ADVANTAME

Prepared at the 80th JECFA (2015), published in FAO JECFA Monographs 17 (2015), superseding tentative specifications prepared at 77th JECFA (2013). An ADI of 0-5 mg/kg body weight was established at the 77th JECFA (2013).

SYNONYMS INS No. 969

DEFINITION Advantame is manufactured by *N*-alkylation of aspartic acid portion of aspartame (L-α-aspartyl-L-phenylalanine methylester) with 3-(3-hydroxy-4-methoxyphenyl) propionaldehyde produced by selective catalytic hydrogenation from 3-hydroxy-4-methoxycinnamaldehyde. The product is purified through re-crystallisation and dried.

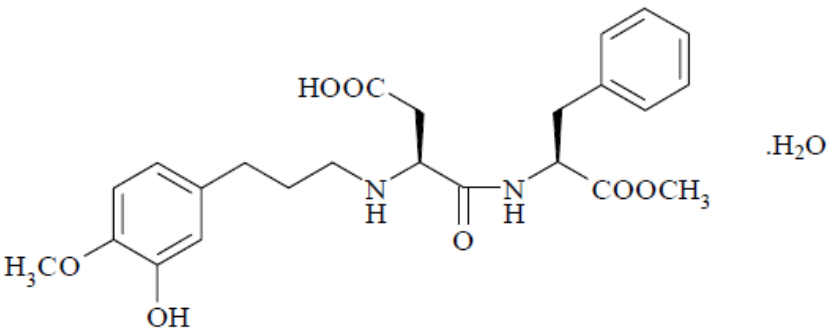
Only the following solvents may be used for the production: methanol and ethyl acetate.

Chemical names (3S)-3-[3-(3-hydroxy-4-methoxyphenyl)propylamino]-4-[[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-4-oxobutanoic acid hydrate, *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-α-aspartyl]-L-phenylalanine 1-methyl ester, monohydrate

C.A.S. number 714229-20-6

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

Structural formula



Formula weight 476.52

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

DESCRIPTION White to yellow powder

FUNCTIONAL USES Sweetener, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very slightly soluble in water, sparingly soluble in ethanol

Infrared spectrum	The infrared spectrum of a potassium bromide dispersion of the sample corresponds to the standard infrared spectrum in Appendix A.

PURITY

Water (Vol. 4)	Not more than 5% (Karl Fischer)
Residue on ignition (Vol. 4)	Not more than 0.2% (use 5 g of the sample)
N-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenyl-alanine (acid of advantame)	Not more than 1% See description under TESTS
Other related substances	Not more than 1.5% (expressed as acid of advantame) See description under TESTS
Specific rotation (Vol. 4)	$[\alpha]_D^{20}$: Between -46° and -39° (0.2% solution in ethanol, on an anhydrous basis)
Residual solvents	Methanol: Not more than 500 mg/kg Ethyl acetate: Not more than 500 mg/kg See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

N-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenyl-alanine (acid of advantame)

Principle

Determination of *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine by HPLC

Mobile phase

Mobile phase A: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 100 ml of acetonitrile to 900 ml of this solution, mix well, and sonicate for about 5 min.

Mobile phase B: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 600 ml of acetonitrile to 400 ml of this solution, mix well, and sonicate for about 5 min.

Standard solution

Dissolve the acid of advantame reference standard (available from Wako Pure Chemicals, Osaka, Japan) in a mixture of water and acetonitrile (7:3 v/v) to concentrations of 15, 10, 5, 2 and 0.2 µg/ml.

Preparation of Sample solution: Dissolve the sample in a mixture of water

and acetonitrile (7:3 v/v) to a concentration of 1 mg/ml.

System suitability solution
Prepare a solution containing 10 µg/mL of advantame reference standard and 10 µg/ml of acid of advantame reference standard (both available from Wako Pure Chemicals, Osaka, Japan) in a mixture of water and acetonitrile (7:3 v/v).

HPLC conditions:

Column:	Inertsil ODS-2 (25 cm x 4.6 mm i.d., 5 µm), GL Sciences, or equiv.
Column temperature:	50°
Mobile phase:	
Mobile phase A:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (9:1 v/v)
Mobile phase B:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (2:3 v/v)
Flow rate:	1.0 ml/min
Injection volume:	20 µl
Detector:	UV at 210 nm
Run Time:	80 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	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

System suitability requirement
The resolution between the advantame and acid of advantame peaks is not less than 3.0 in the chromatogram of the System suitability solution. (Note: The approximate retention times for acid of advantame and advantame are 29.6 min and 56.0, respectively).

Analysis
Separately inject the Standard solution and the Sample solution into the chromatograph, record the chromatograms, and determine the peak area responses for the peaks in the resulting chromatograms.

Calculation
Calculate the percentage of acid of advantame in the sample using the following formula:

$$\text{Acid of advantame (\%)} = (r_U/r_S) \times (C_S/C_U) \times 100$$

where

r_U is the peak area response of advantame acid obtained from the chromatogram of the sample solution
r_S is the peak area response of advantame acid from the chromatogram of the standard solution
C_S is the concentration of the standard solution (µg/ml)
C_U is the concentration of the sample solution (µg/ml)

See Appendix B for example of chromatogram obtained using the method.

