Extension of the Range of Application for the Food Additive β -Carotene**

Name	Function	Food Category No.	Food Name	The largest application amount (g/kg)	Note
β -Carotene	colorant	08.02.01	flavored meat products (flavoring materials for raw meat)	0.02	—

Announcement Concerning 6 New Varieties of Food Additives Including Advantame and Extension of the Amount and Range of Application for 6 Food Additives Including Sodium Cyclamate

2017 No.8

According to Food Safety Law, the review body has organized experts to review the safety evaluation materials for and approved 6 new varieties of food additives including advantame and extension of the amount and range of application for 6 food additives including sodium cyclamate.

It is hereby announced.

Attachment:

1. A New Variety of Food Additive, Advantame (N-[N-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L- α -aspartyl]-L-phenylalanine 1-methylester)
2. Two New Varieties of Food Flavor Ingredients Including 2-Propionylpyrrole
3. A New Variety of Enzymic Preparation for the Food Industry, β -Dextranase
4. 2 New Varieties of Food Nutrient Enhancers Including (6S)-5-methyltetrahydrofolic Acid, Glucosamine Salt
5. Extension of the Amount and Range of Application for 6 Food Additives Including Sodium Cyclamate

National Health and Family Planning Commission of PRC

October 20, 2017

Download link: Attachment 1~5 for **Announcement Concerning 6 New Varieties of Food Additives Including Advantame and Extension of the Amount and Range of Application for 6 Food Additives Including Sodium Cyclamate**

Attachment1**A New Variety of Food Additive, Advantame (N-[N-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L- α -aspartyl]-L-phenylalanine 1-methylester)**

English Name: N-[N-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L- α -aspartyl]-L-phenylalanine 1-methylester

Functional classification: edulcorant

(I) Amount and Range of Application

Food Category No.	Food Name	Largest Application amount (g/kg)	Note	
01.02	fermented milk and flavored fermented milk	0.006	—	
03.0	frozen drinks (except 03.04 edible ice)	0.0005		
04.01.02	processed fruit	0.12		
05.0	cocoa products , chocolate and chocolate products (cocoa butter replacer chocolate and its products) and candies	0.0005		
10.03	egg products (change their physical characteristics)	0.0004		
11.04	sweetness material for dining table	use with an appropriate amount depending on production demands		
11.05	flavoring syrup	use with an appropriate amount depending on production demands		
11.06	other edulcorants	use with an appropriate amount depending on production demands		
12.10	compound seasonings	0.0005		
14.05	tea, coffee and plant drinks	0.003		
14.06	solid drinks	0.004		
16.01	jellies	0.0004		If it is used for jelly powder, the application amount shall be increased according to the dissolving factor.

(II) Quality Specification and Requirements**1. Scope**

The standard applies to the food additive advantame (N-[N-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L- α -aspartyl]-L-phenylalanine 1-methylester) produced through the chemical reaction between vanillin and aspartame.

2. Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

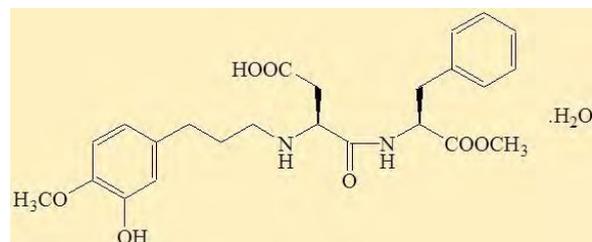
2.1 Chemical Name

N-[N-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L- α -aspartyl]-L-phenylalanine 1-methylester

2.2 Molecular Formula

C₂₄H₃₀N₂O₇·H₂O

2.3 Structural Formula



2.4 Relative Molecular Weight

476.52 (on the basis of 2007 International Relative Atomic Mass)

3. Technical Requirements

3.1 Sensory Requirements

Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	white to yellow powder	Place test samples on a clean, white paper, and observe them by eyes.
State	powder	
Odor	no odor	Take an appropriate amount of sample, and smell it.

3.2 Physical and Chemical Indexes

Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
advantame, w/%	97.0~102.0	A.2 in Appendix A
advantame acid, w/% ≤	1	A.3 in Appendix A
other related substances, w/% ≤	1.5	A.3 in Appendix A
specific rotatory power $\alpha_m(20^\circ\text{C}, D)/[(^\circ)\cdot\text{dm}^2\cdot\text{kg}^{-1}]$	-45~-38	GB/T613 ^a
water content, w/% ≤	5.0	The fourth method in GB5009.3 Karl Fjscher method
ignition residue, w/% ≤	0.2	GB/T9741
arsenic (As)/(mg/kg) ≤	2	GB5009.75
lead (Pb)/(mg/kg) ≤	1	GB5009.12

^a prepare the test sample solution with the mass fraction is 0.2%, and calculate the results on a dry basis.

Appendix A Test Method

A.1 General Provisions

Reagents and water used under the standard are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard titration solutions in the test and standard solutions, preparations and products for determination of impurity are prepared according to provisions of GB/T 601,GB/T602 and GB/T603 unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.2 Determination of Advantame

A.2.1 Method Abstract

Adopt high performance liquid chromatography, under selected working conditions, make constituents in the test sample solution separated through chromatographic column, detect it with UV detector, quantify it with the internal standard method, and calculate the advantame content in the test sample.

A.2.2 Reagents and Materials

A.2.2.1 advantame standard.

A.2.2.2 benzoic acid.

A.2.2.3 acetonitrile: chromatographic pure.

A.2.2.4 monopotassium phosphate.

A.2.2.5 phosphoric acid.

A.2.3 Instrument and Equipment

A.2.3.1 high performance liquid chromatograph (HPLC).

A.2.3.2 constant-flow pump.

A.2.3.3 UV detector.

A.2.4 Reference Conditions of Chromatographic Analysis

For reference conditions of chromatographic analysis, see Table A.1.

Table A.1 Reference Conditions of Chromatographic Analysis

chromatographic column:	reversed phase column (C18), internal diameter 4.6mm×250mm, 5µm particle diameter , or other equivalent chromatographic column	
temperature of chromatographic column:	40°C	
mobile phase A:	mixed solution of phosphate buffer solution (pH 2.8) and acetonitrile (75: 25 volume ratio)	
mobile phase B:	mixed solution of phosphate buffer solution (pH 2.8) and acetonitrile (50:50 volume ratio)	
flow rate:	1.0mL/min	
sample size:	20µL	
detector:	UV detector, detection wavelength: 280nm	
runtime:	55min	
gradient elution process:		
time (min)	mobile phase A (%)	mobile phase B (%)
0	100	0
20	100	0
50	0	100
55	0	100

A.2.5 Analysis Steps

A.2.5.1 Solution Preparation

A.2.5.1.1 preparation of phosphate buffer solution

Accurately weigh 13.61g monopotassium phosphate(A.2.2.4), dissolve it in 1000mL water, adjust the pH to 2.8 with phosphoric acid (A.2.2.5).

A.2.5.1.2 Mobile Phase A

Accurately measure 750mL buffer solution of phosphate (A.2.5.1.1), add 50mL acetonitrile (A.2.2.3), mix them, treat it with ultrasound for about 5 min.

A.2.5.1.3 Mobile Phase B

Accurately measure 500mL buffer solution of phosphate , add 500mL acetonitrile(A.2.2.3), mix them, treat it with ultrasound for about 5 min.

A.2.5.1.4 the mixed solution of water and acetonitrile (7: 3 volume ratio)

Accurately measure 300mL acetonitrile(A.2.2.3), add it into a 1000mL volumetric flask, dilute it with water to the graduation.

A.2.5.1.5 Internal Standard Solution

Accurately weigh about 40mg benzoic acid (A.2.2.2), dissolve it in the mixed solution of water and acetonitrile (A.2.5.1.4) and accurately produce 50mL.

A.2.5.1.6 Standard Stock Solution

Accurately weigh 40mg advantame standard(A.2.2.1), dissolve it in the mixed solution of water and acetonitrile (A.2.5.1.4), produce 50mL.

A.2.5.1.7 Standard Solution

Suck 8, 9, 10, 11,12mL standard stock solution with suction pipet, and transfer them into 5 volumetric flasks respectively, add 5mL internal standard solution into each volumetric flask (A.2.5.1.5), then add the mixed solution of water and acetonitrile (A.2.5.1.4), accurately produce 50mL.

A.2.5.1.8 Sample Solution

Accurately weigh about 40mg test sample, dissolve it in the mixed solution of water and acetonitrile (A.2.5.1.4), accurately produce 50mL. Suck 10mL of the solution, transfer it into a 50mL volumetric flask, accurately add 5mL internal standard solution (A.2.5.1.5), then add the mixed solution of water and acetonitrile (A.2.5.1.4), and produce the constant volume to the graduation.

A.2.5.2 System Applicability

In the chromatogram for standard solution in which the concentration of advantame standard is closest to 160 μ g/mL, the resolution of chromatographic peaks of benzoic acid and advantame is not lower than 10.(note: in the elution order, benzoic acid must be the first, then the advantame.)

When solution is injected for 6 times successively, the standard solution in which the concentration of advantame standard is closest to 160 μ g/mL, the relative standard deviation of peak retention time of advantame doesn't exceed 1.0%.

A.2.6 Determination

Inject the standard solutions to chromatograph (including standard stock solution) respectively, record the chromatogram, determine the peak area response in the generated chromatogram (note: the retention time of advantame is about 16.5 min). For the typical liquid chromatogram of advantame, see Appendix B. For each standard solution, calculate the ratio of peak area response of advantame to peak area response of the internal standard substance benzoic acid . Draw the standard curve between the ratio of peak area response and the concentration of standard solution. Inject the test sample solution to the chromatograph, record the chromatogram, determine the peak area response of the main chromatographic peak in the generated chromatogram . Calculate the ratio of peak area response of advantame peak value to peak area response of the internal standard substance benzoic acid. Determine the advantame concentration (C_A) of the test sample area with the standard curve, in terms of μ g/mL.

A.2.7 Calculation

Advantame percentage in the test sample, W_A , is calculated according to Formula(A.1):

$$W_A = \frac{C_A}{C_U} \times 100 \dots \dots \dots (A. 1)$$

Where:

C_A - concentration of the test sample solution determined with the standard curve($\mu\text{g/mL}$);

C_U - concentration of the test sample solution ($\mu\text{g/mL}$);

100- percentage.

A.3 Determination of Advantame Acid and other Related Substances

A.3.1 Method Abstract

Adopt high performance liquid chromatography, under selected working conditions, make constituents in the test sample solution separated through chromatographic column, detect it with UV detector, quantify it with the internal standard method, and calculate the advantame acid content in the test sample.

A.3.2 Reagents and Materials

A.3.2.1 advantame acid standard.

A.3.2.2 advantame standard.

A.3.2.3 acetonitrile: analytical reagent.

A.3.2.4 monopotassium phosphate.

A.3.2.5 phosphoric acid.

A.3.3 Instrument and Equipment

A.3.3.1 high performance liquid chromatography (HPLC).

A.3.3.2 constant-flow pump.

A.3.3.3 UV detector.

A.3.4 Reference Conditions of Chromatographic Analysis

For reference conditions of chromatographic analysis, see Table A.2

Table A.2 Reference Conditions of Chromatographic Analysis

chromatographic column:	reversed phase column (C18), internal diameter 4.6mm×250mm, 5 μm particle diameter , or other equivalent chromatographic column	
temperature of chromatographic column:	50°C	
mobile phase A:	mixed solution of phosphate buffer solution (pH2.8) and acetonitrile (9:1 volume ratio)	
mobile phase B:	mixed solution of phosphate buffer solution (pH2.8) and acetonitrile (2:3 volume ratio)	
flow rate:	1.0mL/min	
sample size:	20 μL	
detector:	UV detector, Detection wavelength: 210nm	
runtime:	80 min	
gradient elution process:		
time (min)	mobile phase A (%)	mobile phase B (%)
0	85	15
30.0	85	15
55.0	75	25
75.0	0	100

80.0	0	100
80.1	85	15
90.0	85	15

A.3.5 Analysis Steps

A.3.5.1 Solution Preparation

A.3.5.1.1 preparation of phosphate buffer solution

accurately weigh 13.61gmonopotassium phosphate(A.3.2.4), dissolve it in 1000mL water, and adjust the pH to 2.8 with phosphoric acid (A.3.2.5), and prepare it into phosphate buffer solution.

A.3.5.1.2 Mobile Phase A

accurately measure 900mL buffer solution of phosphate (A.3.5.1.1), 加入 100mLacetonitrile(A.3.2.3), mix them, treat it with ultrasound for about 5 min.

A.3.5.1.3 Mobile Phase B

accurately measure 400mL buffer solution of phosphate (A.3.5.1.1), 加入 600mLacetonitrile(A.3.2.3), mix them, treat it with ultrasound for about 5 min.

A.3.5.1.4 Preparation of Standard Solution

Dissolve advantame acid standard (A.3.2.1) in the mixed solution of water and acetonitrile (A.2.5.1.4), and prepare them into solutions with the concentration of 15, 10, 2 and 0.20µg/mL.

A.3.5.1.5 Preparation of Sample Solution

Dissolve the test sample in the mixed solution of water and acetonitrile (A.2.5.1.4), and prepare them into 1mg/mL solution.

A.3.5.1.6 Preparation of System Applicability Solution

In the mixed solution of water and acetonitrile (A.2.5.1.4), prepare solution containing 10µg/m ladvantame standard (A.3.2.2) and solution containing 10µg/mLadvantame acid standard (A.3.2.1).

A.3.5.2 System Applicability Requirement

In the liquid chromatogram, the resolution of chromatographic peaks of advantame and advantame acid is not lower than 3.0.

Note: the retention time of advantame acid and advantame is 29.6min and 56.0min respectively.

The retention time for advantame acid is 29.6 min and advantame 56.0 min.

A.3.6 Determination

Inject the standard solution and test sample solution into the chromatograph respectively, and record the chromatogram; determine the peak area response of the generated chromatogram. For the typical liquid chromatogram of advantame acid and other related substances, see Appendix C.

A.3.7 Calculation

The percentage of advantame acid, W_{AA} , is calculated according to Formula (A.2):

$$W_{AA} = \frac{r_u \times C_S}{r_s \times C_U} \times 100 \dots \dots \dots (A.2)$$

Where:

r_u - peak area response of advantame acid in the chromatogram of test sample solution;

r_s - peak area response of advantame acid in the chromatogram of standard solution;

C_S - concentration of the standard solution, in terms of µg/mL;

C_u - concentration of the test sol sample ution, in terms of µg/mL;

100 - percentage.

