ASPARTAME

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

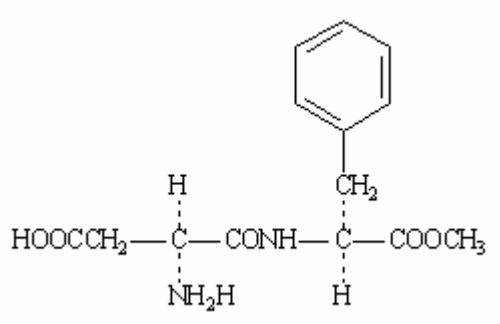
DEFINITION

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, having a strong sweet taste

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Slightly soluble in water and 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, and then add 0.3 ml of 5 N 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 N formic acid; determine within 30 min after preparation of the sample solution)
Spectrophotometry (Vol. 4)	The transmittance of a 1 in 100, 2 N hydrochloric acid solution, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 N 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 1 g of the sample (Method I)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique 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-piperazine acetic acid	Not more than 1.5% See description under TESTS
Other optical isomers	Passes test See description under TESTS

TESTS

5-Benzyl-3,6-dioxo- 2-piperazine acetic acid

Apparatus

Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector and designed for handling glass columns with on-column injection (Micro-Tex 220 or equivalent), containing a 1.83-meter (6 feet) x 4-mm (i.d.) glass column packed with 3% OV-1 on 80/100-mesh Supelcoport (Supelco, Inc. or equivalent). Condition the column overnight at 250° before readjustment and equilibration to the operating conditions. To preclude build-up of silicon oxide, clean the detector with acetone frequently.

Operating conditions

The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: Column temperature, 200°; Inlet temperature, 200°; Detector temperature, 275°; Carrier gas, helium, flowing at a rate of 75 ml per min; Hydrogen and air flow to burner, optimized to give maximum sensitivity;

Recorder, 1 mV full scale.
Note: For the Micro-Tex, the attenuation is 16 x 10.

Silation reagent
Just before use, dilute 3 parts, by volume, of N,O-bis-(trimethylsilyl) acetamide with 2 parts of dimethylformamide.

Standard preparation
Transfer about 25 mg of 5-Benzyl-3, 6-dioxo-2-piperazineacetic acid Reference Standard (available from Food Chemicals Codex, NAS/NRC, 2101 Constitution Avenue, N.W., Washington, D.C. 20418, USA), accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Pipet 10 ml of this solution into a second 100-ml volumetric flask, dilute to volume with methanol, and mix. Pipet 3 ml of the second solution into a 2-dram vial, with Teflon-lined cap, and evaporate to dryness on a steam bath. Add 1 ml of the Silylation reagent to the residue, cap the vial tightly, shake and heat in an oven at 80^{o} for 30 min. Remove the vial from the oven, shake for 15 sec, and cool to room temperature.

Sample preparation
Transfer about 10 mg of the aspartame sample, accurately weighed, into a 2-dram vial, with Teflon-lined cap, add 1 ml of the Silylation reagent, cap tightly, shake, and heat in an oven at 80^{o} for 30 min. Remove the vial from the oven, shake for 15 sec, and cool to room temperature.

Procedure
Inject a 3-µl portion of the Standard preparation into the gas chromatograph, obtain the chromatogram, measure the height of the peak produced by the 5-benzyl-3,6-dioxo-2-piperazineacetic acid, and record it as P. Under the stated conditions, the elution time is about 7-9 min. Similarly, inject a 3-µl portion of the Sample preparation, obtain the chromatogram, measure the height of the peak produced by any 5-benzyl-3,6-dioxo-2-piperazineacetic acid contained in the sample, and record it as p.

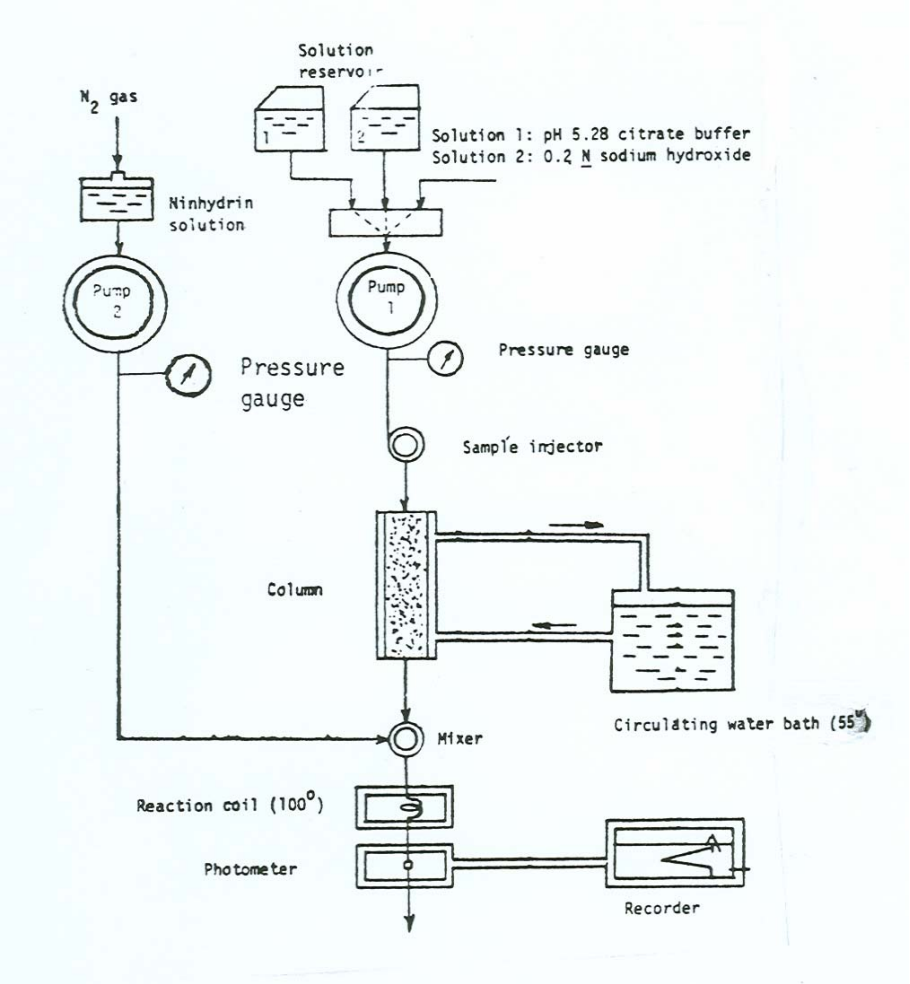
Calculation
Calculate the percent of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the sample by the formula:

