Amendment to the Enforcement Ordinance of the Food Sanitation Law and the Standards and Specifications for Foods and Food Additives.

The government of Japan will designate Advantame as an authorized food additive.

Summary

Under Article 10 of the Food Sanitation Law, food additives shall not be used or marketed without authorization by the Minister of Health, Labour and Welfare (hereinafter referred as "the Minister"). In addition, when specifications or standards are established for food additives based on Article 11 of the act and stipulated in the Ministry of Health, Labour and Welfare Notification (Ministry of Health and Welfare Notification No. 370, 1959), those additives shall not be used or marketed unless they meet the standards or specifications.

In response to a request from the Minister, the Committee on Food Additives of the Food Sanitation Council that is established under the Pharmaceutical Affairs and Food Sanitation Council has discussed the adequacy of the designation of Advantame as a food additive. The conclusion of the committee is outlined below.

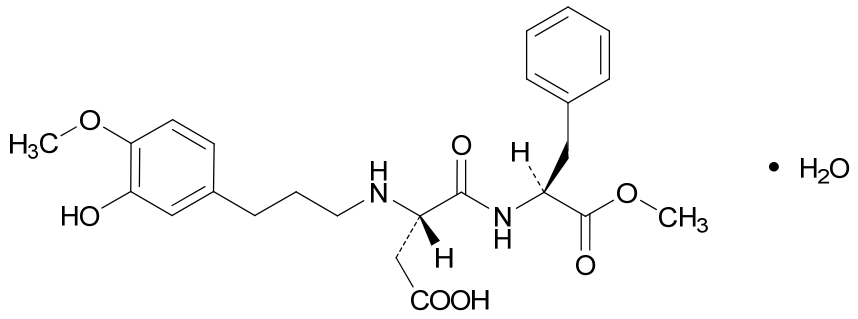
Outline of conclusion

The Minister of Health, Labour and Welfare, based on Article 10 of the Food Sanitation Law, should designate Advantame as a food additive unlikely to harm human health, and establish standard for use and compositional specifications, based on Article 11 of the law (see Attachment).

Attachment

Advantame

アドバンテーム



Standards for use

Not established.

Compositional specifications

Substance name Advantame

Molecular formula $C_{24}H_{30}N_2O_7 \cdot H_2O$

Molecular weight 467.52

Chemical name [CAS number]

Methyl *N*-[3-(3-Hydroxy-4-methoxyphenyl)propyl]-L-α-aspartyl-L-phenylalaninate monohydrate [714229-20-6]

Content Adantame, when calculated on the anhydrous basis, contains 97.0–102.0% of advantame ($C_{24}H_{30}N_2O_7$＝458.50).

Description Advantame is a white to yellowish white powder.

Identification Determine the infrared absorption spectrum of Advantame as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having about the same intensity at the same wavenumbers.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: –39 to –46° (0.2 g, ethanol (99.5), 100 ml, on the

anhydrous basis).

(2) Lead Not more than 1.0 μg/g as Pb.

Test Solution Weigh 4.0 g of Advantame in a platinum, quartz, or porcelain crucible or a quartz beaker. Moisten the sample entirely with diluted sulfuric acid (1 in 4), and heat by increasing the temperature gradually until it is carbonized and white fumes are no longer evolved. If necessary, add diluted sulfuric acid (1 in 4) again, and heat until it is almost carbonized. Loosely lid the crucible if necessary, heat in an electric furnace by increasing the temperature gradually, and incinerate at 450–600°C. If carbonized matter still remains, crush it with a glass rod, moisten with 1 ml of diluted hydrochloric acid (1 in 4) and 1 ml of nitric acid, heat until white fumes are no longer evolved, and ignite in the electric furnace to incineration. To the residue, add 10 ml of diluted hydrochloric acid (1 in 4), and evaporate on a water bath to dryness. To the residue, add a little amount of diluted nitric acid (1 in 100), and dissolve it while heating. After cooling, add diluted nitric acid (1 in 100) again to make exactly 10 ml. When the incineration is done at a temperature of 500°C or less, a heat-resistant glass beaker may be used.