The total percentage of other related substances, W_Q , is calculated according to Formula (A.3):

$$W_Q = \frac{r_T \times C_S}{r_S \times C_U} \times 100 \dots \dots \dots (A.3)$$

Where:

r_T - the sum of peak area response of other constituents other than advantame and advantame acid in the chromatogram of test sample solution.

r_S - peak area response of advantame acid in the chromatogram of standard solution;

C_S - concentration of the standard solution, in terms of $\mu\text{g/mL}$;

C_U - concentration of the test sol sample solution, in terms of $\mu\text{g/mL}$;

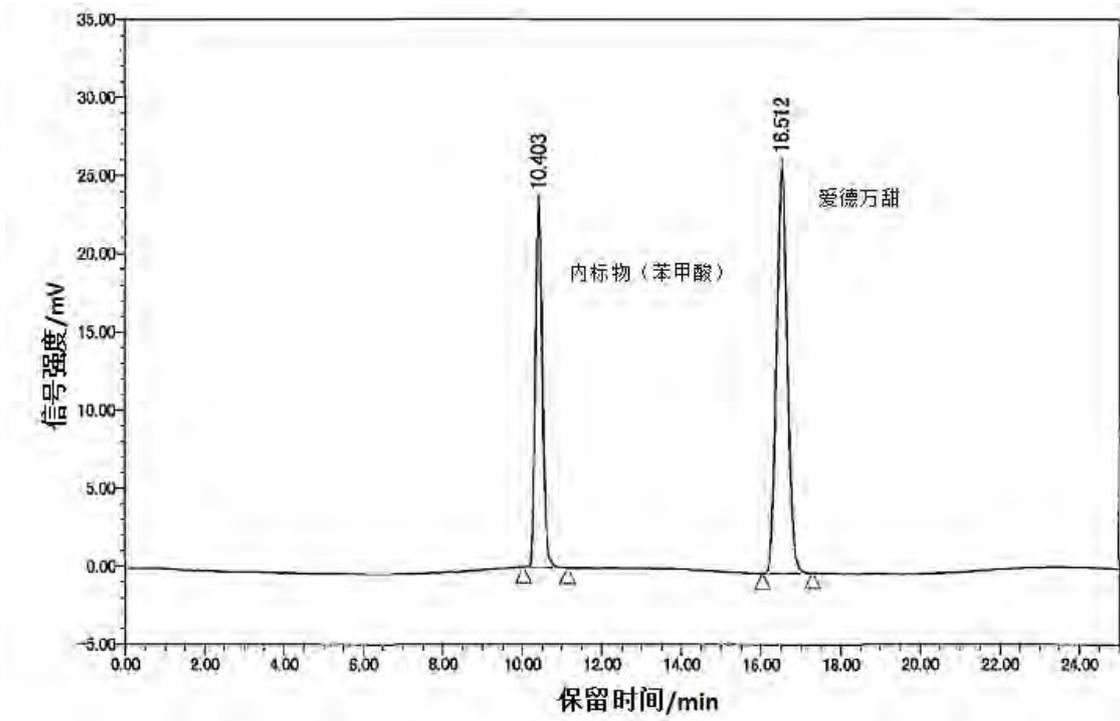
100 - percentage.

Appendix B

Typical Liquid Chromatogram of Advantame

B.1 Typical Liquid Chromatogram of Advantame

For the typical liquid chromatogram of advantame, see Fig. B.1.



信号强度/mV

signal intensity/mV

内标物(苯甲酸)

Internal standard substance (benzoic acid)

爱德万甜

Advantame

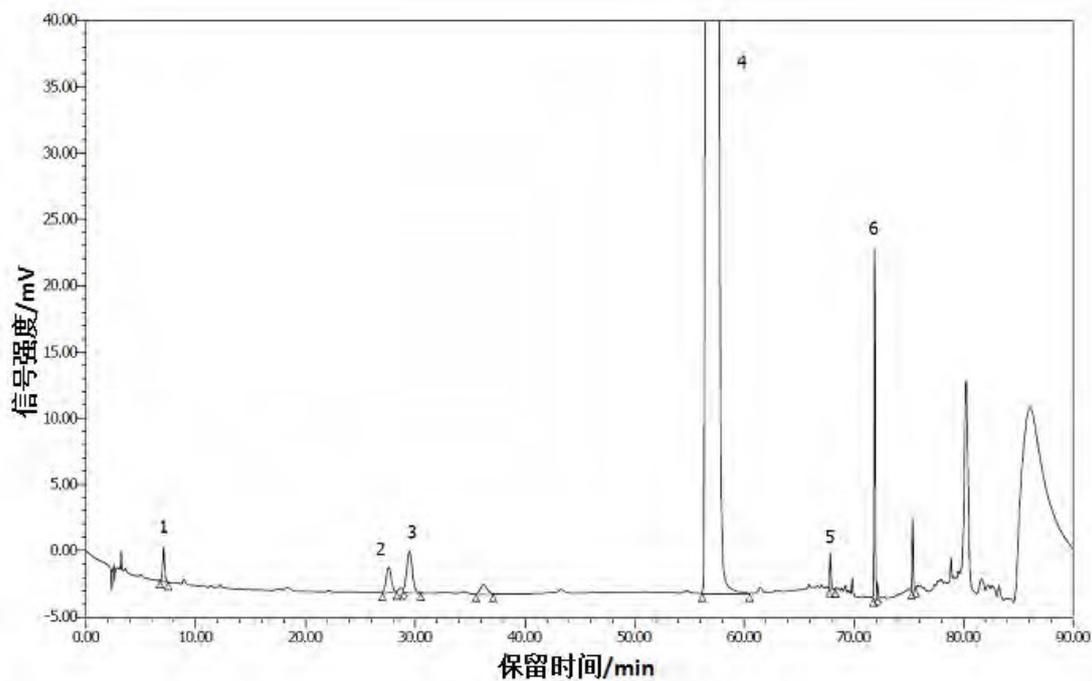
Fig. B.1 Typical Liquid Chromatogram of Advantame

Appendix C

Typical Liquid Chromatogram of Advantame Acid and Other Related Substances

C.1 Typical Liquid Chromatogram of Advantame Acid and Other Related Substances

For the typical liquid chromatogram of advantame acid and other related substances, see C.1.



信号强度/mV

signal intensity/mV

保留时间/min

retention time/min

C.1 Typical Liquid Chromatogram of Advantame Acid and Other Related Substances

1. aspartame 7.156
2. N-[N-[3-(3-hydroxy-4-methoxyphenyl) propyl]- α -L-aspartyl] α -L-aspartyl -L-phenylalanine 1-methyl ester (N-Alkyl-AAPM) 27.664
3. advantame acid 29.250
4. advantame 56.894
5. N-[N-[3-(3-hydroxy-4-methoxyphenyl) amyl]- α -L- asparaginase]-L-phenylalanine 1-methyl ester (9801-D) 67.925
6. N-[N-[3-(3-hydroxy-4-methoxyphenyl) heptyl]- α -L- asparaginase]-L-phenylalanine 1-methyl ester (9801-T) 71.972

Attachment 2

Two New Varieties of Food Flavor Ingredients Including 2-Propionylpyrrole

I. 2-Propionylpyrrole

English Name: 2-Propionylpyrrole

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The standard applies to the food additive 2-propionylpyrrole derived from pyrrole subject to chemical reaction.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

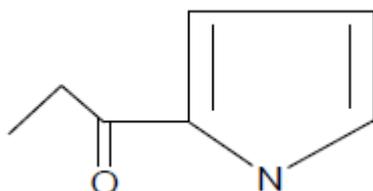
2.1 Chemical Name

2-Propionylpyrrole

2.2 Molecular Formula

C₇H₉NO

2.3 Structural Formula



2.4 Relative Molecular Weight

123.16 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements

Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	white to yellow	Place test samples on a clean, white paper, and observe them by eyes.
State	solid	
Aroma	rubber, leather and quinoline-like odor	GB/T14454.2

3.2 Physical and Chemical Indexes

Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
2-Propionylpyrrole content, w/%	≥ 99.0	Appendix A
Melting point (°C)	49.0~52.0	GB/T14457.3

Appendix A

Determination of 2-Propionylpyrrole Content

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

Preparation of test sample: Weigh 0.1g test sample, dissolve it in 10mL absolute ethyl alcohol, and shake up for future use.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of food additive 2-Propionylpyrrole, see Appendix B.

Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive 2-Propionylpyrrole (normalization method)

B.1 Gas Chromatogram of the Food Additive 2-Propionylpyrrole

For the gas chromatogram of 2-Propionylpyrrole , see Fig. B.1.



Description:

1 - ethanol (solvent);

2 - 2-Propionylpyrrole .

Fig. B.1 Gas Chromatogram of the Food Additive 2-Propionylpyrrole

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 25 m and an internal diameter of 0.20 mm.

B.2.2 Stationary phase: methylsilane.

B.2.3 Membrane thickness: 0.33 μ m.

B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75 $^{\circ}$ C for 4 min, then raise the temperature to 220 $^{\circ}$ C from 75 $^{\circ}$ C with a linear temperature program at a rate of 2 $^{\circ}$ C/min, and at last keep the constant temperature of 225 $^{\circ}$ C for 8 min.

B.2.5 Injection-port temperature: 250 $^{\circ}$ C.

B.2.6 Detector temperature: 250 $^{\circ}$ C.

B.2.7 Detector: hydrogen flame ionization detector.

B.2.8 Carrier gas: nitrogen.

B.2.9 Inlet pressure: 0.06MPa.

B.2.10 Sample size: 0.1 μ L.

B.2.11 Split ratio: 75: 1.

II. Ally 1-propenyl Disulfide

English Name: Ally 1-propenyl disulfide

Functional classification: food flavor ingredients

(I) Amount and Range of Application

The substance can be prepared into food flavors used for various kinds of food (except food categories listed in Table B.1 of GB 2760), and the application amount is depending on the production demands.

(II) Quality Specification and Requirements

1.Scope

The standard applies to the food additive ally 1-propenyl disulfide derived from allyl mercaptan subject to chemical reaction.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

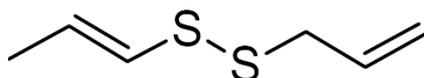
2.1 Chemical Name

Ally 1-Propenyl Disulfide

2.2 Molecular Formula

C₆H₁₀S₂

2.3 Structural Formula



2.4 Relative Molecular Weight

146.27 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements

Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	light yellow	place the test samples in comparison tubes, and observe them by eyes
State	liquid	
Aroma	garlic-like odor	GB/T14454.2

3.2 Physical and Chemical Indexes

Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
ally 1-propenyl disulfide content, w/% ≥	95.0 (the sum of two isomers)	Appendix A
refraction index (20°C)	1.5412~1.5512	GB/T14454.4
relative density (20°C/20°C)	1.004~1.014	GB/T11540

Appendix A

Determination of Ally 1-Propenyl Disulfide Content

A.1 Instrument and Equipment

A.1.1 Chromatograph: comply with provisions of Chapter 5 in GB/T11538-2006.

A.1.2 Column: capillary column.

A.1.3 Detector: hydrogen flame ionization detector.

A.2 Determination Method

normalization method: Determine the content according to Chapter 10.4 of GB/T11538-2006.

A.3 Repeatability and Result Expression

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive ally 1-propenyl disulfide, see Appendix B.

It shall conducted according to provisions of Article 11.4 in GB/T11538-2006 and meet relevant requirements.

For the gas chromatogram and operation conditions of the food additive ally 1-propenyl disulfide , see Appendix B.

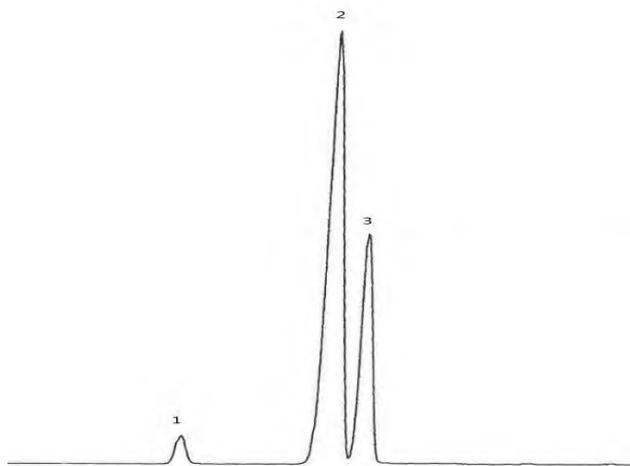
Appendix B

Gas Chromatogram and Operation Conditions of the Food Additive Ally 1-Propenyl Disulfide

(normalization method)

B.1 Gas Chromatogram of the Food Additive Ally 1-Propenyl Disulfide

For the gas chromatogram of Ally 1-Propenyl Disulfide, see Fig. B.1.



Description:

1 - dially sulfide;

2 - cis-ally 1-propenyl disulfide;

3 - trans-ally 1-propenyl disulfide;

Fig. B.1 Gas Chromatogram of the Food Additive Ally 1-Propenyl Disulfide

B.2 Operation Conditions

B.2.1 Column: capillary column, with a length of 50 m and an internal diameter of 0.32 mm.

B.2.2 Stationary phase: methylsilane.

B.2.3 Membrane thickness: 0.5 μ m.

B.2.4 Temperature of chromatographic stove: keep a constant temperature of 75°C for 4 min, then raise the temperature to 220°C from 75°C with a linear temperature program at a rate of 2°C/min, and at last keep the temperature of 220°C for 8 min.

B.2.5 Injection-port temperature: 250°C.

- B.2.6 Detector temperature: 250°C.
- B.2.7 Detector: hydrogen flame ionization detector.
- B.2.8 Carrier gas: nitrogen.
- B.2.9 Inlet pressure: 0.06MPa.
- B.2.10 Sample size: 0.1µL.
- B.2.11 Split ratio: 75: 1.

Attachment 3

A New Variety of Enzymic Preparation for the Food Industry, β -Dextranase

Enzyme	Source	Donor
β -Dextranase	<i>Penicillium funiculosum</i>	—

The quality specification and requirements of β -dextranase shall meet provisions in Food Additive Enzymic Preparation for the Food Industry (GB1886.174-2016).

Attachment 4

2 New Varieties of Food Nutrient Enhancers Including (6S)-5-Methyltetrahydrofolic Acid, Glucosamine Salt

I. (6S)-5-Methyltetrahydrofolic Acid, Glucosamine Salt

English Name: (6S)-5-methyltetrahydrofolic acid, glucosamine salt

Functional classification: food nutrient enhancer

(I) Amount and Range of Application

Food Category No.	Food Name	application amount	Note
14.06	solid drinks	600µg/kg~6000µg/kg	on the basis of folic acid

(II) Quality Specification and Requirements

1.Scope

The standard applies to the food nutrient enhancer (6S)-5-methyltetrahydrofolic acid, glucosamine salt derived from folic acid subject to methylation, salinization, crystallization, lyophilization and other processes.

