Food and Agriculture Organization of the United Nations

World Health Organization

Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 82nd meeting 2016

Aspartame

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ASPARTAME

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016) superseding specifications prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001)
An ADI of 0-40 mg/kg bw was established at the 25th JECFA (1981)

SYNONYMS Aspartyl phenylalanine methyl ester: APM; INS No. 951

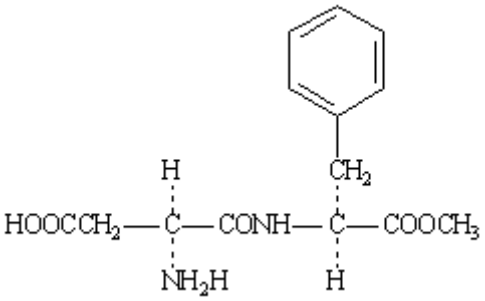
DEFINITION

Chemical names 3-Amino-N-(alpha-carbomethoxy-phenethyl)-succinamic acid, N-L-alpha-aspartyl-L-phenylalanine-1-methyl ester

C.A.S. number 22839-47-0

Chemical formula $C_{14}H_{18}N_2O_5$

Structural formula



Formula weight 294.31

Assay Not less than 98% and not more than 102% on the dried basis

DESCRIPTION White, odourless, crystalline powder

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Slightly soluble in water and practically insoluble or insoluble in ethanol

Test for amine group Dissolve 2 g of ninhydrin in 75 ml of dimethylsulfoxide, add 62 mg of hydrindantin, dilute to 100 ml with 4 M lithium acetate buffer solution (pH

	9), and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple colour is formed.
Test for ester	Dissolve about 20 mg in 1 ml of methanol, add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, then add 0.3 ml of 5 M potassium hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the pH to between 1 and 1.5 with hydrochloric acid TS, and add 0.1 ml of ferric chloride TS. A burgundy colour is produced.

PURITY

Loss on drying (Vol. 4)	Not more than 4.5% (105°, 4 h)
pH (Vol. 4)	4.5 - 6.0 (1 in 125 soln)
Specific rotation (Vol. 4)	[alpha] 20, D: Between + 14.5 and + 16.5° (4% solution in 15 M formic acid; determine within 30 min after preparation of the sample solution)
Spectrophotometry (Vol. 4)	The transmittance of a 1 in 100, 2 M hydrochloric acid solution, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 M hydrochloric acid as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.
Sulfated ash (Vol. 4)	Not more than 0.2% Test 5 g of the sample (Method I)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
5-Benzyl-3,6-dioxo-2-piperazineacetic acid (DKP)	Not more than 1.5% See description under TESTS
Other optical isomers	Not more than 0.02% (As sum of L-alpha-aspartyl-D-phenylalanine methyl ester (L,D-APM) and D-alpha-aspartyl-L-phenylalanine methyl ester (D,L-APM)) See description under TESTS

TESTS

PURITY TESTS

DKP	Apparatus

High performance liquid chromatograph equipped with a UV detector.

Reagents and solutions

*Mobile phase:*Dissolve 5.6 g of potassium dihydrogen phosphate in 820 ml of water before adjusting the pH to 4.3 with 10% phosphoric acid solution. Add 180 ml of methanol to 820 ml of this solution and mix well.

Standard Preparation

Standard stock solution: Dissolve 25 mg DKP Reference Standard (available from The United States Pharmacopeial Convention, Inc.) in 10 ml of methanol and dilute to 100 ml with water.

Standard solutions: Dilute the standard stock solution with 10% methanol to concentrations of 100, 75, 50, 25 and 5.0 µg/ml.

Sample Preparation

Accurately weigh 100 mg of the sample and dissolve in 10% methanol to make exactly 20 ml (5 mg/ml). This solution can be used for the purity test of other optical isomers.

Procedure

HPLC conditions:

Column: L-column2 ODS column (4.6 mm I.D. × 150 mm, particle size: 5 µm, Chemical Evaluation and Research Institute, Japan) or equivalent.

Column temperature: 40°

Mobile phase: Mixture of phosphate buffer solution (0.05 mol/l, pH 4.3) and methanol (82:18 v/v)

Flow rate: 1.0 ml/min

Injection volume: 20 µl

Detector: UV at 210 nm

Run Time: 50 min

Inject the sample and read the concentration of the sample from the standard curve.

Calculation

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

Content (wt%) = (C x V x 0.1)/W

where

C is the concentration of DKP in the sample solution (µg/ml);

V is the volume of the sample solution (20 ml);

W is the weight of the sample (mg);

Other optical isomers

Apparatus

High performance liquid chromatograph equipped with a UV detector.

Reagents and solutions

Mobile phase:
Mobile phase A: (Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (87:13 v/v)): Dissolve 3.0 g of sodium dihydrogen phosphate and 3.55 g of disodium hydrogen phosphate in 1000 ml of water. Add 130 ml of acetonitrile to 870 ml of this solution and mix well.
Mobile phase B: (Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (80:20 v/v)): Dissolve 3.0 g of sodium dihydrogen phosphate and 3.55 g of disodium hydrogen phosphate in 1000 ml of water. Add 200 ml of acetonitrile to 800 ml of this solution and mix well.

Standard Preparation
Standard stock solution: Accurately weigh 20 mg of L,D-APM (Available from Wako Pure Chemical Industries, Ltd., Japan) and dissolve in 10% methanol to make exactly 50 ml (400 µg/ml). (L,D-APM alone is used as the standard since L,D-APM and D,L-APM (enantiomers) have the same retention time and molar absorbance coefficient)

Standard solutions: Dilute the standard stock solution with 10% methanol to concentrations of 10, 5.0, 2.0, 1.0 and 0.5 µg/ml.
Sample Preparation
Accurately weigh 100 mg of the sample and dissolve in 10% methanol to make exactly 20 ml (5 mg/ml). This solution can be used for the purity test of DKP.

Procedure
HPLC conditions:
Column: L-column2 ODS column (4.6 mm I.D. × 250 mm,
particle size: 5 µm, Chemical Evaluation and Research Institute, Japan) or equivalent.
Column temp.: 40°
Mobile phase:
Mobile phase A: Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (87:13 v/v)
Mobile phase B: Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (80:20 v/v)
Flow rate: 0.8 ml/min
Injection volume: 10 µl
Detector: UV at 220 nm
Run Time: 40 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	100	0
25.00	100	0
25.01	0	100
40.00	0	100

Inject the sample and read the concentration of the sample from the standard curve.

Calculation
Calculate the content (%) of L,D-APM using the following formula:
Content (wt%) = (C x V x 0.1)/W
where
C is the concentration of L,D-APM in the sample solution (µg/ml);
V is the volume of the sample solution (20 ml);
W is the weight of the sample (mg);

METHOD OF ASSAY Weigh accurately about 150 mg of the sample, previously dried at 105° for 4 h dissolve in 35 ml of dimethylformamide, add 5 drops of thymol blue TS, and titrate with a microburette to a dark blue end-point with 0.1 M lithium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1 M lithium methoxide is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.
Caution: Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminium foil while dissolving the sample and during the titration.