ADVANTAME
(TENTATIVE)

New tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI of 0-5 mg/kg body weight was established at the 77th JECFA (2013).

Information required on:

- *Suitability of the head space GC method (using appropriate dissolution solvent) for determination of residual solvents published in the "Combined Compendium of Food Additives Specifications, Vol. 4" and data, in a minimum of 5 batches, using the method,*
- *An alternative/improved HPLC method for the assay of advantame and acid of advantame using a standard curve,*
- *Additional data and analytical methods for determination of palladium and platinum,*
- *Information on the purity and availability of the commercial reference standards used in the assay of advantame and acid of advantame*

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.

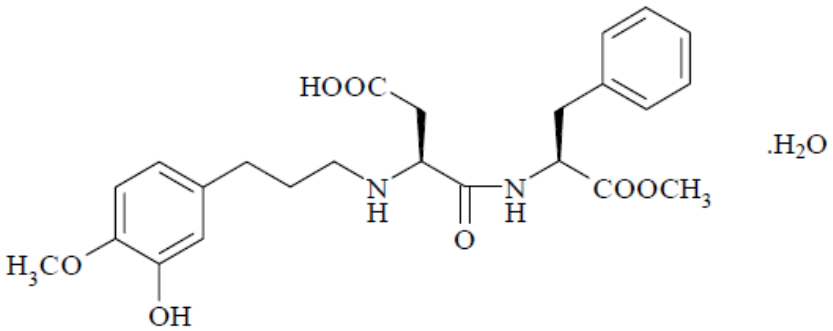
Only the following solvents may be used for the production: methanol and ethyl acetate.

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 -45° and -38°(0.2% solution in ethanol)
Residual solvents	Methanol: Not more than 500 mg/kg Ethyl acetate: Not more than 500 mg/kg See description under TESTS
Palladium	Information required
Platinum	Information required
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)

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

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.

Preparation of Standard Solution: To prepare the standard stock solution of acid of advantame, accurately weigh about 100 mg of acid of advantame standard (ANS9801-acid Standard Reagent, available from Ajinomoto Co., Inc., Japan) and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 100 ml, in order to prepare acid of advantame standard solution, pipet 2 ml of standard stock solution and add a mixture of water and acetonitrile (7:3 v/v) to make an exact volume of 20 ml.

Preparation of Sample Solution: Accurately weigh about 100 mg of advantame and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 100 ml.

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 detector 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

Calculate the content (%) of acid of advantame using the following formula:

Acid of advantame (%) = $[(W_S \times C_S)/W_T] \times [A_{T1}/A_S] \times [1/100]$

where
A_{T1} is peak area of acid of advantame from the sample solution;
A_s is peak area of acid of advantame from the standard solution;
W_T is weight (g) of advantame;
W_S is weight (g) of acid of advantame standard reagent; and
C_S is purity (%) of acid of advantame standard reagent.

Other related substances

Calculate from the results of the Test for acid of advantame using the following formula:

Total content of other related substances (%) = $[(W_S \times C_S)/W_T] \times [A_{T2}/A_S] \times 1/100$

where
A_{T2} is total peak area other than advantame and other than acid of advantame from the sample solution. The peak areas below the quantitation limit (i.e., 0.02%) are not used in the calculation.
A_S is peak area of acid of advantame from the standard solution;
C_S is purity (%) of acid of advantame Standard Reagent;
W_T is weight (g) of advantame; and
W_S is weight (g) of acid of advantame Standard Reagent.

Residual solvents

Determine by GC using the following conditions:
(Tentative)
Preparation of Sample Solution: Accurately weigh about 0.1 g of advantame, and add 1-butanol to make exactly 10 ml.

Preparation of Standard Solution: Accurately weigh 0.1 g methanol, and add 1-butanol to make exactly 50 ml (stock solution 1). Accurately weigh 0.1 g ethyl acetate, and add 1-butanol to make exactly 50 ml (stock solution 2). Pipet and transfer 2.5 ml each of stock solution 1 and stock solution 2 into a 50-ml volumetric flask, and add 1-butanol to make exactly 50 ml (mixture stock solution). Pipet and transfer 2 ml of mixture stock solution into a 20 ml volumetric flask, and add 1-butanol to make exactly 20 ml.

GC conditions:

Column:	DB-WAX (30 m x 0.53 mm i.d., 1.0 µm), J & W Scientific, or equiv.
Column temperature:	40^o – 7 min - 80^o/min - 220^o – 5 min
Detector:	Flame-ionization detector
Carrier gas:	Helium
Carrier gas flow rate:	36 cm/sec (2.8 ml/min)
Injection volume:	1 µl
Detector temperature:	220°
Injector temperature:	120°

Calculation: Calculate the content (µg/g) of each residual solvent using the following formula:

Content of methanol or ethyl acetate (µg/g) = $[W_S \times C_S/W_T] \times [A_T/A_S] \times 10$
where
A_T is a peak area of methanol or ethyl acetate from the sample solution;

A_S is a peak area of methanol or ethyl acetate from the standard solution;
W_S is weight (mg) of methanol or ethyl acetate in the standard solution;
W_T is weight (mg) of advantame sampled;
C_S is purity (%) of methanol or ethyl acetate; and
10 is correction factor for dilution.

METHOD OF ASSAY (Tentative)

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.