2.Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

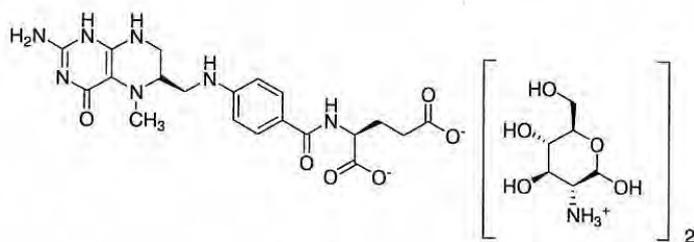
2.1 Chemical Name

N-[4-({[(6S)-2-Amino-5-methyl-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridiny]methyl}amino)benzoyl]-L-glutamic acid, glucosamine salt

2.2 Molecular Formula

C₃₂H₅₁N₉O₁₆

2.3 Structural Formula



2.4 Relative Molecular Weight

817.80 (on the basis of 2007 International Relative Atomic Mass)

3.Technical Requirements

3.1 Sensory Requirements

Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Requirement	Test Method
Color and luster	milk white to light brown	Place appropriate amount of samples on a clean, dry, white porcelain plate in natural light, observe their color, luster and state and smell them.
State	powder, no visible impurities	
Odor	odorless	

3.2 Physical and Chemical Indexes

Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method	
(6S)-5-methyltetrahydrofolic acid, glucosamine salt, w/%	96~105	A.2 in Appendix A	
(6S)-5-methyltetrahydrofolic acid, (on a dry basis), w/%	54~59	A.2 in Appendix A	
Glucosamine (on a dry basis), w/%	34~46	A.3 in Appendix A	
Diastereoisomer ((6S)-5-methyltetrahydrofolic acid), w/%	≥ 99.0	A.4 in Appendix A	
Water content, w/%	≤ 8	GB5009.3 the fourth method	
Ash content, w/%	≤ 0.2	GB5009.4	
Heavy metal (on the basis of Pb)(/mg/kg)	≤ 10	GB5009.74	
Lead (Pb)(/mg/kg)	≤ 2.0	GB5009.12	
Cadmium (Cd)(/mg/kg)	≤ 1.0	GB5009.15	
Mercury (Hg)(/mg/kg)	≤ 0.1	GB5009.17	
Impurity	4-Aminobenzoyl glutamic acid, w/%	≤ 0.3	A.5 in Appendix A
	4α- hydroxy-5-methyltetrahydrofolate, w/%	≤ 1.0	A.5 in Appendix A
	(6S)- pyrazine -s- triazine derivatives([6S]- Mefox], w/%	≤ 0.3	A.5 in Appendix A
	5-methyl tetrahydroptericoic acid, w/%	≤ 0.3	A.5 in Appendix A
	Total impurities, w/%	≤ 2.5	A.5 in Appendix A

3.3 Microbial Index

The microbial index shall meet provisions in Table 3.

Table 3 Microbial Index

Item	Index	Test Method
total numbers of colony /(CFU/g)	≤ 100	GB4789.2
coli group/(MPN/g)	< 3.0	GB4789.3
mould and yeast /(CFU/g)	≤ 100	GB4789.15
pathogenic bacteria (salmonella, Shigella, Staphylococcus aureus)	not detected	GB4789.4, GB4789.5, GB4789.10

Appendix A Test Method

A.1 General Provisions

Reagents and water used under the standard are analytical agents and Grade I water specified in GB/T 6682 respectively, unless otherwise specified. Standard solutions in the test and standard solutions, preparations and products for determination of impurity are prepared according to provisions of GB/T 601, GB/T602 and GB/T603 unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.2 Determination of (6S)-5-methyltetrahydrofolic acid, glucosamine salt and (6S)-5-methyltetrahydrofolic acid (on a dry basis)

A.2.1 Reagents and Materials

A.2.1.1 water.

A.2.1.2 acetonitrile, chromatographic pure.

- A.2.1.3 monopotassium phosphate.
- A.2.1.4 potassium hydrate.
- A.2.1.5 (6S)-5-methyltetrahydrofolate calcium standard: molar mass $MC_{20}H_{23}CaN_7O_6=497.52g/mol$.
- A.2.1.6 potassium hydrate solution: $c(KOH)=20g/100mL$.

A.2.2 Instrument and Equipment

High performance liquid chromatograph: is equipped with UV-VIS detector.

A.2.3 Reference Chromatographic Conditions

For reference chromatographic conditions, see Table A.1.

Table A.1 Reference Chromatographic Conditions

chromatographic column	Reversed-phase C_{18} column, 4.6mm×250mm, particle diameter 5 μ m; or other equivalent chromatographic column.
mobile phase	mobile phase A: weigh 6.8g monopotassium phosphate, dissolve it in 1L water, adjust the pH to 6.5 with potassium hydrate solution. Filter it and treat it with ultrasound.
	mobile phase B: weigh 4.08g monopotassium phosphate, dissolve it in 650mL water, mix it with 350mL acetonitrile, adjust the pH to 8.0 with potassium hydrate solution. Filter it and treat it with ultrasound.
flow rate	1.0mL/min
detection wavelength	280nm
column temperature	25°C
runtime	36min
volume of injection	10 μ L

A.2.4 Situation of Linear Gradient

For the linear gradient, see Table A.2.

Table A.2 Situation of Linear Gradient

time (min)	mobile phase B%	steps
0	0	isocratic
15	40	linear gradient
17	70	linear gradient
22	70	isocratic
31	0	linear gradient
36	0	linear gradient

The retention time of (6S)-5-methyltetrahydrofolic acid (Rt): about 13 min

The retention time of 5-methyl tetrahydropteroic acid (Rt): about 15min

A.2.5 Analysis Steps

A.2.5.1 Preparation of Standard Solution

Weigh an appropriate amount of 6S-5-methyltetrahydrofolate calcium standard (is equivalent to 0.040g (6S)-5-methyltetrahydrofolic acid), with a precision of 0.0001g, place it in 100mL volumetric flask, dissolve it with a small amount of water, then dilute it to the graduation with water, and shake up. Treat the obtained solution with ultrasound (put ice cake in the

ultrasound bath) for 2 min in an environment at a temperature of lower than 20°C, make it pass through 0.45µm filter membrane and then inject the sample immediately.

A.2.5.2 Preparation of Sample Solution

Weigh 0.070 g test sample with a precision of 0.0001g, place it in a 100mL volumetric flask, dissolve it with a small amount of water, then distill it to the graduation, and shake up. Treat the obtained solution with ultrasound (put ice cake in the ultrasound bath) for 2 min in an environment at a temperature of lower than 20°C, make it pass through 0.45µm filter membrane and then inject the sample immediately.

For the reference chromatogram of test sample solution of (6s)-5-methyltetrahydrofolic acid, see Appendix B.

A.2.5.3 Test of System Applicability

Carry out the test of system applicability according to the following steps. Use an automatic sample injector with cooling function, set the temperature to lower than 8°C; if a sample injector without cooling function, the solution shall be stored at 2°C~8°C before injection of sample. Inject standard solution for five times, and for the calculation, see Tale A.3.

Table A.3 Parameters for Test of System Applicability

Parameter		Limit
RSD(peak area), %	≤	2.0
RSD (retention time), %	≤	1.0
tailing factor	≤	2
number of theoretical plates	≥	40000

A.2.5.4 Determination

Under the chromatographic conditions of Table A.1, inject the water (no solute contained) , operate the chromatograph according to the above time. Then conduct chromatographic analysis for the standard solution and test sample solution respectively.

[Note: after completion of the analysis, use the mixed solution of acetonitrile and water (65:35) to rinse the chromatographic column, then seal the column with the mixed solution with of acetonitrile and water (65:35).]

A.2.6 Result Calculation

The mass fraction of (6s)-5-methyltetrahydrofolic acid (on a dry basis) w_1 , is calculated according to Formula (A.1):

$$w_1 = \frac{A_C \times m_{Std} \times T\%}{A_{Std} \times m_C \times (100\% - M)} \dots\dots\dots (A.1)$$

Where:

A_C - peak area of (6s)-5-methyltetrahydrofolic acid in the chromatogram of test sample solution;

m_{Std} - mass of the standard substance, in terms of gram (g);

$T\%$ - mass fraction of (6s)-5-methyltetrahydrofolic acid in (6S)-5-methyltetrahydrofolate calcium standard (%);

A_{Std} - peak area of (6s)-5-methyltetrahydrofolic acid in the chromatogram of standard solution;

m_C - the mass of sample, with a unit of gram (g);

M - water content in the test sample(%).

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 2% of the arithmetic mean value.

The mass fraction of (6S)-5-methyltetrahydrofolic acid, glucosamine salt, w_2 , is calculated according to Formula (A.2):

$$w_2 = \frac{w_1 \times M_1}{M_2} \quad \dots\dots\dots (A.2)$$

Where:

w_1 – mass fraction of (6s)-5-methyltetrahydrofolic acid (on a dry basis) (%);

M_1 -(6S)-5-methyltetrahydrofolic acid, glucosamine salt 的 molar mass, in terms of gram/mol (g/mol)($MC_{32}H_{51}N_9O_{16}=817.80$);

M_2 - molar mass of (6s)-5-methyltetrahydrofolic acid, in terms of gram/mol (g/mol)($MC_{20}H_{25}N_7O_6=459.45$).

A.3 Determination of Glucosamine (on a dry basis)

A.3.1 Reagents and Materials

- A.3.1.1 water.
- A.3.1.2 85% phosphoric acid.
- A.3.1.3 acetonitrile, chromatographic pure.
- A.3.1.4 monopotassium phosphate.
- A.3.1.5 potassium hydrate.
- A.3.1.6 D-(+)- glucosamine hydrochloride standard: molar mass $MC_6H_{13}NO_5 \cdot HCl=215.63$ g/mol.
- A.3.1.7 acetonitrile-aqueous solution (1+1, volume ratio): Weigh 500mL water and 500mL acetonitrile, mix them.
- A.3.1.8 potassium hydrate solution: $c(KOH)=20$ g/100mL.
- A.3.1.9 20mmol/L buffer solution of phosphate: accurately weigh 2.72g monopotassium phosphate, dissolve it in water, accurately adjust the pH to 7.5 with potassium hydrate solution, add water to produce 1000mL, filter it, and treat it with ultrasound.

A.3.2 Instrument and Equipment

High performance liquid chromatograph: is equipped with UV-VIS detector.

A.3.3 Reference Chromatographic Conditions

For reference chromatographic conditions, see Table A.4.

Table A.4 Reference Chromatographic Conditions

chromatographic column	NH ₂ column, 4.6mm×250mm, particle diameter 5μm; or other equivalent chromatographic column.
mobile phase	acetonitrile: 20mmol/L phosphate buffer solution =75: 25
flow rate	1.5mL/min
detection wavelength	195nm
column temperature	35°C
injection volume	10μL
time	30min

Retention time of glucosamine: about 18min.

A.3.4 Analysis Steps

A.3.4.1 Preparation of Standard Solution of Glucosamine Hydrochloride

Weigh 0.375g glucosamine hydrochloride standard, with a precision of 0.0001g, place it in a 100mL volumetric flask, add 50mL acetonitrile-aqueous solution to dissolve it, then add

acetonitrile-aqueous solution to produce a constant volume to the graduation, shake up, filter it immediately and inject the sample.

A.3.4.2 Preparation of Sample Solution

Weigh 0.350g test sample, with a precision of 0.0001g, place it in a 100mL volumetric flask, add 50mL acetonitrile-aqueous solution to dissolve it, then add acetonitrile-aqueous solution to produce a constant volume to the graduation, shake up, filter it immediately and inject the sample.

For the reference chromatogram of test sample solution of glucosamine, see Appendix B.

A.3.4.3 Test of System Applicability

Inject the standard solution of glucosamine hydrochloride for five times, determine the relative standard deviation (RSD), tailing factor and number of theoretical plates of peak area. Criterion of acceptability: $RSD \leq 2.0\%$, tailing factor ≤ 2.0 , number of theoretical plates ≥ 1500 .

A.3.4.4 Determination

According to chromatographic conditions of Table A.4, first inject the standard solution of glucosamine hydrochloride, conduct chromatographic determination according to the above duration, record the chromatogram, take other sample solution and determine with the same method.

A.3.5 Result Calculation

The mass fraction of glucosamine (on a dry basis), w_3 , is calculated according to Formula (A.3):

$$w_3 = \frac{A_c \times m_{Std} \times T\%}{A_{Std} \times m_c \times (100\% - M)} \quad \dots\dots\dots (A.3)$$

Where:

A_c - peak area of glucosamine in the chromatogram of the test sample solution;

m_{Std} - mass of the standard substance, in terms of gram (g);

$T\%$ - mass fraction of D-(+)-glucosamine in D-(+)-glucosamine hydrochloride standard (%);

A_{Std} - peak area of glucosamine in the chromatogram of standard solution;

m_c - the mass of sample, with a unit of gram (g);

M - water content in the test sample (%).

The test result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 2% of the arithmetic mean value.

A.4 Determination of Diastereoisomers (6s)-5-methyltetrahydrofolic Acid

A.4.1 Reagents and Materials

A.4.1.1 water.

A.4.1.2 isopropanol, chromatographic pure.

A.4.1.3 sodium dihydrogen phosphate.

A.4.1.4 sodium hydrate.

A.4.1.5 (6S)-5-methyltetrahydrofolate calcium.

A.4.1.6 Sodium hydrate solution: $c(\text{NaOH})=10\text{g}/100\text{mL}$

A.4.1.7 100mmol/L trisodium phosphate buffer: dissolve 12.0g sodium dihydrogen phosphate in water, adjust the pH to 7.0 with sodium hydrate solution, add water into it to produce 1000mL, filter it, and treat it with ultrasound.

A.4.2 Instrument and Equipment

High performance liquid chromatograph: is equipped with UV-VIS detector.

A.4.3 Reference Chromatographic Conditions

For reference chromatographic conditions, see Table A.5.

Table A.5 Reference Chromatographic Conditions

chromatographic column	HSA chiral column, 4.0mm×100mm, particle diameter 5µm;or other equivalent chromatographic column.
mobile phase	Isopropanol: 100mmol/L trisodium phosphate buffer solution=6:94
flow rate	0.7mL/min, adjust the flow rate, make the retention time of (6s)-5-methyltetrahydrofolic acid be about 4.7min
detection wavelength	225nm
column temperature	30°C
runtime	20min
injection volume	5µL
resolution of (6R) and (6S)	Not less than 2

A.4.4 Analysis Steps

A.4.4.1 Preparation of Standard Solution (used for identification of peak and calculation of resolution)

Weigh about 0.025g (6R,S)-5-methyltetrahydrofolate calcium, with a precision of 0.0001g, place it in a 100mL volumetric flask, dissolve it with 90mL water, treat it with ultrasound at 20°C for 1 min, produce the constant volume to the graduation with water. Transfer 5mL of the solution into a 10mL volumetric flask, produce the constant volume with mobile phase, make it pass through 0.45µm filter membrane and then inject the sample immediately.

