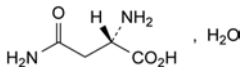
07/2010:2086

ASPARAGINE MONOHYDRATE

Asparaginum monohydricum



$C_4H_8N_2O_3,H_2O$ M_r 150.1
[5794-13-8]

DEFINITION

(2*S*)-2,4-Diamino-4-oxobutanoic acid monohydrate.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: slightly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *asparagine monohydrate CRS*.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances.
Results: the principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (c).

TESTS

Solution S. Dissolve with heating 2.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 4.0 to 6.0 for solution S.

Specific optical rotation (*2.2.7*): + 33.7 to + 36.0 (dried substance).

Dissolve 2.50 g in a 309.0 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same acid.

Ninhydrin-positive substances. Thin-layer chromatography (*2.2.27*).

Test solution (a). Dissolve 0.25 g of the substance to be examined in *water R*, heating to not more than 40 °C, and dilute to 10 mL with the same solvent.

Test solution (b). Dilute 1 mL of test solution (a) to 10 mL with *water R*.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 200 mL with *water R*.

Reference solution (b). Dissolve 25 mg of *glutamic acid R* in *water R*, add 1 mL of test solution (a) and dilute to 10 mL with *water R*.

Reference solution (c). Dissolve 25 mg of *asparagine monohydrate CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *glacial acetic acid R, water R, butanol R* (25:25:50 *V/V/V*).

Application: 5 µL.

Development: over half of the plate.

Drying: at 110 °C for 15 min.

Detection: spray with *ninhydrin solution R* and heat at 110 °C for 10 min.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated principal spots.

Limit: test solution (a):

– *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 12.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*): maximum 200 ppm.

To 0.75 g add 2.5 mL of *dilute hydrochloric acid R* and dilute to 15 mL with *distilled water R*. Examine after 30 min.

Ammonium (*2.4.1, Method B*): maximum 0.1 per cent, determined on 10 mg.

Iron (*2.4.9*): maximum 10 ppm.

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 10 mL with the same acid. Shake 3 times with 10 mL of *methyl isobutyl ketone R1* for 3 min. Wash the combined organic phases with 10 mL of *water R* for 3 min. The aqueous phase complies with the limit test for iron.

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in a mixture of 3 mL of *dilute hydrochloric acid R* and 15 mL of *water R* with gentle warming if necessary. Dilute to 20 mL with *water R*. 12 mL of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*): 10.5 per cent to 12.5 per cent, determined on 1.000 g by drying in an oven at 130 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

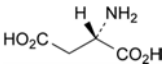
ASSAY

Dissolve 0.110 g in 5 mL of *anhydrous formic acid R*. Add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

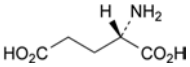
1 mL of *0.1 M perchloric acid* is equivalent to 13.21 mg of $C_4H_8N_2O_3$.

IMPURITIES

Specified impurities: A, B.



A. (2*S*)-2-aminobutanedioic acid (aspartic acid),

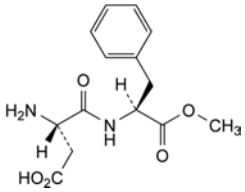


B. (2*S*)-2-aminopentanedioic acid (glutamic acid).

01/2008:0973
corrected 6.0

ASPARTAME

Aspartamum



$C_{14}H_{18}N_2O_5$ M_r 294.3
[22839-47-0]

DEFINITION

(3*S*)-3-Amino-4-[[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-4-oxobutanoic acid (methyl α-L-aspartyl-L-phenylalaninate).

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, slightly hygroscopic, crystalline powder.

Solubility: sparingly soluble or slightly soluble in water and in ethanol (96 per cent), practically insoluble in hexane and in methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 0.1 g in *ethanol (96 per cent) R* and dilute to 100 mL with the same solvent.

Spectral range: 230-300 nm.

Absorption maxima: at 247 nm, 252 nm, 258 nm and 264 nm.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *aspartame CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 15 mg of the substance to be examined in 2.5 mL of *water R* and dilute to 10 mL with *acetic acid R.*

Reference solution. Dissolve 15 mg of *aspartame CRS* in 2.5 mL of *water R* and dilute to 10 mL with *acetic acid R.*

Plate: *TLC silica gel G plate R.*

Mobile phase: *water R, anhydrous formic acid R, methanol R, methylene chloride R* (2:4:30:64 *V/V/V/V*).

Application: 20 µL.

Development: over a path of 15 cm.

Drying: in air.

Detection: spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

Results: the spot in the chromatogram obtained with the test solution is similar in position, colour and size to the spot in the chromatogram obtained with the reference solution.

D. Dissolve about 20 mg in 5 mL of *methanol R* and add 1 mL of *alkaline hydroxylamine solution R1.* Heat on a water-bath for 15 min. Allow to cool and adjust to about pH 2 with *dilute hydrochloric acid R.* Add 0.1 mL of *ferric chloride solution R1.* A brownish-red colour is produced.

TESTS

Solution S. Dissolve 0.8 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

Conductivity (*2.2.38*): maximum 30 µS·cm^{-1}.

Dissolve 0.80 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent. Measure the conductivity of the solution (C_1) and that of the water used for preparing the solution (C_2). The readings must be stable within 1 per cent over a period of 30 s.
Calculate the conductivity of the solution of the substance to be examined using the following expression:

$$C_1 - 0.992\,C_2$$

Specific optical rotation (*2.2.7*): + 14.5 to + 16.5 (dried substance).