Control Solution Add water to exactly measured 1 ml of Lead Standard Stock Solution to make exactly 100 ml. To exactly measured 4 ml of this solution, add diluted nitric acid (1 in 100) to make exactly 10 ml.

Procedure Proceed as directed under Method 1 in the Lead Limit Test.

(3) Advantame-acid Not more than 1.0%.

Test Solution Weigh accurately about 0.1 g of Advantame, and add a 7:3 mixture of water/acetonitrile to dissolve and make exactly 100 ml.

Standard Solution Weigh accuretaly about 0.1 g of advantame-acid, and add a 7:3 mixture of water/ acetonitrile to dissolve and make exactly 100 ml. Measure exactely 2 ml of this solution, and add a 7:3 mixture of water/acetonitrile to make exactly 20 ml. Measure exactely 2 ml of the second solution, and add a 7:3 mixture of water/acetonitrile to make exactly 20 ml.

Procedure Analyze 20 μl potions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of advantame-acid for the test solution and the standard solution, and determine its amount by the formula:

Amount (%) of advantame-acid

$$= \frac{W}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S}$$

W= weight (g) of advantame-acid taken

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 210 nm).

Column: A stainless steel tube of about 4.6 mm internal diameter and about 25 cm length.

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature around 50°C.

Mobile phase

A: Dissolve 13.6 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. To 900 ml of this solution, add 100 ml of acetonitrile.

B: Dissolve 13.6 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. To 400 ml of this solution, add 600 ml of acetonitrile.

Concentration gradient (A/B): Maintain (85/15) for 30 minutes, and run a linear gradient from 85/15 to 75/25 over 25 minutes, then 75/25 to 0/100 over 20 minutes, and maintain 0/100 for 15 minutes.

Flow rate: 1.0 ml/minute.

(4) Related substances other than advantame-acid Not more than 1.5%.

Test Solution and Standard Solution Use the test solution and the standard solution prepatred in Purity (3). Analyze 20 μl potions of these solutions by liquid chromatography using the operating conditions given below. Continues the chromatography for about three times the retention time of advantame-acid. Measure the sum (A_{sum}) of peak areas of all components, other than advantame and advantame-acid, in the test solution. Also measure the peak area (A_S) of advantame-acid in the standard solution. Determine the amount of the related substances other than advantame-acid by the formula:

$$\text{Amount (\%) of related substances} = \frac{W}{\text{Weight (g) of the sample}} \times \frac{A_{sum}}{A_S}$$

W= weight (g) of advantame-acid taken.

Operating Conditions Follow the operating conditions specified in Purity (3).

Water Not more than 5.0% (0.1g, Direct Titration).

Residue on Ignition Not more than 0.2% (550°C, 3 hours)

Assay

Test Solution Weigh accurately about 0.04 g of Advantame, and add a 7:3 mixture

of water/acetonitrile to dissolve and make exactely 50 ml. To exactly measured 10 ml of this solution, add exactly 5 ml of the internal standard and a 7:3 mixture of water/acetonitrile to make exactly 50 ml.

Standard Solution Weigh accurately about 0.04 g of advantame for assay, and proceed as directed for the test solution.

Internal Standard To 0.04 g of benzoic acid, exactly weighed, add a 7:3 mixture of water/acetonitrile to make 50 ml.

Procedure Analyze 20 μl potions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of advantame to benzoic acid for both solutions to calculate the Content of advantame by the formula:

$$\text{Content (\%) of advantame } (C_{24}H_{30}N_2O_7) = \frac{\text{Anhydrous based weight (g) of advantame for assay}}{\text{Anhydrous based weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 280 nm).

Column: A stainless steel tube of about 4.6 mm internal diameter and about 25 cm length.

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature around 40°C.

Mobile phase

A: Dissolve 13.6 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. To 750 ml of this solution, add 250 ml of acetonitrile.

B: Dissolve13.6 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. To 500 ml of this solution, add 500 ml of acetonitrile.