A.4.4.2 Preparation of Sample Solution

Weigh about 0.035g test sample, with a precision of 0.0001g, place it in a 1000mL volumetric flask, dissolve it with 90mL water, treat it with ultrasound at 20°C for 1 min, produce the constant volume to the graduation with water. Transfer 5mL of the solution into a 10mL volumetric flask, produce the constant volume with mobile phase, make it pass through 0.45µm filter membrane and then inject the sample immediately.

A.4.4.3 Determination

First inject the standard solution, check the system applicability. The resolution of (6s)-5-methyltetrahydrofolic acid and (6R)-5-methyltetrahydrofolic acid shall not exceed 2. Then inject the test solution.

The resolution R , is calculated according to Formula (A.4):

$$R = \frac{1.18 \times (T_2 - T_1)}{W_1 + W_2} \dots\dots\dots (A.4)$$

Where:

T_2 - the retention time of the previous peak in the two adjacent chromatographic peaks, in terms of minute (min);

T_1 - the retention time of the latter peak in the two adjacent chromatographic peaks, in terms of minute (min);

W_1 - the half-height peak width of the previous peak in the two adjacent chromatographic peaks;

W_2 - the half-height peak width of the latter peak in the two adjacent chromatographic peaks;

1.18- resolution fraction

A.4.4.4 Retention Time

(6s)-5-methyltetrahydrofolic acid: about 4.7min.

(6R)-5-methyltetrahydrofolic acid: about 8.7min.

Note: the standard solution and test sample solution must be injected immediately after being prepared.

A.4.5 Result Calculation

The mass fraction of diastereoisomer (6s)-5-methyltetrahydrofolic acid, w_4 , is calculated according to Formula (A.5):

$$w_4 = \frac{A_S}{A_S + A_R} \times 100\% \quad \dots\dots\dots (A.5)$$

Where:

A_S - peak area of (6s)-5-methyltetrahydrofolic acid in the chromatogram of test sample solution;

A_R - peak area of (6R)-5-methyltetrahydrofolic acid in the chromatogram of test sample solution;

The report result is subject to the arithmetic mean value of parallel determination results. The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 2% of the arithmetic mean value.

A.5 Determination of Impurities

A.5.1 Reagents and Materials

A.5.1.1 water

A.5.1.2 monopotassium phosphate

A.5.1.3 potassium hydrate

A.5.1.4 acetonitrile, chromatographic pure

A.5.1.5 (6R,S)-5-methyltetrahydrofolate calcium standard: molar
mass $MC_{20}H_{23}CaN_7O_6=497.52g/mol$.

A.5.1.6 potassium hydrate solution: $c(KOH)=20g/100mL$

A.5.2 Instrument and Equipment

High performance liquid chromatograph: is equipped with UV-VIS detector

A.5.3 Reference Chromatographic Conditions

For reference chromatographic conditions, see Table A.6

TableA.6 Reference Chromatographic Conditions

chromatographic column	Reversed-phase C_{18} column, 4.6mm×250mm, particle diameter 5 μ m; or other equivalent chromatographic column.
mobile phase	mobile phase A: weigh 6.8g monopotassium phosphate, dissolve it in 1L water, adjust the pH to 6.5 with potassium hydrate solution. Filter it, and treat it with ultrasound.
	mobile phase B: weigh 4.08g monopotassium phosphate, dissolve it in 650mL water, mix it with 350mL acetonitrile, adjust the pH to 8.0 with potassium hydrate solution. Filter it, and treat it with ultrasound.
flow rate	1.0mL/min
detection wavelength	280nm
column temperature	25°C
runtime	36min
injection volume	10 μ L

A.5.4 Situation of Linear Gradient

For the situation of linear gradient, see Table A.7.

Table A.7 Situation of Linear Gradient

time (min)	mobile phase B%	steps
0	0	isocratic
15	40	linear gradient
17	70	linear gradient
22	70	isocratic
31	0	linear gradient
36	0	linear gradient

The retention time of (6s)-5-methyltetrahydrofolic acid (Rt): about 13min.

The retention time of 5-methyl tetrahydropteroic acid (Rt): about 15min.

A.5.5 Analysis Steps

A.5.5.1 Preparation of Standard Solution

Weigh an appropriate amount of (6R,S)-5-methyltetrahydrofolate calcium standard [equivalent to 0.040g(6s)-5-methyltetrahydrofolic acid], with a precision of 0.0001g, place it in 100mL volumetric flask, dissolve it with a small amount of water, then dilute it to the graduation with water, and shake up. Treat the obtained solution with ultrasound (put ice cake in the ultrasound bath) for 2 min in an environment at a temperature of lower than 20°C. Make it pass through 0.45µm filter membrane and then inject the sample immediately.

A.5.5.2 Preparation of Sample Solution

Weigh 0.070g test sample, with a precision of 0.0001g, place it in 100mL volumetric flask, dissolve it with a small amount of water, then dilute it to the graduation with water, and shake up. Treat the obtained solution with ultrasound (put ice cake in the ultrasound bath) for 2 min in an environment at a temperature of lower than 20°C. Make it pass through 0.45µm filter membrane and then inject the sample immediately.

A.5.5.3 Retention Time (approximation)

Table A.8 Retention Time of Single Impurity

Impurity	Indicative Retention Time (min)
4-Aminobenzoyl glutamic acid (ABGA)	5.6
4α-hydroxy-5-methyltetrahydrofolate (HOMeTHFA)	6.5
(6S)- pyrazine-s-triazine derivatives ([6S]-Mefox)	8.6
5-methyl tetrahydropteroic acid (5-MTHF)	13.2
5-methyl tetrahydropteroic acid (MeTHPA)	14.7

A.5.5.4 Test of System Applicability

The test of system applicability is conducted according to following steps. Inject standard solution for five times, and calculate following parameters:

Table A.9 Test Parameters of System Applicability

Parameter	Limit
RSD (area), %	≤ 2.0
RSD (retention time), %	≤ 1.0
tailing factor	≤ 2
number of theoretical plates	≥ 40000

A.5.5.5 Determination

Under the test condition, inject water (blank), run the chromatographic system until it reaches the specified time. Analyze standard solution and test sample solution with the same steps.

[Note: after completion of the analysis, use the mixed solution of acetonitrile and water (65:35) to rinse the chromatographic column, then seal the column with the mixed solution with of acetonitrile and water (65:35).]

A.5.6 Result Calculation

Use the chromatogram of the test sample solution to calculate the mass fraction (X_i) of all single impurities, the range include all chromatographic peaks except the main peak, and ignore all peaks whose peak area is 10% of the area of the main peak in the chromatogram of the test solution (0.1%).

The mass fraction of single impurity, X_i , is calculated according to Formula (A.6):

$$X_i = \frac{A_i \times m_{Std} \times T\% \times (RF)_i}{A_{Std} \times m_C} \quad \dots\dots\dots (A.6)$$

Where:

A_i - peak area of single impurity in the chromatogram of test sample solution;

m_{Std} - mass of the standard substance, in terms of gram (g);

$T\%$ - the mass fraction of (6s)-5-methyltetrahydrofolic acid in (6S)-5-methyltetrahydrofolate calcium standard (%);

$(RF)_i$ - response factor of single impurity.

A_{Std} - peak area of (6s)-5-methyltetrahydrofolic acid in the chromatogram of standard solution;

m_C - the mass of sample, with a unit of gram (g);

Note: RF of 5-methyl tetrahydropteroic acid is 0.68, and RF of other single impurity 1.00.

The total impurity is the sum of mass fractions of single impurity, and the mass fraction of total impurity, w_5 , is calculated according to Formula (A.7):

$$w_5 = \sum X_i \quad \dots\dots\dots (A.7)$$

Where:

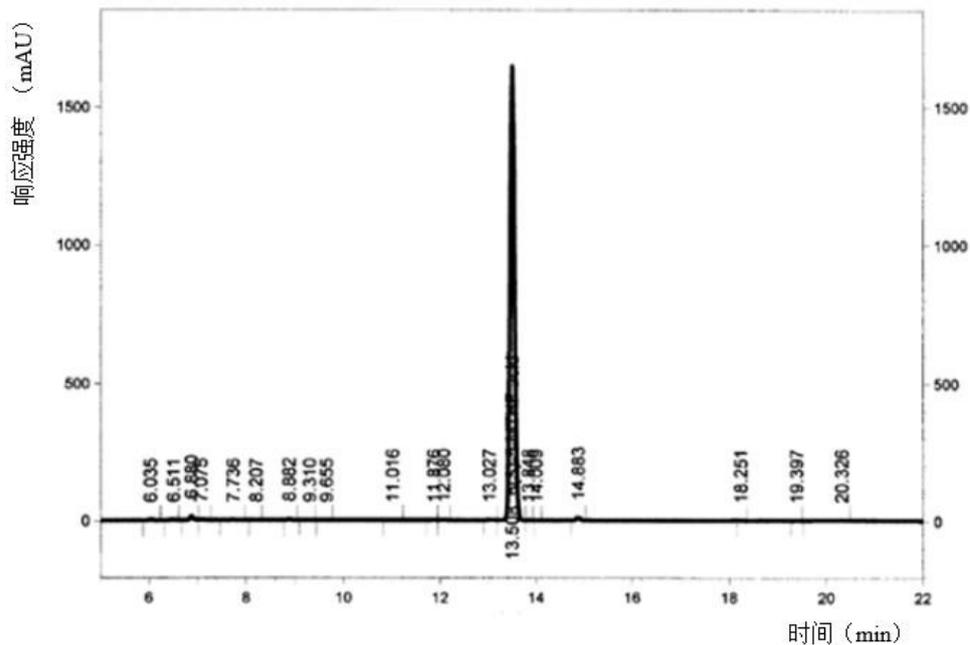
X_i - the mass fraction of single impurity (%).

Appendix B

High Performance Liquid Chromatogram for Determination of (6s)-5-methyltetrahydrofolic Acid Content and Glucosamine Content

B.1 Reference Chromatogram of (6s)-5-Methyltetrahydrofolic Acid

For the reference chromatogram of (6s)-5-methyltetrahydrofolic acid, see Fig. B.1.



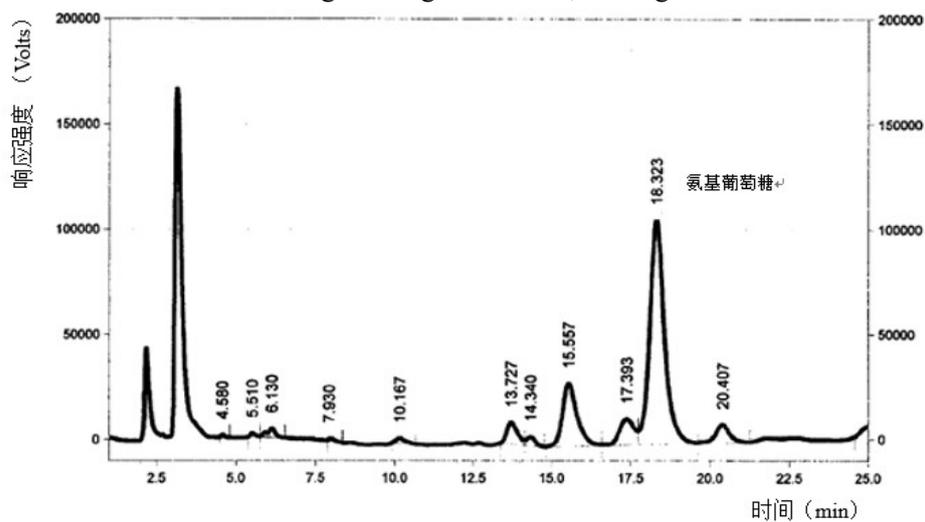
响应强度(mAU)/Response Intensity (mAU)

时间 (min) /time (min)

Fig. B.1 Reference Chromatogram of (6s)-5-Methyltetrahydrofolic Acid

B.2 Reference Chromatogram of Glucosamine

For the reference chromatogram of glucosamine, see Fig. B.2.



响应强度(Volts)/Response intensity(Volts)

时间 (min) /Time (min)

氨基葡萄糖/Glucosamine

Fig. B.2 Reference Chromatogram of Glucosamine

II. Galacto-oligosaccharides (sourced from whey permeate)

English Name: Galacto-oligosaccharides (GOS)(sourced from whey permeate)

Functional classification: food nutrient enhancer

(I) Amount and Range of Application

Food Category No.	Food Name	application amount
13.01	infant formula	It can be used singly or mixed with other substances, and the largest application amount doesn't exceed 64.5g/kg.
13.02.01	cereal-based complementary foods for infants and young children	

(II) Quality Specification and Requirements

1.Scope

The standard applies to the food nutrient enhancer galacto-oligosaccharides derived from filtered whey liquid subject to a process. During the process, β -galactosidase produced from *Aspergillus oryzae* hydrolyze galactoside bonds and hydrolyze lactose into galactose and glucose, at the same time galactoside generated through hydrolysis is transferred into the lactose molecules.

2.Technical Requirements

2.1 Sensory Requirements

Sensory requirements shall meet provisions of Table 1.

Table 1 Sensory Requirements

Item	Index	Test Method
color and luster	white or yellowish powder	Place an appropriate amount of test samples on a clean, dry, white porcelain plate in natural light, observe their color, luster and state and smell them.
odor	no odor	
taste	sweet	

2.2 Physical and Chemical Requirements

Physical and chemical indexes shall meet provisions of Table 2.

Table 2 Physical and Chemical Indexes

Item	Index	Test Method
galacto-oligosaccharides content (on a dry basis), w/% \geq	46	A.2 in Appendix A
lactose content(on a dry basis), w/%	25~45	A.3 in Appendix A
glucose content (on a dry basis), w/% \leq	10	A.4 in Appendix A
galactose content (on a dry basis), w/% \leq	5	A.4 in Appendix A
sialyllactose content (on a dry basis), w/% \geq	0.2	A.5 in Appendix A
protein (on a dry basis), w/% \leq	4.47	GB5009.5
water content, w/% \leq	5.5	GB/T20884
ash content (on dry basis), w/% \leq	4	GB5009.4
pH(10% solution)	5~6	GB/T20885
lead (on the basis of Pb)/(mg/kg) \leq	0.1	GB5009.12

2.3 Microbial Requirements

The microbial index shall meet provisions of Table 3.

Table 3 Microbial Index

Item	Index	Test Method
total numbers of colony /(CFU/g)	≤ 3000	GB4789.2
coli group /(CFU/g)	≤ 10	GB4789.3
mould/(CFU/g)	≤ 50	GB4789.15
saccharomycetes/(CFU/g)	≤ 50	GB4789.15
staphylococcus aureus /25g	not detected	GB4789.10
salmonella/25g	not detected	GB4789.4

Appendix A Test Method

A.1 General Provisions

Reagents and water used under the standard are analytical agents and Grade III water specified in GB/T 6682 respectively, unless otherwise specified. Standard titration solutions in the test and standard solutions, preparations and products for determination of impurity are prepared according to provisions of GB/T 601, GB/T602 and GB/T603 unless otherwise specified. Solutions used in the test for which no specific solvent is specified for preparation mean aqueous solutions.

