IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *amiloride hydrochloride CRS.*

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

Test solution. Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 40 mg of *amiloride hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate: *TLC silica gel plate R.*

Mobile phase: *dilute ammonia R1, water R, dioxan R* (6:6:88 *V/V/V*); freshly prepared mixture.

Application: 5 µl.

Development: over a path of 12 cm.

Drying: in air.

Detection: examine in ultraviolet light at 365 nm.

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

C. Dissolve about 10 mg in 10 ml of *water R*. Add 10 ml of a 200 g/l solution of *cetrimide R*, 0.25 ml of *dilute sodium hydroxide solution R* and 1 ml of *bromine water R*. A greenish-yellow colour is produced. Add 2 ml of *dilute hydrochloric acid R*. The solution becomes deep yellow and shows blue fluorescence in ultraviolet light at 365 nm.

D. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

Free acid. Dissolve 1.0 g in a mixture of 50 ml of *methanol R* and 50 ml of *water R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to reach the end-point.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 20.0 mg of the substance to be examined in a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R.*

Reference solution (c). Dissolve 5.0 mg of *amiloride impurity A CRS* in a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R* and dilute to 5.0 ml with the same mixture of solvents. Dilute 1.0 ml of this solution to 100.0 ml with a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R.*

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 5 volumes of *tetramethylammonium hydroxide solution R,* 250 volumes of *acetonitrile R* and 745 volumes of *water R*; adjust to pH 7.0 with a mixture of 1 volume of *phosphoric acid R* and 9 volumes of *water R.* Adjust the concentration of acetonitrile in the mobile phase so that the retention time of impurity A is 5-6 min (an increase in the concentration of acetonitrile results in a shorter retention time). Adjust the concentration of tetramethylammonium hydroxide and of phosphoric acid keeping the pH at 7.0 so that the retention time of amiloride is 9-12 min (an increase in the concentration results in a shorter retention time for amiloride).

Flow rate: 1 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl.

Run time: 5 times the retention time of amiloride.

System suitability: reference solution (b):

- *signal-to-noise ratio*: minimum 5.0 for the peak due to amiloride.

Limits:

- *total*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*): 11.0 per cent to 13.0 per cent, determined on 0.200 g.

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

ASSAY

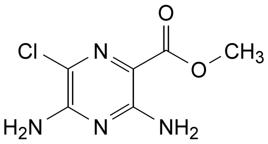
Dissolve 0.200 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 26.61 mg of $C_6H_9Cl_2N_7O$.

STORAGE

Protected from light.

IMPURITIES

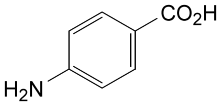


A. methyl 3,5-diamino-6-chloropyrazine-2-carboxylate.

01/2008:1687

4-AMINOBENZOIC ACID

Acidum 4-aminobenzoicum



$C_7H_7NO_2$ M_r 137.1

[150-13-0]

DEFINITION

4-Aminobenzoic acid.

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

CHARACTERS

Appearance: white or slightly yellow, crystalline powder.

Solubility: slightly soluble in water, freely soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (*2.2.14*): 186 °C to 189 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *4-aminobenzoic acid CRS*.

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

Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (a). Dissolve 20 mg of *4-aminobenzoic acid CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *4-nitrobenzoic acid R* in 10 ml of reference solution (a).

Plate: suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm as the coating substance.

Mobile phase: *glacial acetic acid R*, *hexane R*, *methylene chloride R* (5:20:75 *V/V/V*).

Application: 1 μl.

Development: over a path of 10 cm.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

System suitability: the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *alcohol R* and dilute to 20 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. Dissolve 25.0 mg of *4-nitrobenzoic acid R* and 25.0 mg of *benzocaine R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.12 m, Ø = 4.0 mm,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 μm).

Mobile phase: mix 20 volumes of a mixture of 70 volumes of *acetonitrile R* and 80 volumes of *methanol R*, and 80 volumes of a solution containing 1.5 g/l of *potassium dihydrogen phosphate R* and 2.5 g/l of *sodium octanesulphonate R* adjusted to pH 2.2 with *phosphoric acid R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 270 nm.

Injection: 20 μl.

Run time: 11 times the retention time of 4-aminobenzoic acid.

Relative retention with reference to 4-aminobenzoic acid (retention time = about 3 min): impurity A = about 4; impurity B = about 9.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.2 per cent),
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.2 per cent),
- *any other impurity*: not more than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with the reference solution (0.1 per cent),
- *total*: not more than 2.5 times the area of the peak due to impurity A in the chromatogram obtained with the reference solution (0.5 per cent),
- *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with the reference solution (0.02 per cent).