Concentration gradient (A/B): Maintain 100/0 for 20 minutes, and run a linear gradient from 100/0 to 0/100 over 5 minutes, then 0/100 for 15 minutes.

Flow rate: 1.0 ml/minute.

Reagents and Solutions

Advantame-acid $C_{28}H_{28}N_2O_7$

N-[3-(3-Hydroxy-4-methoxyphenyl)propyl]-L-α-aspartyl-L-phenyl Alanine

A white to yellow powder.

Content Not less than 94% of advantame-acidon anhydrous basis.

Purity (1) Chlorides Not more than 1.0% as Cl.

Prepare a test solution by adding a 7:3 mixture of water/acetonitrile to about 0.01 g of Advantame-acid, accurately weighed, to dissolve and make exactely 100 ml. Prepare two standard solutions. First, add water to about 0.016 g of sodium chloride, accurately weighed, to disslove and make exactely 100 ml. Designate this as standard solution A. Prepare standard solution B by dilutuing exactely measured 2 ml of standard solution A with water to make exactely 20 ml. Anlyze 30-μl potions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas of cloride anion for standard solutions A and B to make a calibration curve. Measure the peak area of cloride anion for the test solution. Determine the concentration of chlorides in the test solution from the clibration curve and calculate the amount of chlorides by the formula:

$$\text{Amount (\%)of chlorides} = \frac{\text{Concentration (g/ml) of chlorides in the test solution}}{\text{Weight (g) of the sample}} \times 10{,}000$$

Operating conditions

Detector: Electric conductivity detector.

Column: A polyether ketone tube of about 4.6 mm internal diameter and about 15 cm length.

Column packing material: 6-μm strongly basic anion-exchange resin.

Column temperature: A constant temperature around 40°C.

Mobile phase: A mixture prepared by dissolving 201.62 mg of sodium hydrogen carbonate and 264.98 mg of anhydrous sodium carbonate in 1000 ml of water.

Flow rate: Adjust so that the retention time of chloride anion is about 7 minutes.

(2) Sodium Not more than 5.0% as Na.

Prepare a test solution by adding a 7:3 mixture of water/acetonitrile to about 0.01 g ofAdvantame-acid, accurately weighed, to dissolve and make exactely 100 ml. Prepare two standard solutions. First, add water to about 0.006 g of sodium chloride, accurately weighed, to disslove and make exactely 100 ml. Designate this as standard solution A. Prepare standard solution B by dilutuing exactely measured 2 ml of standard solution A with water to make exactely 20 ml. Anlyze 30-μl potions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below.

Measure the peak areas of sodium for standard solutions A and B to make a calibration curve. Measure the peak area of sodium cation for the test solution. Determine the concentration of sodium cation in the test solution from the clibration curve and calculate the amount of sodium by the formula:

$$\text{Amount (\%) of sodium} = \frac{\text{Concentration (g/ml) of sodium in the test solution}}{\text{Weight (g) of the sample}} \times 10{,}000$$

Operating conditions

Detector: Electric conductivity detector.

Column: A polyether ketone tube of about 4.6 mm internal diameter and about 15 cm length.

Column packing material: 3-μm weakly acidic cation-exchange resin for liquid chromatography.

Column temperature: A constant temperature around 40°C.

Mobile phase: A mixture prepared by dissolving 77.58 mg of L-histidine in 1.25 ml of 2 mol/L methanesulfonic acid and adding 1000 ml of water.

Flow rate: Adjust so that the retention time of sodium cation is about 4 minutes.

Water Not more that 1.0% (0.1 g, Direct Titration)

Assay Prepare a test solution by adding a 7:3 mixture of water/acetonitrile to 0.01 g of Advantame-acid to dissolve and make exactely 100 ml. Anlyze 20-μl potions of the test solution by liquid chromatography using the operating conditions given below. Continue chromatography for about six times the retention time of advantame-acid. Measure the peak areas, and determine the area percentage, C (%), of the main peak by normalizing the sum of the peak areas of all components in the tets solution to 100. Calculate the content by the formula:

Content (%) of advantame-acid

$$= (100 - \text{chlorides amount} - \text{sodium amount} - \text{water}) \times \frac{C(\%)}{100}$$

Operating conditions Follow the conditions given in the Assay for Advantame in the Monographs.