Other related substances

Calculation

Calculate the total percentage of other related substances from the results of the Test for *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine using the following formula:

$$\text{Total content of other related substances (\%)} = (r_T/r_S) \times (C_S/C_U) \times 100$$

where

r_T is the total peak response of all peaks, except those of advantame and advantame acid obtained from the chromatogram of the sample solution (disregard any peak areas less than 0.02%)
r_S is the peak area response of advantame acid from the chromatogram of the standard solution
C_S is the concentration of the standard solution (µg/ml)
C_U is the concentration of the sample solution (µg/ml)

Residual solvents

Principle

Proceed as directed in Residual Solvents by Headspace Gas Chromatography (Vol. 4) using the following:

Sample solution

Accurately weigh about 0.08 g of advantame to an appropriate headspace vial, and add 2 ml of DMF, apply the stopper, cap, and mix.

Standard Solution

Accurately weigh 0.1 g methanol, and add DMF to make exactly 10 ml (stock solution 1). Accurately weigh 0.1 g of ethyl acetate, and add DMF to make exactly 20 ml (stock solution 2). Transfer 1 ml of stock solution 1 and 1 ml of stock solution 2 into a 10-ml volumetric flask, and add DMF to make exactly 10 ml. Transfer 1 ml of this solution into a 10 ml volumetric flask, and add DMF to make exactly 10 ml (mixture stock solution). Transfer 1 ml of mixture stock solution into a 25 ml volumetric flask, and add DMF to make exactly 25ml. Transfer 2 ml of this solution to an appropriate headspace vial, apply the stopper, cap, and mix.

Procedure

Analyse using the analytical conditions for Residual Solvents by Headspace Gas Chromatography as described in Vol. 4.

Calculation

Calculate the content (mg/kg) of each residual solvent using the following formulae:

$$\text{Content of methanol (mg/kg)} = W_{SA} / W_T \times A_{TA} / A_{SA} \times 80$$
$$\text{Content of ethyl acetate (mg/kg)} = W_{SB} / W_T \times A_{TB} / A_{SB} \times 40$$

where

A_{TA} is the peak area of methanol from the Sample solution;
A_{TB} is the peak area of ethyl acetate from the Sample solution
A_{SA} is the peak area of methanol from the Standard solution;
A_{SB} is the peak area of ethyl acetate from the Standard solution;
W_T is the weight (g) of Advantame in the Sample solution;
W_{SA} is the weight (g) of methanol in the Standard solution; and
W_{SB} is the weight (g) of ethyl acetate in the Standard solution.

METHOD OF ASSAY

Principle

Determine by HPLC using the following conditions:

Mobile phase

Mobile phase A: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 250 ml of acetonitrile to 750 ml of this solution, mix well, and sonicate for about 5 min.

Mobile phase B: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 500 ml of acetonitrile to 500 ml of this solution, mix well, and sonicate for about 5 min.

Iinternal standard

Accurately weigh about 40 mg of benzoic acid and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Standard stock solution

Accurately weigh about 40 mg of advantame reference standard (available from Wako Pure Chemical Industries, Osaka, Japan), dissolve in a mixture of water and acetonitrile (7:3 v/v) to make 50 ml.

Standard solution

Pipet 8, 9 10, 11, 12 ml of standard stock solution into five volumetric flasks. Add 5 ml of the Internal standard solution to each volumetric flask, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Sample solution

Accurately weigh about 40 mg of advantame and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml. Pipet 10 ml of this solution, transfer into 50 ml volumetric flask, add exactly 5 ml of the internal standard solution, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

HPLC conditions

Column:	Inertsil ODS-2 (25 cm x 4.6 mm i.d., 5 µm) GL Sciences, or equiv.
Column temperature:	40°
Mobile phase:	
Mobile phase A:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (75:25 v/v)
Mobile phase B:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (50:50 v/v)
Flow rate:	1.0 ml/min
Injection volume:	20 µl
Detector:	UV at 280 nm
Run Time:	55 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
20	100	0
50	0	100
55	0	100

System suitability

Suitability requirement 1: The resolution between the benzoic acid and advantame peaks is not less than 10 in the chromatogram of the standard solution having the concentration of advantame reference standard closest to 160 µg/ml. (Note: The elution order must be benzoic acid then advantame).

Suitability requirement 2: When injected six consecutive times, the relative standard deviation for the retention time of the advantame peak is not more than 1.0% for the standard solution having the concentration of advantame reference standard closest to 160 µg/ml.

Analysis

Separately inject the Standard solutions into the chromatograph (including the stock solution), record the chromatograms, and determine the peak area responses for the major peaks in the resulting chromatographs (Note: The approximate retention time for advantame is 16.5 min). For each standard solution, calculate the ratio of the peak area response of the advantame peak to that of the benzoic acid internal standard peak. Create a standard curve by plotting the resulting peak area response ratios versus the concentrations of the standard solutions. Inject the Sample solution into the chromatograph, record the chromatogram, and determine the peak area responses for the major peaks in the resulting chromatogram. Calculate the ratio of the peak area response of the advantame peak to that of the benzoic acid internal standard peak. Using the standard curve, determine the concentration of advantame (C_A) in the sample solution, in µg/m.

