Food and Agriculture Organization of the United Nations

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Specifications Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 96th Meeting 2023

Aspartame

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Aspartame

Revised specifications prepared at the 96th JECFA (2023) and published in FAO JECFA Monograph 31 (2023) superseding specifications prepared at the 82nd JECFA (2016). 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, α-aspartame, L-aspartyl-L-phenylalanine methyl ester, N-L-α-aspartyl-L-phenylalanine-1-methyl ester, APM

DEFINITION Aspartame is a dipeptide methyl ester of L-aspartic acid and L-phenylalanine. It is produced chemically or enzymatically. Chemical synthesis of aspartame is accomplished by reacting L-phenylalanine or L-phenylalanine methyl ester with N-protected L-aspartic anhydride. This is followed by separation and crystallization of the major component, α-aspartame, from its non-sweet isomer, β-aspartame.

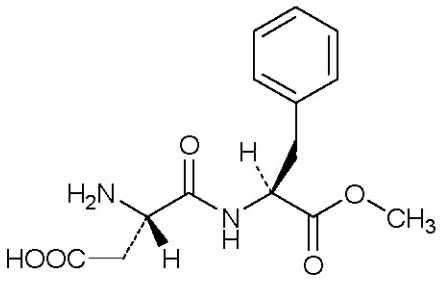
Chemical names 3-Amino-N-(alpha-carboxy-phenethyl)-succinamic acid, L-alpha-aspartyl-L-phenylalanine-1-methyl ester

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

INS number 951

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

Structural formula



Formula weight 294.30

Assay Not less than 98.0% and not more than 102.0% on the dried basis

See description under TESTS

DESCRIPTION White, odourless, crystalline powder

FUNCTIONAL USES Sweetener, flavour enhancer

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 °C, 4 h)
pH (Vol. 4)	4.5 - 6.0 (1 g in 125 ml solution)
Specific rotation (Vol. 4)	$[\alpha]_D^{20}$: Between + 14.5° and + 16.5° (4% solution in 15 M formic acid; determine within 30 min after preparation of the sample solution)
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 (diketopiperazine or DKP)	Not more than 1.5% See description under TESTS
Other related impurities	Not more than 2.0% See description under TESTS

TESTS

PURITY TESTS

DKP

Apparatus

High performance liquid chromatography system 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 (Aspartame Related Compound A from The United States Pharmacopeial Convention, or equivalent) 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 µg/ml.

Sample Preparation

Accurately weigh 100 mg of the sample and dissolve in 10% methanol to make exactly 20 ml (5 mg/ml).

Procedure
HPLC conditions:
Column: L-column2 ODS (4.6 mm I.D. × 150 mm, particle size: 5 µm, Chemical Evaluation and Research Institute, Japan) or equivalent
Column temperature: 40 °C
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:

$$\%DKP = \frac{C \times V \times 0.1}{W}$$

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 related impurities

Apparatus

High performance liquid chromatograph equipped with a UV detector.

Reagents and Solutions:
Use the Reagents and Solutions, Diluent, System suitability solution, and HPLC Conditions described in the METHOD OF ASSAY.

Sample stock solution: 5 mg/ml in Diluent. [Prepare immediately before injection.]
Sample solution: Using the *Sample stock solution*, prepare a 0.1 mg/ml solution in Diluent.

System suitability
Suitability requirement 1: The resolution, R, between L-phenylalanine and DKP in the *System suitability solution* is not less than 8.

Analysis: Separately inject equal volumes of the *Sample stock solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the sum of responses for the major peaks on the resulting chromatograms.

The sum of all peak responses of the *Sample stock solution*, excluding DKP and aspartame, is not more than the aspartame peak response of the *Sample solution* (not more than 2.0%).

METHOD OF ASSAY

Apparatus

High performance liquid chromatograph equipped with a UV detector.

Reagents and Solutions:
Buffer solution: 0.05 M monobasic potassium phosphate adjusted with phosphoric acid to pH 4.3
Mobile phase: Methanol and Buffer solution (18:82)
System suitability, Standard and Sample Preparation:
Diluent: Methanol and water (1:9)
System suitability solution: 0.1 mg/ml each of DKP Reference Standard and L-phenylalanine Reference Standard in Diluent
Standard solution: 0.5 mg/ml of aspartame Reference Standard in Diluent.
Sample solution: 0.5 mg/ml in Diluent.

[NOTE— Prepare Standard and Sample solutions immediately before injection, avoiding exposure to heat. Use aspartame Reference Standard and DKP Reference Standard (Aspartame related compound A) from the United States Pharmacopeial Convention, or equivalent.]

Procedure:
HPLC Conditions:

Column: 250-mm × 4.6-mm column that contains 5-µm octadecylsilane chemically bonded to porous silica or ceramic micro-particles (Lichrospher 100RP-18 (Merck KGaA), or equivalent)
Column temperature: 40 °C
Flow rate: 2 ml/min
Injection volume: 20 µl
Run time: 45 min

System suitability
Suitability requirement 1: The relative standard deviation, RSD, for the aspartame peak area is not more than 1.0% for five replicate injections of the *Standard solution*.

Suitability requirement 2: The resolution, R, between L-phenylalanine and DKP in the *System suitability solution* is not less than 8.
[NOTE: The relative retention times for L-phenylalanine and DKP are 0.6 and 1.0, respectively.]

Suitability requirement 3: The tailing factor for the aspartame peaks in the *Standard solution* is not more than 1.5.

Analysis: Separately inject equal volumes of the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks on the resulting chromatograms.

Calculation:

$$Percentage\ of\ Aspartame\ = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100$$

where
r_U = peak response of aspartame in the Sample solution
r_S = peak response of aspartame in the Standard solution
C_S = concentration of in the Standard solution (mg/ml)
C_U = concentration of the Sample solution (mg/ml)