Advantame for Assay $C_{24}H_{30}N_2O_7 \cdot H_2O$ A white to yellowish white powder.

Content Not less than 99.0% of advantame ($C_{24}H_{30}N_2O_7$) when calculated on the anhydrous basis.

Identification Determine the infrared absorption spectrum of Advantame for Assay as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at about 3405 cm^{-1}, 3320 cm^{-1}, 2945 cm^{-1}, 1717 cm^{-1}, 1661

cm^{-1}, 1582 cm^{-1}, 1376 cm^{-1}, 1242 cm^{-1}, 1131 cm^{-1}, and 703 cm^{-1}.

Purity (1) Specific rotation $[\alpha]_D^{20}$: –39 to –46° (0.2 g, ethanol (99.5), 100 ml, on the anhydrous basis).

(2) Related substances Not more than 1.0% as advantame-acid.

Prepare a test solution by adding a 7:3 mixture of water/acetonitrile to about 0.1 g of Advantame for Assay, accurately weighed, to dissolve and make exactly 100 ml. Prepare a standard solution as follows: Add a 7:3 mixture of water/acetonitrile to about 0.1 g of advantame-acid, accuretaly weighed, to dissolve and make exactly 100 ml. To exactly measured 2 ml of this solution, add a 7:3 mixture of water/acetonitrile to make exactly 20 ml. To exactly measured 2 ml of the second solution, add a 7:3 mixture of water/acetonitrile to make exactly 20 ml. Analyze 20 μl potions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Continues the chromatography for about three times the retention time of advantame-acid. Measure the sum (A_T) of the peak areas of all components, other than advantame, in the test solution and the peak area (A_S) of advantame-acid in the standard solution. Calculate the amount of the related substances by the formula:

$$\text{Amount (\%) of related substances} = \frac{W}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S}$$

W= weight (g) of advantame-acid taken.

Operating Conditions Follow the operating conditions given in Purity (3) for Advantame in the Monographs.

Water Not more than 5.0% (0.1g, Direct Titration).

Residue on Ignition Not more than 0.2% (550°C, 3 hours)

Assay To about 0.5 g of Advantame for Assay, accurately weighed, add 100 ml of ethanol to dissolve, and titrate with 0.1 mol/L sodium hydroxide. Usually, a potentiometer is used to confirm the end point. Perform a blank test to make correction.

Each mL of 0.1 mol/L sodium hydroxide = 45.85 mg of $C_{24}H_{30}N_2O_7$

L-Histidine $C_6H_9N_3O_2$ White crystals or powder.

Content Not less than 98.0% of L-histidine.

Purity Specific rotation $[\alpha]_D^{20}$: +12.0 to +13.0° (1 g, hydrochloric acid, 10 ml).

Assay Dissolve about 0.15 g of L-Histidine, accurately weighed, in 2 ml of formic acid, add 50 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the end point. Perform a blank test to make correction.

Each ml of 0.1 mol/L perchloric acid = 15.52 mg of $C_6H_9N_3O_2$

Methanesulfonic Acid CH_4O_3S A colorless to light yellow-brown, clear liquid.

Content Not less than 98.0% of methanesulfonic acid.

Assay Mix about 2 g of Methanesulfonic Acid, accurately weighed, with 40 ml of water. Titrate with 1 mol/L sodium hydroxide using 2 drops of bromothymol blue TS as an indicator. Perform a blank test to make correcction.

Each ml of 1 mol/L sodium hydroxide = 96.11 mg of CH_4O_3S

Strongly Basic Anion-exchange Resin for Liquid Chromatography Use a product prepared for liquid chromatography.

Weakly Acidic Cation-exchange Resin for Liquid Chromatography Use a product prepared for liquid chromatography.

Infrared spectrum

Advantame