Impurity C and impurity D. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 20.0 mg of *lauric acid R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Test solution. Dissolve 1.000 g of the substance to be examined in 10.0 ml of an 84 g/l solution of *sodium hydroxide R* and extract with 2 quantities, each of 10 ml, of *methylene chloride R*. Combine and wash with 5 ml of *water R*; filter through *anhydrous sodium sulphate R*. Wash the filter with *methylene chloride R*. Evaporate in a water-bath at 50-60 °C to obtain a volume of about 1-5 ml. Add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *methylene chloride R*.

Reference solution (a). Dissolve 20.0 mg of *aniline R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution (b). Dissolve 20.0 mg of *p-toluidine R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution (c). Dilute 0.50 ml of reference solution (a), 0.50 ml of reference solution (b) and 10.0 ml of the internal standard solution to 100.0 ml with *methylene chloride R*.

Column:

- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.32 mm,
- *stationary phase*: *poly[methyl(95)phenyl(5)] siloxane R* (film thickness 0.5 μm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1.0 ml/min.

Split ratio: 1:10.

Temperature:

	Time (min)	**Temperature (°C)**
Column	0 - 4	130
	4 - 6.5	130 → 180
	6.5 - 11.5	180
Injection port		280
Detector		300

Detection: flame ionisation.

Injection: 2 μl; inject the test solution and reference solution (c).

Retention time: internal standard = about 9.5 min.

Limits:

- *impurity C*: calculate the ratio (*R*) of the area of the peak due to impurity C to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (c); calculate the ratio of the area of the peak due to impurity C to the area of the peak due to the internal standard from the chromatogram obtained with the test solution: this ratio is not greater than *R* (10 ppm),
- *impurity D*: calculate the ratio (*R*) of the area of the peak due to impurity D to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (c); calculate the ratio of the area of the peak due to impurity D to the area of the peak due to the internal standard from the chromatogram obtained with the test solution: this ratio is not greater than *R* (10 ppm).

Iron (*2.4.9*): maximum 40 ppm.

Dissolve 0.250 g in 3 ml of *alcohol R* and dilute to 10.0 ml with *water R*.

Heavy metals (*2.4.8*): maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 0.2 per cent, determined on 1.00 g.

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

ASSAY

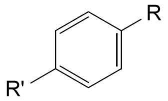
Dissolve 0.100 g with heating in 50 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 13.71 mg of $C_7H_7NO_2$.

STORAGE

Protected from light.

IMPURITIES



A. R = CO_2H, R′ = NO_2: 4-nitrobenzoic acid,

B. R = CO-O-C_2H_5, R′ = NH_2: benzocaine,

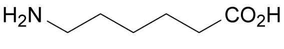
C. R = H, R′ = NH_2: aniline,

D. R = CH_3, R′ = NH_2: 4-methylaniline (*p*-toluidine).

01/2008:0874
corrected 6.0

AMINOCAPROIC ACID

Acidum aminocaproicum



$C_6H_{13}NO_2$ M_r 131.2
[60-32-2]

DEFINITION

Aminocaproic acid contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 6-aminohexanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in alcohol.

It melts at about 205 °C with decomposition.

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

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

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

C. Dissolve 0.5 g in 4 ml of a mixture of equal volumes of *dilute hydrochloric acid R* and *water R*. Evaporate to dryness by heating on a water-bath. Dry the residue in a desiccator. Dissolve the residue in about 2 ml of boiling *ethanol R*. Allow to cool and maintain at 4 °C to 8 °C for 3 h. Filter under reduced pressure. The residue washed with about 10 ml of *acetone R* and dried at 60 °C for 30 min, melts (*2.2.14*) at 131 °C to 133 °C.

D. Dissolve about 5 mg in 0.5 ml of *distilled water R*. Add 3 ml of *dimethylformamide R* and 2 ml of *ascorbic acid solution R*. Heat on a water-bath. An orange colour develops.

TESTS

Solution S. dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution. Solution S is colourless (*2.2.2, Method II*) and remains clear (*2.2.1*) on standing for 24 h.

pH (*2.2.3*). The pH of solution S is 7.5 to 8.0.

Absorbance (*2.2.25*).

A. The absorbance of solution S at 287 nm is not more than 0.10 and at 450 nm is not more than 0.03.

B. Place 2.0 g in an even layer in a shallow dish 9 cm in diameter, cover and allow to stand at 98 °C to 102 °C for 72 h. Dissolve in *water R* and dilute to 10.0 ml with the same solvent. The absorbance of the solution at 287 nm is not more than 0.15 and at 450 nm is not more than 0.03.

Ninhydrin-positive substances. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

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

Reference solution (a). Dissolve 10 mg of *aminocaproic acid CRS* in *water R* and dilute to 50 ml with the same solvent.

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

Reference solution (c). Dissolve 10 mg of *aminocaproic acid CRS* and 10 mg of *leucine CRS* in *water R* and dilute to 25 ml with the same solvent.

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*. Dry the plate in a current of warm air. Spray with *ninhydrin solution R*