A.2 Determination of Galacto-oligosaccharides Content

A.2.1 Double-column High Performance Liquid Chromatography

A.2.1.1 Method Abstract

After extracting the test sample with water, determine it with silver-type cation exchange column, amino column separation, determine it with high performance chromatography-differential detector, and quantify it with the normalization method.

A.2.1.2 Reagents and Materials

A.2.1.2.1 Acetonitrile: chromatographic pure.

A.2.1.2.2 galactose, glucose, lactose, isolactose, galactooligodisaccharide, galactooligotrisaccharide, galactooligotetrasaccharide, galactooligopentasaccharide, galactooligohexasaccharide, galactooligoheptasaccharide, galactooligooctasaccharide standard(purity ≥95%).

A.2.1.2.3 standard solution of each constituent, including galactose, glucose, lactose, galactooligodisaccharide, galactooligotrisaccharide, galactooligotetrasaccharide, galactooligopentasaccharide, galactooligohexasaccharide, galactooligoheptasaccharide, galactooligooctasaccharide.

Weigh an appropriate amount of galactose, glucose, lactose, galactooligodisaccharide, galactooligotrisaccharide, galactooligotetrasaccharide, galactooligopentasaccharide, galactooligohexasaccharide, galactooligoheptasaccharide, galactooligooctasaccharide standard, dissolve them with an appropriate amount of water respectively to prepare them into standard solutions of each constituent with a concentration of 20mg/mL.

A.2.1.3 Instrument and Equipment

A.2.1.3.1 High performance liquid chromatograph, with a differential detector.

A.2.1.3.2 Ultrasonic oscillator.

A.2.1.4 Analysis Steps

A.2.1.4.1 Preparation of Sample Solution

Weigh 1.0g test sample, add an appropriate amount of water to dissolve it, oscillate it in an ultrasonic oscillator for 10min, produce 100mL with water, mix them, and filter it with 0.2μm micro-porous filter membrane, for the purpose of determination of silver-type cation

exchange column. Weigh 5.0 g test sample, add an appropriate amount of water to dissolve it, oscillate it in an ultrasonic oscillator for 10 min, produce 100mL with water, mix them, and filter it with 0.2 μm micro-porous filter membrane, for the purpose of determination of animo column.

A.2.1.4.2 reference chromatographic conditions

A.2.1.4.2.1 reference chromatographic conditions of silver-type cation exchange column

A.2.1.4.2.1.1 silver-type cation exchange column (10mm×200mm); or chromatographic column with the same performance.

A.2.1.4.2.1.2 Detector temperature: 50°C.

A.2.1.4.2.1.3 Flow rate of mobile phase: 0.3mL/min.

A.2.1.4.2.1.4 Column temperature: 75°C.

A.2.1.4.2.1.5 Sample size: 20μL.

A.2.1.4.2.1.6 Mobile phase: high-purity water.

A.2.1.4.2.2 Reference Chromatographic Conditions of Animo Column

A.2.1.4.2.2.1 animo column(250mm×4.6mm, 5μm); or chromatographic column with the equivalent performance.

A.2.1.4.2.2.2 mobile phase: acetonitrile: water=70: 30.

A.2.1.4.2.2.3 flow rate of mobile phase: 1.0mL/min.

A.2.1.4.2.2.4 detector temperature: 40°C.

A.2.1.4.2.2.5 column temperature: 35°C.

A.2.1.4.2.2.6 sample size: 20μL.

A.2.1.5 Qualitative Determination

Under the reference chromatographic conditions (A.2.1.4.2.1) and (A.2.1.4.2.2), qualify it according to the retention time of each monosaccharide standard and the retention time of test sample to be determined, and for the qualitative chromatogram, see Fig. B.1 and Fig B.2 in Appendix B.

A.2.1.6 Quantitative Determination

A.2.1.6.1 according to reference chromatographic conditions of silver-type cation exchange column (A.2.1.4.2.1), stabilize the high performance liquid chromatograph, inject the prepared sample (A.2.1.4.1) into the high performance liquid chromatograph , determine the peak area of each constituent in the sample, and calculate the relative percentage of each constituent with the normalization method.

A.2.1.6.2 according to reference chromatographic conditions of animo column A.2.1.4.2.2), stabilize high performance liquid chromatograph, inject the prepared sample (A.2.1.4.1) into high performance liquid chromatograph, determine the peak area of each constituent in the sample, and calculate the relative percentage of each constituent with the normalization method.

A.2.1.7 Result Calculation

A.2.1.7.1 silver-type cation exchange column, the relative percentage of each constituent i in the total saccharide in the test sample, DP_i, is calculated according to Formula (A.1):

$$DP_i = \frac{A_i}{\sum A_i} \times 100 \dots\dots\dots (A.1)$$

Where:

A_i- peak area of each constituent i in the test sample;

∑A_i- the total of peak areas of all constituents in the test sample;

100- unit conversion factor.

A.2.1.7.2 animo column, percentage of lactose in total disaccharide in the test sample, X_{lac}, is calculated according to Formula (A.2).

$$X_{lac} = \frac{A_{lac}}{A_{gd} + A_{is} + A_{lac}} \dots\dots\dots (A.2)$$

Where:

A_{gd} - peak area of galactooligosaccharide in the sample

A_{is} - peak area of isolactose in the sample

A_{lac} - peak area of lactose in the sample

100 - unit conversion factor.

A.2.1.7.3 the percentage of galacto-oligosaccharides in the sample, G_n , is calculated according to (A.3).

$$G_n = 100 - DP_{gl} - DP_{glu} - X_{lac} \times PD_2 \dots\dots\dots (A.3)$$

Where:

DP_{gl} - percentage of galactose in the total saccharide in the test sample, %;

DP_{glu} - percentage of glucose in the total saccharide in the test sample, %;

PD_2 - percentage of disaccharide (galactooligosaccharide, lactose and isolactose) in the total saccharide in the test sample, %;

100 - unit conversion factor.

A.2.1.8 Precision

The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 5.0% of the arithmetic mean value.

A.2.2 High Efficiency Liquid Chromatography

A.2.2.1 Method Abstract

After labeling it with anthranilic acid amide, use internal standard method to determine the molar concentration of different oligosaccharides, and with molecular weights of different oligosaccharides, convert the molar concentration into the mass concentration to quantify them.

A.2.2.2 Reagents and Materials

A.2.2.2.1 dimethyl sulfoxide, purity >99.9%.

A.2.2.2.2 anthranilic acid amide, purity >98%.

A.2.2.2.3 sodium cyanoborohydride, purity >95%.

A.2.2.2.4 purity of formic acid: 98~100%.

A.2.2.2.5 acetonitrile, purity >99%.

A.2.2.2.6 25% ammonium hydroxide solution.

A.2.2.2.7 laminaritriose, purity >90%.

A.2.2.2.8 maltotriose, purity >95%.

A.2.2.2.9 glacial acetic acid, purity: 100%.

A.2.2.2.10 standard stock solution of maltotriose: 3.0 μmol/mL.

Weigh and take 75.0 ± 5.0 mg maltotriose (A.2.2.2.8), with a precision of 0.1 mg, dissolve it in a 50 mL volumetric flask with 40 mL water, and titrate it to the graduation for the constant volume with water. The solution can be stored at 4°C for a week.

A.2.2.2.11 standard working solution of maltotriose: 0.30 μmol/mL.

Take 10.0 mL standard stock solution of maltotriose (A.2.2.2.10) with a transfer liquid gun, place it in a 100 mL volumetric flask, and titrate it to the graduation for the constant volume with water. The solution can be stored at 4°C for a week.

A.2.2.2.12 internal standard stock solution of laminaritriose: 2.0 μmol/mL.

Quantificationally take 50mg laminaritriose(A.2.2.2.7) and about 15mL water, place them in a 50mL volumetric flask, and titrate it to the graduation for the constant volume with water. The solution can be stored at -18°C for year.

A.2.2.2.13 internal standard working solution of laminaritriose: 0.4μmol/mL.

Take 4mLl internal standard stock solution of aminaritriose (A.2.2.2.12) with a transfer liquid gun, place it in 20mL volumetric flask, and titrate it to the graduation for the constant volume with water. The solution can be stored at -18°C for year.

A.2.2.2.14 water-acetonitrile(25%-75%) solution.

Weigh 50mL±1mL water and 150mL±1mL acetonitrile(A.2.2.2.5), place them in a glass bottle, and mix them. The solution can be stored at the room temperature for 3 months.

A.2.2.2.15 eluant B: ammonium formate , 50mmol/L, pH4.4.

Add 2.3g±0.1g(1.89mL) formic acid (A.2.2.2.4) into a flask containing 800mL water. Adjust the pH to 4.40±0.05 with ammonium hydroxide (A.2.2.2.6). Transfer the solution into a 1000mL volumetric flask, and titrate it to the graduation for the constant volume with water. The solution can be store for a week at the room temperature.

A.2.2.2.16anthranilic acid amide labeling reagent: anthranilic acid amide[0.35mol/L]-sodium cyanoborohydride [1.0mol/L]dimethyl sulfoxide- acetic acid [30%] solution

Determine the maximum sample quantity according to demands in experiment, take a corresponding amount of dimethyl sulfoxide (A.2.2.2.1) and acetic acid (A.2.2.2.9) with a transfer liquid gun, place them in a 10mL glass tube, and mix them with a turbine mixer (see Table A.1). Place anthranilic acid amide and sodium cyanoborohydride with corresponding mass into a glass tube, them add dimethyl sulfoxide containing 30% acetic acid, mix them with a turbine mixer and use ultrasonic cleaner until they are fully dissolved (about 10min).

Table A.1 Quantity of the Labeling Reagent anthranilic acid amide

The largest test quantity	dimethyl sulfoxide containing 30% acetic acid		anthranilic acid amide (0.35M) and sodium cyanoborohydride (1M) are dissolved in dimethyl sulfoxide containing 30% acetic acid		
	dimethyl sulfoxide(mL)	acetic acid (mL)	dimethyl sulfoxide containing 30% acetic acid (mL)	anthranilic acid amide (mg)	sodium cyanoborohydride (mg)
11	2.10	0.90	2.50	118±5	157±5
22	4.20	1.80	5.00	236±10	314±10
35	6.30	2.70	7.50	354±10	471±10
47	7.70	3.30	10.00	708±10	942±10

A.2.2.3 Instrument and Equipment

A.2.2.3.1 High performance liquid chromatograph equipped with fluorescence detector.

A.2.2.3.2 2mL centrifuge tube with auto-lock.

A.2.2.3.3 miniature pipe frame.

A.2.2.3.4 centrifuge.

A.2.2.3.5 water bath or heated plane.

A.2.2.3.6 turbine mixer.

A.2.2.3.7 transfer liquid gun .

A.2.2.3.8 analytical balance: precision of 0.1mg.

A.2.2.3.9 ultrasonic wave cleaner.

A.2.2.4 Reference Conditions of Chromatography

- A.2.2.4.1 chromatographic column: acylamino 80 column 3 μ m; 4.6mm x 150mm, or other equivalent chromatographic column.
- A.2.2.4.2 pre-separation column: acylamino 80 protection column; 3 μ m;3.2mm x 15mm.
- A.2.2.4.3 column temperature 23°C \pm 2°C.
- A.2.2.4.4 Sample size: 10 μ L.
- A.2.2.4.5 Mobile Phase A: acetonitrile(A.2.2.2.5).
- A.2.2.4.6 Mobile Phase B: ammonium formate (A.2.2.2.15).
- A.2.2.4.7 Gradient elution: for the elution program, see Table A.2.

Table A.2 Elution Program Table

time (min)	flow rate (mL/min)	mobile phase %		10-position valve positions	6-pass switching
		A	B		
0	1.0	98	2	6/10-1(sample injection)	
4.0	1.0	98	2	6/10-1(sample injection)	
7.5	1.0	98	2	1-2 (analysis)	
8.0	1.0	84	16	1-2 (analysis)	
16.0	1.0	84	16	1-2 (analysis)	
50.0	1.0	61	39	1-2 (analysis)	
51.0	0.80	20	80	1-2 (analysis)	
54.0	0.80	20	80	1-2 (analysis)	
55.0	0.80	90	10	1-2 (analysis)	
61.0	1.0	90	10	1-2 (analysis)	

A.2.2.4.8 excitation wavelength: 330nm.

A.2.2.4.9 emission wavelength: 420nm.

A.2.2.5 Analysis Steps

A.2.2.5.1 Preparation of samples and solution

A.2.2.5.1.1 Preparation of test solution

accurately weigh 0.250g \pm 0.050g galacto-oligosaccharides, place it in a volumetric flask, add 70mL \pm 5mL water. Place volumetric flask in 70°C \pm 5°C water bath, keep it for 20min~25min and stir it. Cool the solution until it is at the room temperature, and titrate it to the graduation for the constant volume with water.

A.2.2.5.1.2 blank reagent

In each series of tests, add labels in 500 μ L water to be used as blank reagent to replace the test sample.

A.2.2.5.1.3 labeling with anthranilic acid amide

A.2.2.5.1.3.1 addition of internal standard

Measure 500 μ L test sample solution (A.2.2.5.1.1) or maltotriose standard working solution (A.2.2.2.11) with a transfer liquid gun, place it in a 2mL miniature tube, then add 200 μ L internal standard working solution of laminaritriose (A.2.2.2.13) in each sample or standard solution, and mix them on the swirl mixer.

A.2.2.5.1.3.2 addition of anthranilic acid amide reagent

Measure 20 μ L test solution containing internal standard, place it into a 2mL miniature tube, and add 200 μ L anthranilic acid amide labeling reagent (A.2.2.2.16) into each miniature tube, mix them on the vortex mixer, then place it in 65°C \pm 1°C water bath for reaction for 2h \pm 5min.

mix them in a swirling way every 20 min. mix the test solutions 2h after the reaction, and place them in the environment of 4°C for at least 10min.

A.2.2.5.1.4 Sample Dilution

After labeling by anthranilic acid amide, add 1.5mL water-acetonitrile(25%-75%) solution (A.2.2.2.14) to each miniature tube. After mixing (in a swirling way) them, centrifugate them for 5 min, and transfer 1mL liquid supernatant into the sampling bottle. Place the sampling bottle in an automatic sample injector (10°C), and inject 10µL standard solution and test sample.

A.2.2.5.2 test of instrument stability

Make the chromatographic system balanced under the initial condition. Ensure that the base line the system pressure keep stable before test, and inject reference sample and standard working solution at least once before test. Check the retention time and separation and compare them with those of the prior test. Check the response fraction of maltotriose-anthranilic acid amide standard solution with different concentrations to check the linear response of fluorescence detector in the whole range.