Preparation of Internal 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.

Preparation of Standard Solution: Accurately weigh about 40 mg of advantame reference standard (available from Ajinomoto Co., Inc., Japan), dissolve in a mixture of water and acetonitrile (7:3 v/v) to make 50 ml. Pipet 10 ml of this solution, add 5 ml of the internal standard solution, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Preparation of 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, add 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 detector 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

Calculate the content (%) of advantame using the following formula:

Advantame (%) = $[W_S/W_T] \times [Q_T/Q_S] \times [(100 - W_{std} - S_{std})/(100 - W_{smp} - S_{smp})] \times 100$

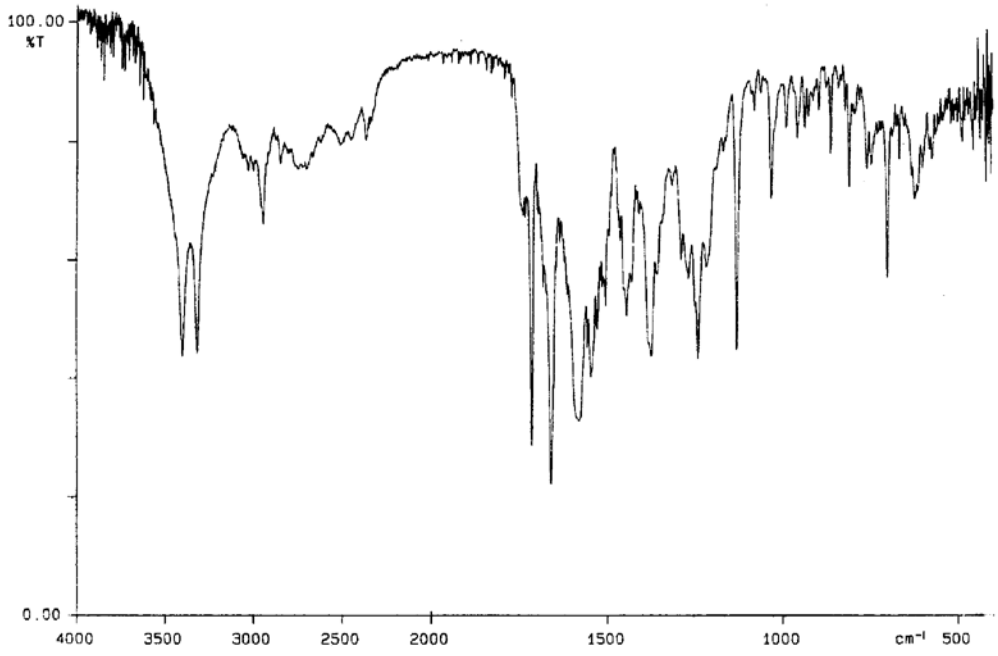
where

Q_T is ratio of the peak area of advantame to that of the internal standard from the sample solution;
Q_S is ratio of the peak area of advantame to that of the internal standard from the standard solution;
S_{std} is residual solvent content (%) of advantame reference standard;
S_{smp} is residual solvent content (%) of advantame sample;
W_S is weight (g) of advantame reference standard sampled;
W_T is weight (g) of advantame sample sampled;
W_{std} is water content (%) of advantame reference standard sample determined by Karl Fischer (Vol.4);
W_{smp} is water content (%) of advantame sample determined by Karl Fischer (Vol.4); and
100 is correction factor

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

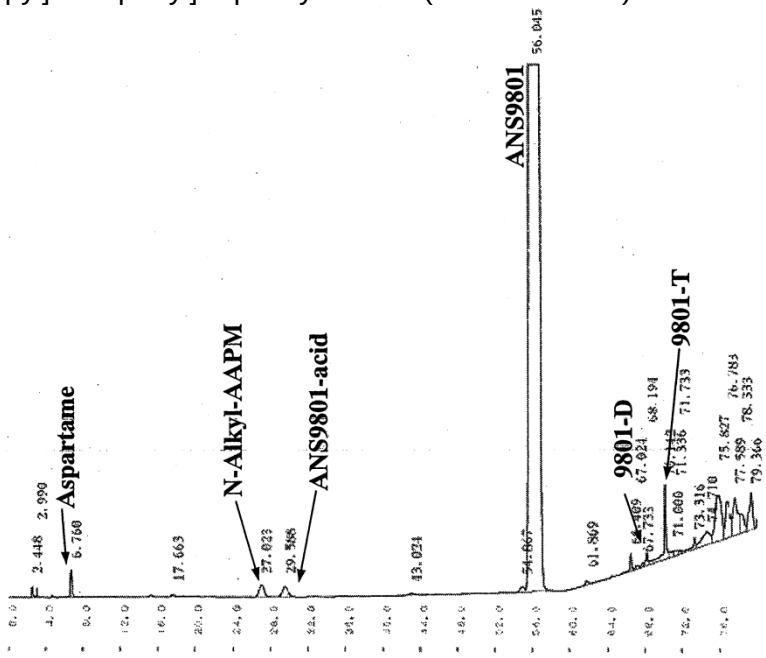
Appendix A

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



Appendix B

Representative chromatogram for advantame (ANS9801) and N-[N-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (ANS9801-acid) 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); and
- *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)heptyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (9801-T);