$$\frac{3 \times W \times p}{500 \times w \times p} \times 100$$

where
W = the exact weight in mg of the reference standard taken
w = the exact weight in mg of the aspartame taken

Other optical isomers

Apparatus
Use a suitable amino acid analyzer (such as Hitachi KLA-5, or equivalent) which is equipped with a 550-mm x 9-mm (i.d.) column packed with approximately 50 g of strong cation exchange resin (Hitachi Custom Ion-Exchange Resin No. 2613, or equivalent), 29-m x 0.5 mm (i.d.) reaction coil, a ninhydrin supply, and a photometer with an interference filter for 570 nm and selenium photocell detector (see Figure).



Operating conditions

The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

- Column temperature: 55°
- Reaction coil temperature: 100°
- Eluant: pH 5.28 citrate buffer solution
- Pressure of eluant: 8-10 kg/cm^2
- Flow rate of eluant: 60 ml/h
- Pressure of ninhydrin solution: 2-5 kg/cm^2
- Flow rate of ninhydrin solution: 30 ml/h
- Photometric detector: measuring wavelength: 570 nm
- Recorder full scale: absorbance: 0-0.1

Reagents and solutions

- pH 5.28 citrate buffer solution: Dissolve 34.3 g of sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in about 400 ml of water, add 7.5 ml of hydrochloric acid TS (35%) and 5 ml of benzyl alcohol, and add sufficient water to make 1,000 ml.
- pH 2.2 citrate buffer solution: Dissolve 1.4 g of sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), 13.0 g of citric acid ($C_6H_8O_7H_2O$) and 10.9 g of sodium chloride in about 400 ml of water and add sufficient water to make 1,000 ml.

- Ninhydrin solution: Pour 140 ml of water into a 500-ml beaker, add 82.0 g of sodium acetate ($C_2H_3NaO_2$), stir to dissolve completely, add 25 ml of glacial acetic acid TS and dilute to 250 ml with water. Adjust the pH of the solution to 5.51 ± 0.03 with glacial acetic acid TS or sodium acetate TS (Acetate Buffer Solution). Pour 750 ml of methyl-cellosolve into a 1,000-ml brown glass bottle and add 250 ml of Acetate Buffer Solution. Supply nitrogen gas through the solution, while mixing, dissolving 20 g of ninhydrin and then 0.38 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in the solution. Allow the solution to stand for at least 24 h before use.

Preparation

- Standard Preparation: Transfer 2.50 mg of L-alpha-aspartyl-D-phenylalanine methyl ester Reference Standard (Available from Ajinomoto Co. Inc., 1-5-8 Kyobashi, Chuo-ku, Tokyo 104, Japan.) into a 100-ml volumetric flask, dissolve and dilute to volume with water (Solution A).Transfer 250 mg of L-alpha-aspartyl-L-phenylalanine methyl ester Reference Standard into another 100-ml volumetric flask, dissolve in pH 2.2 citrate buffer solution, add 10.0 ml of Solution A and dilute to volume with pH 2.2 citrate buffer solution. Store this preparation below 5^o.
- Sample Preparation: Transfer 250 mg of the sample into a 100-ml volumetric flask, dissolve and dilute to volume with pH 2.2 citrate buffer solution.

Procedure

Regenerate the column with 0.2 N sodium hydroxide TS and then buffer with pH 5.28 citrate buffer solution. After conducting ninhydrin solution to the system, inject a 500-µl portion of the Standard Preparation into the amino acid analyzer and obtain the chromatogram. Under the stated conditions, the retention time is about 100 min for L-alpha-aspartyl-D-phenylalanine methyl ester and about 115 min for L-alpha-aspartyl-L-phenylalanine methyl ester, respectively.

Similarly, inject a 500-µl portion of the Sample Preparation and obtain the chromatogram. Compare this chromatogram with that of the Standard Preparation and identify the component by comparing the retention time. No peak corresponding to L-alpha-aspartyl-D-phenylalanine methyl ester is observed. (The same method is applicable to D-alpha-aspartyl-L-phenylalanine methyl ester.

The detection limit for the sum of L-alpha-aspartyl-D-phenylalanine methyl ester and D-alpha-aspartyl-L-phenylalanine methyl ester in this method is about 1 µg/ml.)

METHOD OF ASSAY

Weigh accurately about 150 mg of the sample, previously dried at 105^o 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 N lithium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1 N 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.