A.2.2.5.3 Calibration

In each analytic sequence, determine maltotriose standard solution containing the internal standard identical to the test sample. Standard substance shall be injected for at least every 8 test samples. Take the average value of $\left(\frac{Area_{maltotriose}}{Area_{IS}}\right)$ as the Y axis, and the molar concentration of standard solution $\left(\frac{Conc_{maltotriose}}{Conc_{IS}}\right)$ as the X axis to draw the internal standard calibration curve passing through the origin of the coordinate.

Use the response fraction of maltotriose standard curve to quantify the molar concentration of each chromatographic peak (or chromatographic peak set) in the chromatogram.

A.2.2.5.4 Identification and Confirmation

Integrate and determine the nature of each chromatographic peak (or chromatographic peak set with identical molecular weight). Determine molecular weights of different chromatographic peaks through comparison with the reference liquid chromatogram (Fig B.3 in Appendix B).

A.2.2.6 Result Calculation

A.2.2.6.1 molar concentration of oligosaccharide

The molar concentration of oligosaccharide in the test sample, C_{os} , is expressed in terms of µmol/mL and is calculated according to Formula (A.4).

$$C_{os} = \frac{A_{OS_{sple}}}{A_{IS_{sple}}} \times \frac{C_{std}}{Amt_{IS_{std}}} \times \frac{A_{IS_{std}}}{A_{std}} \times Amt_{IS_{sple}} \times \frac{V}{m_{sple}} \dots\dots\dots (A.4)$$

Where:

C_{std} - concentration of maltotriose in the standard solution, in terms of µmol/mL;

$Amt_{IS_{sple}}$ - quantity of the internal standard solution of laminaritriose added in the sample test

$Amt_{IS_{std}}$ - quantity of the internal standard solution of laminaritriose added in the standard test;

$A_{OS_{sple}}$ - peak area of galacto-oligosaccharides in the injected sample;

A_{std} - peak area of maltotriose hydrate in the standard solution;

$A_{IS_{sple}}$ - peak area of the internal standard in the injected sample;

$A_{IS_{std}}$ - peak area of the internal standard in the standard solution

V - volume of the sample, in terms of mL;

m_{sple} - mass of the test sample, in terms of mg.

A.2.2.6.2 mass fraction of galacto-oligosaccharides

galacto- the mass fraction of oligosaccharides (disaccharide included or not included), W , in terms of g/100g, is calculated according to Formula (A.5).

$$W = \sum(C_{os} \times M) \times 0.0001 \dots \dots \dots (A.5)$$

Where:

C_{os} - the molar concentration of oligosaccharide in the test sample, in terms of $\mu\text{mol/g}$, is calculated according to Formula (A.4).

M - the molar mass of different analytes (see B.3 in Appendix B)

0.0001- conversion factor from $\mu\text{g/g}$ to g/100g.

A.2.2.7 Result Expression

The result is expressed in terms of the mass fraction of galacto-oligosaccharides (disaccharide included or not included).

If the test value is higher than 1.00g/100g, 3-digit significance figure is kept for the result of total oligosaccharide (g/100g).

If the test value is lower than 1.00g/100g, 2-digit significance figure is kept for the result of total oligosaccharide (g/100g).

A.2.2.8 Accuracy

The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 0.65g/100g.

A.3 Determination of Lactose Content

A.3.1 Double-column High Performance Liquid Chromatography

A.3.1.1 Analysis Steps

It the same with A.2.1.4.

A.3.1.2 Quantitative Determination

It the same with A.2.1.6.

A.3.1.3 Result Calculation

The mass fraction of lactose in the sample (on a dry basis), in terms of %, is calculated according to Formula (A.6).

$$W_{lac} = X_{lac} \times DP_2 \dots \dots \dots (A.6)$$

Where,

W_{lac} - content of lactose in the sample, %;

X_{lac} - percentage of lactose in total disaccharide in the test sample;

DP_2 - percentage of total disaccharide in total saccharide (galactooligosaccharide, lactose and isolactose) in the test sample;

A.3.1.4 Precision

The absolute difference of two independent determination results obtained under the condition of repeatability shall not exceed 5%.

A.3.2 Direct Calculation Method

Lactose in the test sample can be obtained through direct calculation, and the lactose content W_{lac} (on a dry basis), in terms of %, is calculated according to Formula (A.7).

$$W_{lac} = 100 - W_{gos} - W_{glu} - W_{glc} - W_{ash} - W_{pro} \dots \dots \dots (A.7)$$

where,

W_{lac} -content of lactose in the sample, %;

W_{gos} - percentage of galacto-oligosaccharides in the test sample, %;

W_{glu} - the percentage of glucose in the test sample, %;

W_{gos} - the percentage of galactose in the test sample, %;

W_{ash} - the percentage of ash in the test sample, %;

W_{pro} - the percentage of protein in test sample, %.

A.4 Determination on the Content of Glucose and Galactose

A.4.1 Double-column High Performance Liquid Chromatography

A.4.1.1 Analysis Steps

It the same with A.2.1.4.

A.4.1.2 Quantitative Determination

It the same with A.2.1.6.

A.4.1.3 Result Calculation

It the same with A.2.1.7.

A.4.2 high performance anion exchange chromatography - pulsed amperometric detection

A.4.2.1 Method Abstract

Extract saccharide with hot water, inject it into high performance anion exchange chromatography-pulsed amperometric detector (HPAEC-PAD) with a pulsed amperometric detector for analysis. Saccharide is ionized partially under the condition of strong basicity, and then separate it with polymeric column of anion exchange chromatography. Conduct calculation and analysis through the current generated by oxidation reaction of saccharide occurring on the gold electrode surface. Addition of alkali in post-column can increase the sensitivity and linear range and stabilize the base line.

A.4.2.2 Reagents and Materials

A.4.2.2.1 sodium hydrate solution, 50mmol/L.

A.4.2.2.2 50%(w/w)sodium hydrate solution.

A.4.2.2.3 sodium acetate anhydrous , purity>99%.

A.4.2.2.4 D-(+)-anhydrous glucose, purity>99.5%.

A.4.2.2.5 D-(+)- galactose, purity>99.0%.

A.4.2.2.6 methanol.

A.4.2.2.7 helium, purity>99.996%.

A.4.2.2.8 silica gel containing indicator.

A.4.2.2.9 Sodium hydrate solution: 0.05mol/L.

Measure 10.0mL 5.0mol/Lsodium hydrate solution(A.4.2.2.1) with a transfer liquid gun, place it in a 1000mL volumetric flask, and titrate it to the graduation for the constant volume with water. Store it in a polythene bottle at the room temperature, which can be stored stably for 6 months.

A.4.2.2.10 eluant A: sodium hydrate solution: 300mmol/L.

Measure 985mL deionized water with a 1000 mLgraduated cylinder, inject it into instrument liquid storage tank A, and degas it with helium (A.4.2.2.7) for 20 min. Add 15.6mL50%(w/w)sodium hydrate solution(A.4.2.2.2) with a disposable plastic suction pipet, slowly mix them in a swirling way. Spay helium (A.4.2.2.7) for 15 min. Seal and store it helium (A.4.2.2.7)(34.47kPa~55.16kPa) at the room temperature.The solution can be stored for 4 days.

A.4.2.2.11 eluant B: deionized water.

Measure 2000mL deionized water, inject it into instrument liquid storage tank A, and degas it with helium (A.4.2.2.7) for 20 min. The eluant must be prepared on the same day for use. Seal and store it helium (A.4.2.2.7)(34.47kPa~55.16kPa).

A.4.2.2.12 eluant C: sodium hydrate: 150mmol/L, sodium acetate: 500mmol/L.

Weigh 41.0g±0.1g sodium acetate anhydrous (A.4.2.2.3), place it in a 1000mL volumetric flask, dissolve it with 800mL water and mix them. Add water to produce the constant volume to the graduation. Add 7.8mL 50%(w/w)sodium hydrate solution(A.4.2.2.2) with a disposable

plastic suction pipet. Slowly mix them in a swirling way and then spay helium (A.4.2.2.7) for 15 min. Seal and store it helium (A.4.2.2.7)(34.47kPa~55.16kPa) at the room temperature. The solution can be stored for 4 days.

A.4.2.2.13 post-column reagent, sodium hydrate: 300mmol/L.

Accurately measure 985mL water with with a graduated cylinder, inject it in the post-column liquid storage tank. Add 15.6mL 50%(w/w)sodium hydrate solution(A.4.2.2.2) with a disposable plastic suction pipet, slowly mix them in a swirling way. The solution can be stored at the room temperature for 4 weeks. Add 15.6mL 50%(w/w)sodium hydrate solution(A.4.2.2.2) with a disposable plastic suction pipet, slowly mix them in a swirling way. The solution can be stored at the room temperature for a week.

A.4.2.2.14 standard stock solution of sugar.

Use a flask with plug to hold standard solution, store it in a drier, and place it over the silica gel (A.4.2.2.8) with indicator. Weigh an appropriate amount of saccharide according to Table A.3, place it in a 100mL volumetric flask. Record the mass, with a precision of 0.1 mg, produce the constant volume to the graduation with water.

Table A.3 Weigh and Measurement Plan for Preparation of Standard Solution

Saccharide	Mass (mg)	Volumetric flask(mL)	Concentration (mg/mL)
glucose	100±5	100	1.0
galactose	100±5	100	1.0

A.4.2.2.15 calibration working solution of polysaccharide

Prepare calibration solution according to the dilution standard in Table A.4.

Table A.4 Preparation Plan for Calibration Solution

standard solution	application amount of stock solution		final volume (mL)	concentration of each saccharide constituent in the calibration standard solution	
	glucose (μL)	galactose (μL)		glucose (μg/mL)	galactose (μg/mL)
A	100	50	100	1.50	0.375
B	250	100	100	3.75	0.750
C	500	200	100	7.50	1.50
D	750	400	100	11.25	3.00
E	1000	600	100	15.00	4.50
F	1250	800	100	18.75	6.00

The concentrations in the above table are the recommended values. The actual solution concentration shall be obtained through calculation and calibration. distribute the solution into vessels and store them at -20°C for 12 months.

A.4.2.3 Instrument and Equipment

A.4.2.3.1 Inert ion chromatography without interference of metal ions, equipped with pulsed electrochemical detector.

A.4.2.3.2 suction pipet.

A.4.2.3.3 vacuum filter system.

A.4.2.3.4 nylon membrane.

A.4.2.3.5 miniature pipe for water bath

A.4.2.3.6 miniature pipe.

A.4.2.3.7 centrifuge.

- A.4.2.3.8 single-use syringe
- A.4.2.3.9 analytical balance, with a precision of 0.1mg.
- A.4.2.3.10 nylon syringe-type filter.
- A.4.2.3.11 sampling bottle.

A.4.2.4 Chromatographic Condition

- A.4.2.4.1 column: CarboPacPA20 chromatographic column, 3×150mm, 6.5μm, or other columns with equivalent performance.
- A.4.2.4.2 column temperature: 30°C±2°C.
- A.4.2.4.3 sample size: 25μL.
- A.4.2.4.4 Injection-port temperature: room temperature or 10°C(if any cooling system).
- A.4.2.4.5 eluant A: 300mmol/L sodium hydrate solution(A.4.2.2.10).
- A.4.2.4.6 eluant B: deionized water (A.4.2.2.11).
- A.4.2.4.7 eluant C: sodium hydrate: 150mmol/L, sodium acetate: 500mmol/L(A.4.2.2.12).
- A.4.2.4.8 elution program: for the elution program, see Table A, 5.

Table A.5 Elution Program for Determination of Glucose and Galactose

time	flow rate	eluant A	eluant B	eluant C	Note
[min]	[mL/min]	[%]	[%]	[%]	
Initial	0.5	2.0	98.0	0.0	
0.0	0.5	2.0	98.0	0.0	Start to gather signals
1.0	0.5	2.0	98.0	0.0	
12.0	0.5	5.0	95.0	0.0	
21.0	0.5	22.4	65.6	12.0	
21.1	0.5	0.0	0.0	100.0	Start to rinse
26.0	0.5	0.0	0.0	100.0	
26.1	0.5	100.0	0.0	0.0	
31.0	0.5	100.0	0.0	0.0	Stop rinsing
31.1	0.5	2.0	98.0	0.0	Start rebalance
37.0	0.5	2.0	98.0	0.0	Stop rebalance

- A.4.2.4.9 post-column addition: 300 mmol/L sodium hydrate (A.4.2.2.13), flow rate 0.2mL/min.
- A.4.2.4.10 wave form of detector: adopt the optimized pulsed electrochemical conditions, such as the quadruple waveform of saccharide in Table A.6.

Table A.6 Quadruple Waveform of saccharide Detected with Electrochemical Detector

Time [s]	Electric potential [V]	Integration
0.00	+0.1	
0.20	+0.1	Start
0.40	+0.1	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.6	
0.44	-0.1	
0.50	-0.1	

A.4.2.4.11 Estimated retention time: glucose, 9.6 min; galactose, 8.6 min. it's just the reference retention time, and the actual retention time will be set depending on such factors as the instrument and the batches of chromatographic column.

A.4.2.5 Analysis Steps

A.4.2.5.1 Preparation of Sample and Test Solution

A.4.2.5.1.1 Sample Preparation

Weigh 1g~10g even test sample (m_s), with a precision of 0.0001g, place it in a 100mL(V_s) volumetric flask.

A.4.2.5.1.2 Extraction

Add 60mL~70mL water, and measure the pH. If $\text{pH} < 4.0$, add 50mmol/L sodium hydrate solution(A.4.2.2.1) in a dropwise way to adjust the pH to 6~7. Place it in a $70^\circ\text{C} \pm 2^\circ\text{C}$ water bath, stir it continuously and at the same time heat it for 25min~30min. Cool it until it reaches the room temperature, add water to the graduation, and shake it violently.

A.4.2.5.1.3 preparation of test solution

Measure 1.5mL solution (A.4.2.5.1.2), transfer it into a 2mL miniature tube, centrifugate it for 5 min with the 12000g centrifugation force. If necessary, further dilute the sample, and ensure the saccharide concentration in the sample falls in within the standard curve. Filter the sample solution and polysaccharide calibration standard working solution (A.4.2.2.15) with 0.2 μm nylon injection-style filter and let it fall into the automatic sampling bottle.

A.4.2.5.2 Instrument Calibration

Under the initial condition of the above chromatography, balance the chromatographic system for 1h. Ensure that the system pressure and baseline stable without leakage. Before injection of sample, make the standard working solution and sample solution balanced to the temperature of automatic sample injector.

Start the analytic sequence, first inject water (to check base line) to conduct analysis, then inject polysaccharide calibration working solutions (A.4.2.2.15) (at least 3). Ensure the system stability through check the repetitiveness of retention time and response. The coefficient of variation for retention time and peak area shall not be larger than 2% and 3% respectively. If it doesn't meet the requirement, the balance time shall be extended. Compare it with the results of previous analysis (for the demonstrated chromatogram, see Fig. C.1 in Appendix C) to check the separation effect. The result of initial injection analysis is not included in the statistical range of data.