Calculation

Calculate the percentage of advantame ($C_{24}H_{30}N_2O_7$) in the sample taken:

$$\text{Advantame (\%)} = (C_A/C_U) \times 100$$

where

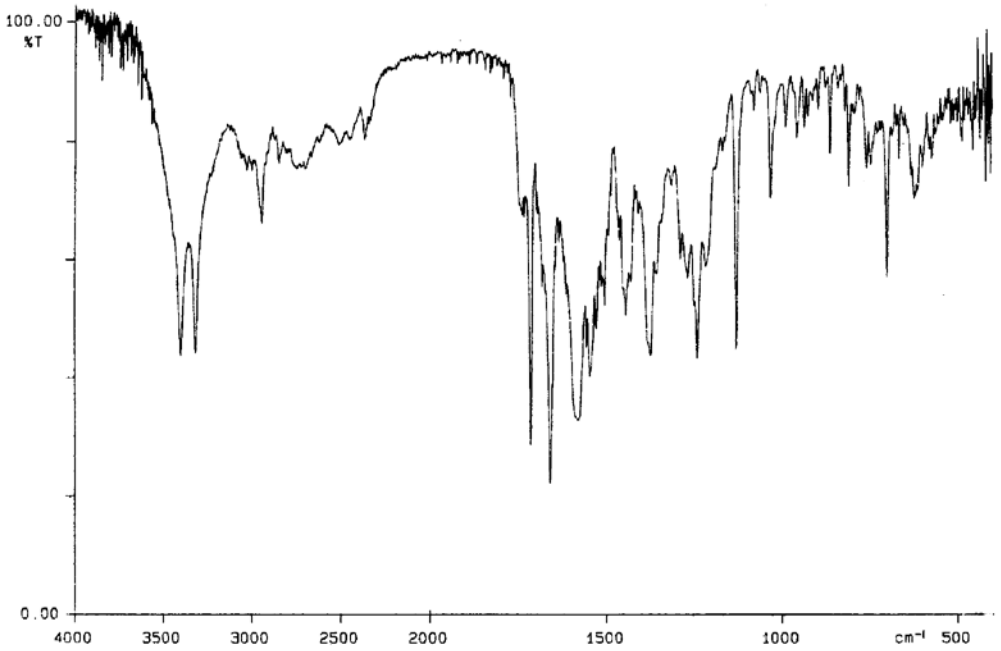
C_A is the concentration of advantame in the sample solution as determined from the standard curve (µg/mL)

C_U is the concentration of the sample solution (µg/mL)

See Appendix C for example of chromatogram obtained using the method.

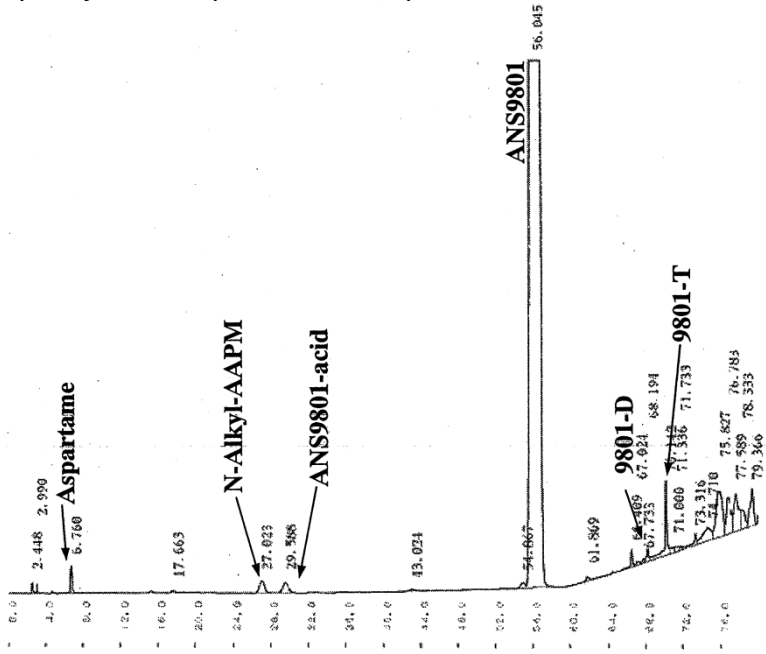
Appendix A

IR spectrum of advantame standard (Ajinomoto Co., Inc.)



Appendix B

Representative chromatogram for advantame (ANS9801), N-[N-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (ANS9801-acid) and other related substances at 210 nm.



Other identified compounds:

- L-α-aspartyl-L-phenylalanine methylester (Aspartame)
- *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (ANS9801-acid)
- *N*-[*N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-α-L-aspartyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (N-Alkyl-AAPM)
- N-[*N*-[3-(3-hydroxy-4-methoxyphenyl)pentyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (9801-D)
- *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)heptyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (9801-T)

Appendix C

Representative chromatogram for Advantame using the Method of Assay at 280 nm