Dissolve 2.00 g in a 690 g/L solution of *anhydrous formic acid R* and dilute to 50.0 mL with the same solution. Measure within 30 min of preparation.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.60 g of the substance to be examined in a mixture of 1.5 volumes of *glacial acetic acid R* and 98.5 volumes of *water R* and dilute to 100.0 mL with the same mixture of solvents.

Reference solution (a). Dissolve 4.5 mg of *aspartame impurity A CRS* in a mixture of 1.5 volumes of *glacial acetic acid R* and 98.5 volumes of *water R* and dilute to 50.0 mL with the same mixture of solvents.

Reference solution (b). Dissolve 30.0 mg of *phenylalanine R* (impurity C) in a mixture of 15 volumes of *glacial acetic acid R* and 85 volumes of *water R* and dilute to 100.0 mL with the same mixture of solvents. Dilute 1.0 mL of this solution to 10.0 mL with *water R.*

Reference solution (c). Dilute 5.0 mL of the test solution to 10.0 mL with *water R.* Dilute 3.0 mL of this solution to 100.0 mL with *water R.*

Reference solution (d). Dissolve 30.0 mg of *L-aspartyl-L-phenylalanine R* (impurity B) in a mixture of 15 volumes of *glacial acetic acid R* and 85 volumes of *water R* and dilute to 100.0 mL with the same mixture of solvents. Dilute 1.0 mL of the solution to 10.0 mL with *water R.* Mix 1.0 mL of this solution with 1.0 mL of reference solution (b).

Column

– *size*: *l* = 0.25 m, Ø = 4.0 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase: mix 10 volumes of *acetonitrile R* and 90 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.7 with *phosphoric acid R.*

Flow rate: 1 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µL.

Run time: twice the retention time of aspartame.

System suitability: reference solution (d):

– *resolution*: minimum 3.5 between the peaks due to impurities B and C.

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
– *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
– *sum of impurities other than A and C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent);
– *disregard limit*: disregard any peak due to the solvent.

Heavy metals (*2.4.8*): maximum 10 ppm.

1.0 g complies with test C. Prepare the reference solution using 1 mL of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*): maximum 4.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 1.5 mL of *anhydrous formic acid R* and 60 mL of *anhydrous acetic acid R.* Titrate immediately with *0.1 M perchloric acid,* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

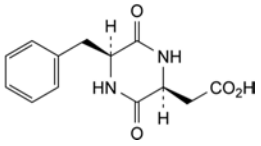
STORAGE

In an airtight container.

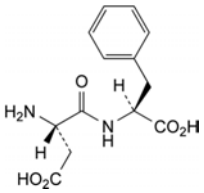
IMPURITIES

Specified impurities: *A, C.*

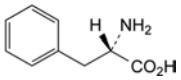
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B.*



A. 2-[(2*S*,5*S*)-5-benzyl-3,6-dioxopiperazin-2-yl]acetic acid,



B. (3*S*)-3-amino-4-[[(1*S*)-1-carboxy-2-phenylethyl]amino]-4-oxobutanoic acid (α-L-aspartyl-L-phenylalanine),



C. (2*S*)-2-amino-3-phenylpropanoic acid (L-phenylalanine).

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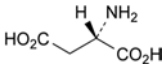
corrected 6.0

ASPARTIC ACID

Acidum asparticum



$C_4H_7NO_4$ M_r 133.1
[56-84-8]

DEFINITION

Aspartic acid contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of (2*S*)-2-aminobutanedioic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A, C.

Second identification: A, B, D.

A. Specific optical rotation (see Tests).

B. A suspension of 1 g in 10 mL of *water R* is strongly acid (*2.2.4*).

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *aspartic acid CRS*. Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution. Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 10 mL with the same acid. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dissolve 2.000 g in *hydrochloric acid R1* and dilute to 25.0 mL with the same acid. The specific optical rotation is + 24.0 to + 26.0, calculated with reference to the dried substance.

Ninhydrin-positive substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a). Dissolve 0.10 g of the substance to be examined in 2 mL of *ammonia R* and dilute to 10 mL with *water R*.

Test solution (b). Dilute 1 mL of test solution (a) to 50 mL with *water R*.

Reference solution (a). Dissolve 10 mg of *aspartic acid CRS* in 2 mL of *dilute ammonia R1* and dilute to 50 mL with *water R*.

Reference solution (b). Dilute 5 mL of test solution (b) to 20 mL with *water R*.

Reference solution (c). Dissolve 10 mg of *aspartic acid CRS* and 10 mg of *glutamic acid CRS* in 2 mL of *dilute ammonia R1* and dilute to 25 mL with *water R*.

Apply separately to the plate 5 µL of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R*. Heat at 100-105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated principal spots.

Chlorides (*2.4.4*). Dissolve 0.25 g in 3 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*. The solution, to which 1 mL of *water R* is added instead of *dilute nitric acid R*, complies with the limit test for chlorides (200 ppm).

Sulfates (*2.4.13*). Dissolve 0.5 g in 4 mL of *hydrochloric acid R* and dilute to 15 mL with *distilled water R*. The solution complies with the limit test for sulfates (300 ppm). Carry out the evaluation of the test after 30 min.

Ammonium.(*2.4.1*) 50 mg complies with limit test B (200 ppm). Prepare the standard using 0.1 mL of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*). In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*). 2.0 g complies with limit test D (10 ppm). Prepare the standard using 2 mL of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.