A.4.2.5.3 Sequence Setting

At the beginning and end of each analytic sequence and after injection of every 8 samples, inject 25 μL polysaccharide calibration working solution (A.4.2.2.15) respectively to conduct analysis. It can ensure necessary extra calibration.

A.4.2.5.4 Calibration

Draw the standard curve with the concentration and peak area of the standard. Quantify the sample with Bracket calibration of the software (Bracket calibration, inject the identical standards before and after sample test, and use the average peak area of the standards injected before and after sample test). The quantification way can make up the difference of retention time and detector response. Use the concentration obtained by backward calculation of peak area and standard curve to calculate the concentration of each kind of saccharide in the test sample solution.

A.4.2.5.5 nature determination and confirmation

A.4.2.5.5.1 determine the nature with retention time

Through comparison with the retention time of corresponding peaks in the polysaccharide calibration working solution, determine the nature of the chromatographic peak of each saccharide in the test sample solution. For the chromatogram, see Fig. C.1 in Appendix C.

A.4.2.5.5.2 sample labeling and confirmation

If there is uncertainty to determine the nature of peaks, labeling shall be conducted for samples, and the chromatogram shall be compared with that of the unlabeled sample.

A.4.2.5.5.3 analysis time

At least 20 samples (secondary repeated injection) include a reference sample. The above number of samples need 48h of analysis time.

A.4.2.6 Calculation

draw the standard curve of each kind of saccharide with the sample concentration and peak area as the axes, and obtain the calibration curve formula according to linear regression. Parameters of the curve formula are calculated according to Formula (A.8).

$$A_{Std} = mx + C \dots\dots\dots (A.8)$$

Where:

- A_{Std} - peak area of the standard working solution (A.4.2.2.15);
- x - concentration of saccharide constituent, in terms of $\mu\text{g/mL}$;
- C - intercept of the calibration curve;
- m - slope of the calibration curve.

The mass fraction of each saccharide (w), in terms of g/100g sample, is calculated according to Formula (A.9).

$$W = \frac{A_s - C}{m} \times \frac{V_s \times D_s}{10^6} \times \frac{100}{m_s} \dots\dots\dots (A.9)$$

Where:

- A_s - peak area of saccharide in the test sample solution;
- V_s - the volume of test sample solution, in terms of mL;
- D_s - the dilution factor (A.4.2.5.1.3) of the test sample solution;
- 10^6 - conversion factor from μg to g;
- 100 - conversion factor to transfer the results into g/100g;
- m_s - the mass of sample (A.4.2.5.1.1) , in terms of g;
- C - intercept of the calibration curve;
- m - slope of the calibration curve.

A.4.2.7 Precision

The absolute difference of the results of two dependant single tests carried out by the same operator in a short time for the same test materials with the same equipment and method in

the same lab (on the basis of $\frac{|X_1 - X_2|}{\bar{x}} \times 100$) shall not exceed 5% of the average value of sample.

A.5 Test for Sialyllactose

A.5.1 Method Abstract

Extract sialyllactose (SL) with 70°C water. Add the internal standard (glucuronyl-lacto-N-tetraose). After the solution is eluted with amino-column extraction column, separate SL (electriforous) and other oligosaccharides (OS) (non-electriforous). Then label sialyllactose with fluorescer (2AB). After dilution with acetonitrile, separate sialyllactose with high performance liquid chromatography, conduct detection by monitoring its fluorescence, and at last conduct quantitative analysis compare it with the external standard calibration curve subject to treatment of the same fluorescent reagent and correction of internal standard.

A.5.2 Reagents and Materials

- A.5.2.1 water.
- A.5.2.2 dimethyl sulfoxide, purity \geq 99.7%.
- A.5.2.3 2-aminobenzamide (anthranilamide), purity \geq 98%.

A.5.2.4 sodium cyanoborohydride , purity: 95%.

A.5.2.5 formic acid, purity98~100%.

A.5.2.6 acetic acid, purity: 100%.

A.5.2.7 aqueous ammonia, purity: 25%.

A.5.2.8 methanol.

A.5.2.9 acetonitrile.

A.5.2.10 3'-sialyllactose sodium salt .

A.5.2.11 6'-sialyllactose sodium salt .

A.5.2.12 glucuronyl-lacto-N-tetraose sodium salts.

A.5.2.13 acetic acid solution , 1m.

Add 57mL±2mL acetic acid into a 1000mL volumetric flask containing 800mL deionized water, produce a constant volume to the graduation with deionized water .

A.5.2.14 aqueous ammonia(NH₄OH), 5%(v/v).

Add 100mL±1mLaqueous ammonia into a 500mL volumetric flask containing 300mL deionized water, and then produce a constant volume to the graduation with deionized water .

A.5.2.152AB labeling reagent: DMSO (dimethyl sulfoxide) containing 0.35m 2AB-1.0m NaBH₃CN 30% acetic acid solution

According to the number of tests, suck an appropriate amount of dimethyl sulfoxide (DMSO) and acetic acid according to the description in Table A.7, place them in a 10mL tube (with screw cap). Mix the mixed solution with a turbine mixer.

Weigh an appropriate amount of 2-Aminobenzamide (2AB) and sodium cyanoborohydride (NaBH₃CN), place them in another 10mL tube (with screw cap), and then add 30% acetic acid –DMSO solution with the corresponding volume.

Mix them with a turbine mixer, and make them totally dissolved with ultrasound cleaner (for about 10 min).

Table A.7 Preparation of 2AB Labeling Reagents

The maximum number of tests	30% acetic acid – DMSO solution		DMSO solution of 30% acetic acid solution containing 0.35M 2AB–1M NaBH ₃ CN		
	DMSO [mL]	100% acetic aid [mL]	30% acetic acid - DMSO solution [mL]	2AB [mg]	NaBH ₃ CN [mg]
11	2.10	0.90	2.50	118±5	157±5
22	4.20	1.80	5.00	236±10	314±10
35	6.30	2.70	7.50	354±10	471±10
47	7.70	3.30	10.00	472±10	628±10
72	11.20	4.80	15.00	708±10	942±10

A.5.2.16 water - acetonitrile 25+75 solution.

Add 50mL±1mL water into a glass bottle containing 150mL±1m Lacetonitrile, mix them.

A.5.2.17 Standard Solution.

A.5.2.17.1 stock solution of glucuronyl-Lacto-N-tetraos nternal standard (IS), about 700µg/mL (free acid).

Weigh 20mg±2mg glucuronyl-lacto-N-tetraose sodium salts, with a precision of 0.1mg. use deionized water to quantificationally transfer it into a 25mL volumetric flask, and use the same solvent to produce the constant volume to the graduation.

A.5.2.17.2working solution of glucuronyl-lacto-N-tetraose internal standard (IS), about 140µg/mL (free acid).

Suck 4.0mL glucuronyl-lacto-N-tetraose stock solution (A.5.2.17.1), place it in a 20mL volumetric flask中. Use deionized water to produce the constant volume to the graduation.

A.5.2.17.3 3'-sialyllactose stock solution, about 1040μg/mL (free acid).

Weigh 30mg±3mg 3'-sialyllactose sodium salt, with a precision of 0.1mg, use deionized water to quantitatively transfer it into a 25mL volumetric flask, and use the same solvent to produce the constant volume to the graduation.

A.5.2.17.4 6'-sialyllactose stock solution, about 660μg/mL (free acid).

Weigh 18mg±2mg 6'-sialyllactose sodium salt, with a precision of 0.1mg, use deionized water to quantitatively transfer it into a 25mL volumetric flask, and use the same solvent to produce the constant volume to the graduation.

A.5.2.17.5 3'-sialyllactose /6'-sialyllactose standard working solution.

As the description in Table A.8., measure an appropriate amount of 3'-sialyllactose stock solution (A.5.2.17.3) and 6'-sialyllactose stock solution(A.5.2.17.4), place it in six 25mL volumetric flasks. Use 5%(v/v)aqueous ammonia(A.5.2.14) to produce the constant volume to the graduation.

Table A.8 Dilution Plan of Grade-6 Calibration Curve

	volumetric flask [mL]	3'-sialyllactose [μL]	6'-sialyllactose [μL]	concentration of 3'-sialyllactose (free acid) [μg/mL]	concentration of 6'-sialyllactose (free acid)浓度 [μg/mL]
#1	25	50	50	2.1	1.3
#2	25	200	75	8.4	2.0
#3	25	350	100	14.6	2.6
#4	25	500	125	20.9	3.3
#5	25	650	150	27.1	4.0
#6	25	800	175	33.4	4.6

A.5.3 Instrument and Equipment

A.5.3.1 High performance liquid chromatograph equipped with fluorescence detector.

A.5.3.2 analytical balance, with a precision of 0.1mg.

A.5.3.3 water bath.

A.5.3.4 10mL test tube, with screw cap .

A.5.3.5 solid-phase extraction column.

A.5.3.6 vacuum divided manifold for solid-phase extraction.

A.5.3.7 turbine mixer.

A.5.3.8 ultrasonic wave cleaner.

A.5.3.9 2mL miniature tube with security lock or screw cap.

A.5.3.10 miniature pipe frame.

A.5.3.11 miniature centrifuge.

A.5.3.12 automatic sampling bottle.

A.5.3.13 online pre-column filter.

A.5.4 Chromatographic Condition

A.5.4.1 chromatographic column: acylamino 80 column;3μm;4.6mm x 150mm;or other equivalent chromatographic column.

A.5.4.2 capture column: acylamino 80 guard column;3μm;3.2mm x 15mm.

A.5.4.3 column temperature: 23°C±1°C.

A.5.4.4 sample size: 20μL.

A.5.4.5 mobile phase A: acetonitrile.

A.5.4.6 mobile phase B: ammonium formate, 50mmol/L, pH4.40.

A.5.4.7 elution program: for the elution program, see Table A.9.

Table A.9 Elution Program Table

time [min]	flow rate [mL/min]	eluant (A) [%]	eluant (B) [%]	switching position of 10-position 6-pass valve
0	1.0	98.0	2.0	1-10(sample injection)
4.0	1.0	98.0	2.0	1-10(sample injection)
7.5	1.0	98.0	2.0	1-2 (analysis)
8.0	1.0	84.0	16.0	1-2 (analysis)
16.0	1.0	84.0	16.0	1-2 (analysis)
50.0	1.0	61.0	39.0	1-2 (analysis)
51.0	0.7	20.0	80.0	1-2 (analysis)
55.0	0.7	20.0	80.0	1-2 (analysis)
56.0	0.8	90.0	10.0	1-2 (analysis)
62.0	1.0	90.0	10.0	1-10(sample injection)
62.1	1.0	98.0	2.0	1-10(sample injection)
64.0	1.0	98.0	2.0	1-10(sample injection)

A.5.4.8 excitation wavelength: 330nm.

A.5.4.9 emission wavelength: 420nm.

A.5.4.10 start-up flow rate: 1mL/min.

A.5.5 Analysis Steps

Replace the sample solution and internal standard with 5.50mL water, and the rest steps (including SPE) are the same with sample preparation.

A.5.5.1.4 preparation of test sample solution

A.5.5.1.4.1 addition of internal standard (IS)

Accurately measure 5.00mL sample solution or standard working solution of 3'-sialyllactose /6'-sialyllactose (A.5.2.17.5), and place it in a 10mL tube (with screw cap). Add 500µL internal standard working solution of glucuronyl-lacto-N-tetraose (A.5.2.17.2). After covering it tightly, mix them with a turbine mixer .

A.5.5.1.4.2 steps of solid phase extraction and elution

a) the steps of SPE activation are as follows:

- 1) 5mL methanol.
- 2) 5mL water.
- 3) 2 x 5mL 1m acetic acid solution (A.5.2.13).
- 4) 4 x 5mL water.

b) inject 5.5mL sample solution diluted with internal standard into the upper part of filter) cartridge, and let it pass through slowly. Discard the leachate.

c) rinse the column with 3 x 5mL water, and discard the cleaning water.

- d) slowly elute it with 5 x 1mL5%(v/v)aqueous ammonia(A.5.2.14) in a clean 10mL tube (with screw cap).

A.5.5.1.4.3 2AB Mark

Transfer 20 μ L purified sample solution or standard working solution into a 2mL miniature tube. Add 200 μ L 2AB labeling reagent (A.5.2.15). add 200 μ L 2AB labeling reagent (A.5.2.15). Plug the tube up, after mixing them up with a turbine mixer, and place the tube on the miniature tube frame. Place in a 65°C \pm 1°C water bath for 2h \pm 5min. Mix them 20 min after the water bath. Mix them 2 h after the reaction and place it in a 4°Crefrigerator to rapidly cool it for 10 min.

A.5.5.1.4.4 dilution

After it is cooled, open the miniature tube, add 1.5mL water- acetonitrile25+75 solution (A.5.2.16) to dilute it. Mix them with a turbine mixer, and centrifugate it for 5 min with a 10000g centrifugal force. Transfer 1mL liquid supernatant into the sampling bottle. Keep it cooled before injecting the sample.

A.5.5.2 check and test of instrument

Blance the chromatographic system and preheat fluorescence detector. Before injecting the sample, make the standard solution and sample solution balanced to the temperature of automatic sample injector. Ensure that the system pressure and baseline stable without leakage.

Before analysis, inject water-acetonitrile25+75 solution into the chromatographic syste (to check the base line), and then inject the first standard solution for at least two times. Check the retention time, separation and response and compare them with the previous analysis.

A.5.5.3 Sequence Setting

Horizontal standard calibration curve : when the analytic sequence starts, inject and analyze three horizontal standard working solutions (#1-3-5), and inject and analyze three standard working solutions (#2-4-6) before completion. During the period, at most 20 samples can be injected to ensure equivalent calibration.

A.5.5.4 Calibration and Sample Analysis

Draw the linear regression curve of 3'-sialyllactose and 6'-sialyllactose for the corresponding sialyllactose concentration (in terms of μ g/mL) according to the ratio of peak area of standard substance and that of the internal standard (obtained by injection analysis of the calibration curve). Calculate the slope and intercept of each regression curve, and calculate the concentrations of two kinds of sialyllactose in the sample solution.

Calculate the slopes and intercepts of all regression curves. Calculate the concentrations of two kinds of sialyllactose in the sample solution.

A.5.5.5 Identification and Confirmation

Prepare single standard solution and single internal standard solution of two kinds of sialyllactose respectively, and analyze them respectively. Under the optimum chromatographic conditions, after the retention time for each chemical compound, mixed standard solutions can be used safely.

Identify three peaks of derived sample solutions (3'-sialyllactose , 6'-sialyllactose and internal standard glucuronyl-lacto-N-tetraose) through comparison with the retention time of the corresponding peak obtained from the standard solution. For the demonstrated chromatogram, see Fig. D.1 in Appendix D.

A.5.6 Calculation

The mass fraction of 3'-sialyllactose or 6'- sialyllactose (w), in terms of mg/100g sample, is calculated according to Formula A.10.

$$W = \frac{(\frac{A_s}{A_{IS}} - f) \times V_s \times 100}{5 \times m_s \times 10^3} \dots \dots \dots (A.10)$$

Where:

A_S - peak area of sialyllactose in the test sample solution (A.5.5.3.4)

A_{IS} - peak area of internal standard in the test sample solution (A.5.5.3.4)

I - intercept of calibration curve;

V_S - volume of test solution (A.5.5.1.1) (usually, it is 50), in terms of mL;

100- conversion factor on the basis of 100g;

S - slope of calibration curve;

m_S - mass of the sample (A.5.5.1.1), in terms of g;

10^3 - conversion factor from μg to mg.

A.5.7 Result Expression

Report the results of 3'-sialyllactose and 6'-sialyllactose in terms of mg/100g, rounding to one decimal place.

A.5.8 Precision

For 3'-sialyllactose, the absolute difference of the results of two dependant single tests carried out by the same operator in a short time for the same test materials with the same equipment and method in the same lab (calculated through $|x_1-x_2|$) shall not exceed:

- 1) 3mg, for galacto-oligosaccharides whose 3'-sialyllactose content $<200\text{mg}/100\text{g}$
- 2) 6mg, for galacto-oligosaccharides whose 3'-sialyllactose $>200\text{mg}/100\text{g}$.

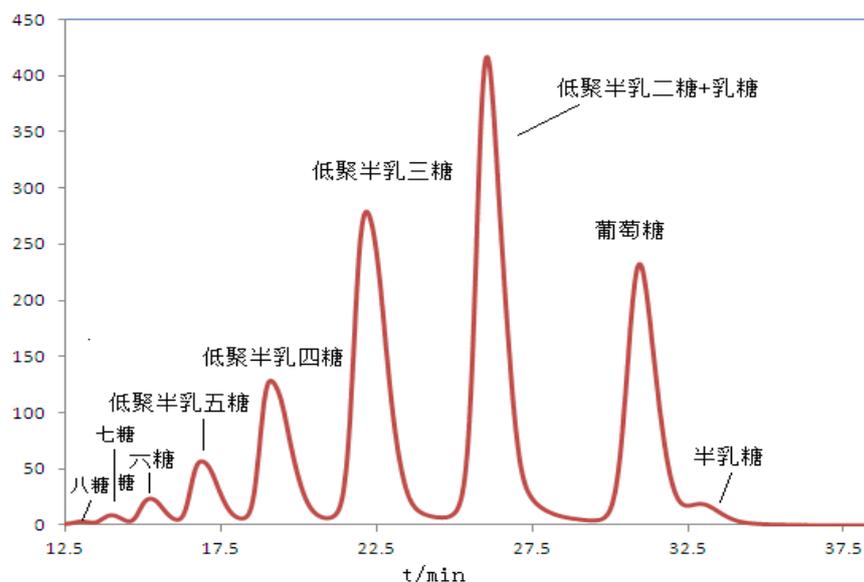
For 6'-sialyllactose, the absolute difference of the results of two dependant single tests carried out by the same operator in a short time for the same test materials with the same equipment and method in the same lab (calculated through $|x_1-x_2|$) shall not exceed 2 mg.

Appendix B

High Performance Liquid Chromatogram of Galacto-oligosaccharides

B.1 Chromatogram of Galacto-oligosaccharides Determined with Double-column High Performance Liquid Chromatography

For the high performance liquid chromatogram of Galacto-oligosaccharides, see Fig. B.1 and B.2.



低聚半乳二糖+乳糖/galactooligodisaccharide+ lactose

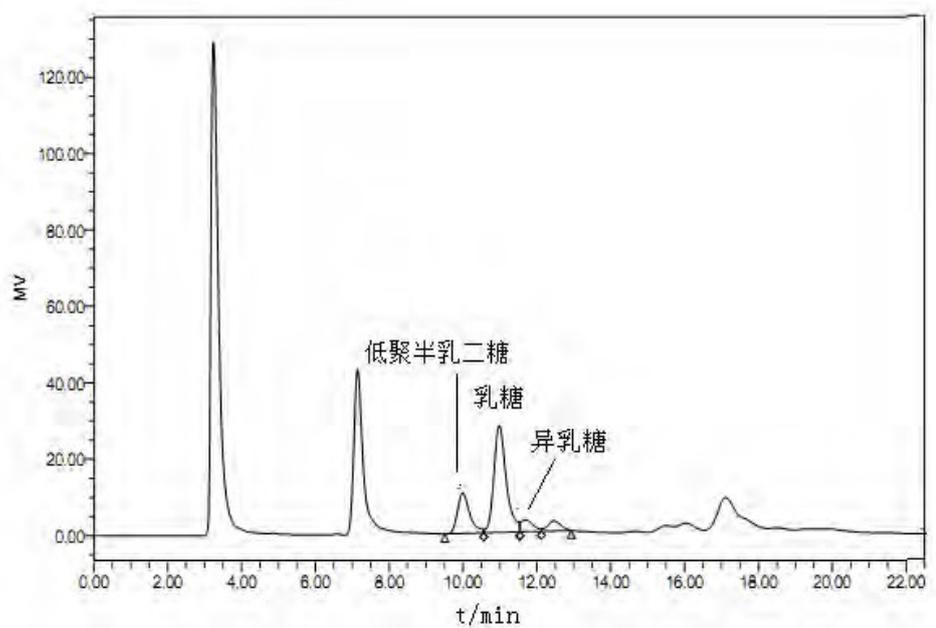
低聚半乳三糖/galactooligotrisaccharide

低聚半乳四糖/galactooligotetrasaccharide

低聚半乳五糖/galactooligopentasaccharide

半乳糖/galactose
七糖/heptasaccharide
六糖/hexasaccharide
八糖/octasaccharide
糖/saccharide
葡萄糖/glucose

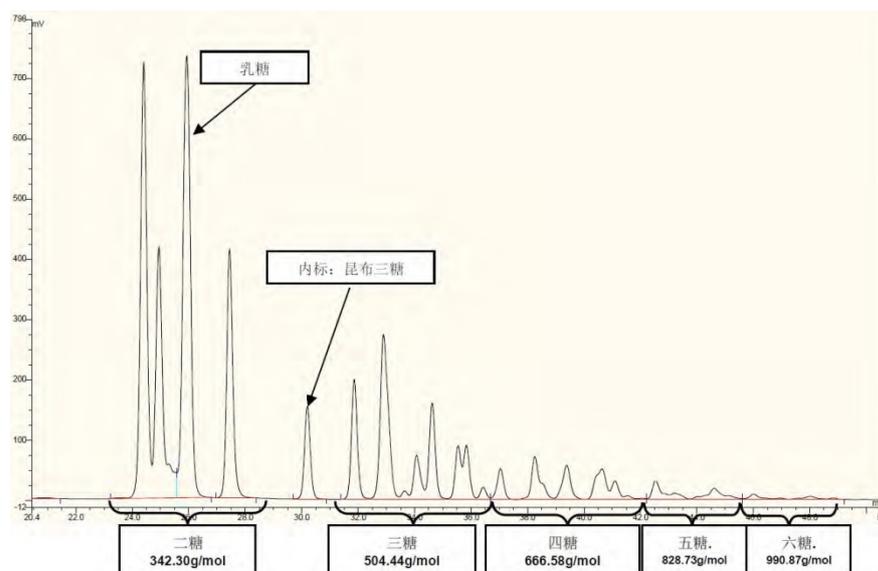
Fig. B.1 Chromatogram of Galacto-Oligosaccharide Determined with Silver-type Cation Exchange Column



低聚半乳二糖/galactooligodisaccharide
乳糖/Lactose
异乳糖/isolactose

Fig. B.2 Chromatogram of Galacto-Oligosaccharide Determined with Animo Column
B.2 Chromatogram of Galacto-Oligosaccharide Determined with High Performance Liquid Chromatograph

For the high performance liquid chromatogram of galacto-oligosaccharides, see FigB.3. .



乳糖/lactose

内标: 昆布三糖/internal standard: laminaritriose

二糖/disaccharide

三糖/trisaccharide

四糖/tetrasaccharide

五糖/pentasaccharides

六糖/hexasaccharide

Fig B.3 High Performance Liquid Chromatogram of Galacto-oligosaccharides

B.3 Quantitative Test for Molecular Mass of Different Oligosaccharides

B.3.1 disaccharide: 342.30g/mol

B.3.2 trisaccharide: 504.44g/mol

B.3.3 tetrasaccharide: 666.58g/mol

B.3.4 pentasaccharides: 828.73g/mol

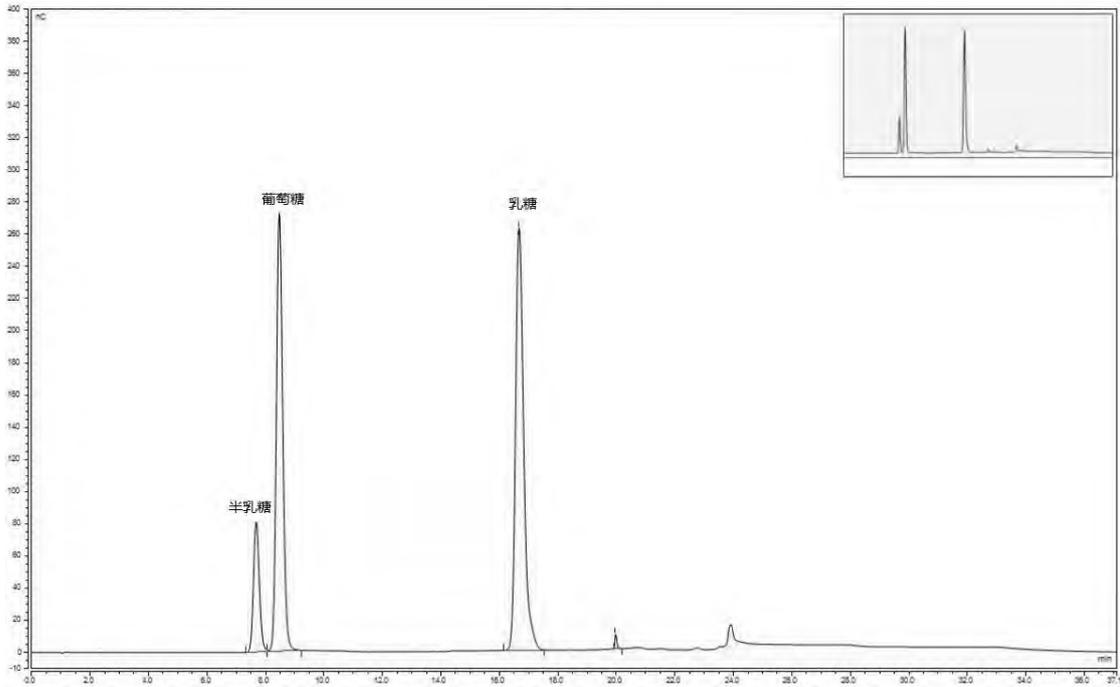
B.3.5 hexaose: 990.87g/mol

Appendix C

High Performance Liquid Chromatogram of Glucose and Galactose

C.1 High Performance Liquid Chromatogram of Glucose and Galactose

For the high performance liquid chromatogram of glucose and galactose, see Fig. C.1.



葡萄糖/Glucose
 半乳糖/Galactose
 乳糖/Lactose

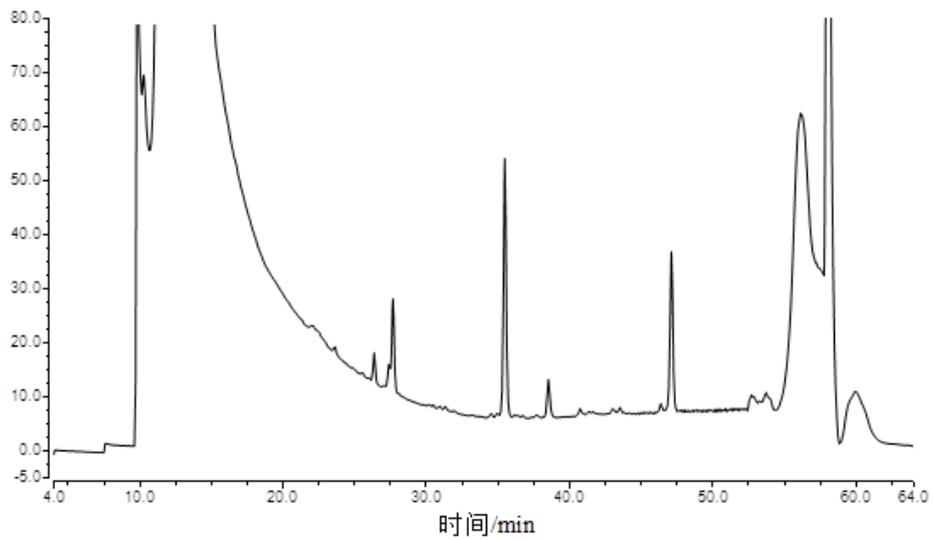
Fig. C.1 High Performance Liquid Chromatogram of Glucose and Galactose (lactose is just used as a quantitative reference)

Appendix D

High Performance Liquid Chromatogram of Sialyllactose

D.1 High Performance Liquid Chromatogram of Sialyllactose

For the high performance liquid chromatogram of 3'-sialyllactose, see Fig. D.1.



时间/min /Time/min

Fig. D.1 High Performance Liquid Chromatogram of Sialyllactose

Attachment 5

Extension of the Amount and Range of Application for 6 Food Additives Including Sodium Cyclamate

No.	Name	Function	Food Category No.	Food Name	The largest application amount(g/kg)	Note
1.	sodium cyclamate	edulcorant	06.07	instant rice and wheat food (only limited to flavored flour products)	1.6	on the basis of cyclohexyl-sulfamic acid
2.	tamarind polysaccharide gum	thickener	12.10.02	semi-solid compound seasoning	7.0	—
			12.10.03	liquid compound seasonings (12.03 and 12.04 not included)	3.0	
3.	rosemary extract	antioxidant	02.02.01	emulsified products with a fat content of over 80%	0.7	—
			02.03	fat emulsified products except Category 02.02, including mixed and (or) flavored fat emulsified products		
4.	sorbitol	humectant	09.04.01	cooked dry aquatic products	use with an appropriate amount depending on production demands	—
			09.04.02	cooked or fried aquatic products		
			09.04.03	smoked and roast aquatic products		
5.	ethylenediaminetetraacetic acid disodium salt	antioxidant	04.03.02.03	pickled edible fungi and algae	0.2	—
6.	diethyl ether	processing	-	processing of	residual	

		agent for the food industry (extraction solvent)		rice bran oil	quantity $\leq 2\text{mg/kg}$	
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