of a 20 g/L solution of *ammonium sulfamate R*, shake, allow to stand for 3 min and add 1 mL of a 5 g/L solution of *naphthylethylenediamine dihydrochloride R*. Any pinkish-violet colour produced in the test solution is not more intense than that in the reference solution.

Chlorides (*2.4.4*): maximum 500 ppm.

To 2 g add a mixture of 1.2 mL of *acetic acid R* and 40 mL of *water R*, boil for 2 min, cool and filter. Dilute 2 mL of the filtrate to 15 mL with *water R*.

Loss on drying (*2.2.32*): 4.5 per cent to 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

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

ASSAY

Dissolve 0.3000 g in 80 mL of a mixture of equal volumes of *acetone R* and *methanol R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

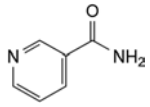
STORAGE

Protected from light.

01/2008:0047
corrected 6.0

NICOTINAMIDE

Nicotinamidum



$C_6H_6N_2O$ M_r 122.1
[98-92-0]

DEFINITION

Nicotinamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of pyridine-3-carboxamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water and in ethanol.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 128 °C to 131 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nicotinamide CRS*.

C. Boil 0.1 g with 1 mL of *dilute sodium hydroxide solution R*. Ammonia is evolved which is recognisable by its odour.

D. Dilute 2 mL of solution S (see Tests) to 100 mL with *water R*. To 2 mL of the solution, add 2 mL of *cyanogen bromide solution R* and 3 mL of a 25 g/L solution of *aniline R* and shake. A yellow colour develops.

TESTS

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (*2.2.3*). The pH of solution S is 6.0 to 7.5.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R*.

Test solution. Dissolve 0.4 g of the substance to be examined in a mixture of equal volumes of *alcohol R* and *water R* and dilute to 5.0 mL with the same mixture of solvents.

Reference solution. Dilute 0.5 mL of the test solution to 200 mL with a mixture of equal volumes of *alcohol R* and *water R*.

Apply to the plate 5 µL of each solution. Develop over a path of 10 cm using a mixture of 4 volumes of *water R*, 45 volumes of *ethanol R* and 48 volumes of *chloroform R*. Allow the plate to dry and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.25 per cent).

Heavy metals (*2.4.8*). Dilute 12 mL of solution S to 18 mL with *water R*. 12 mL of the solution complies with limit test A for heavy metals (30 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.00 g by drying *in vacuo* for 18 h.

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

ASSAY

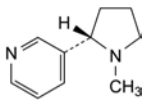
Dissolve 0.250 g in 20 mL of *anhydrous acetic acid R*, heating slightly if necessary, and add 5 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution R* as indicator until the colour changes to greenish-blue.

1 mL of *0.1 M perchloric acid* is equivalent to 12.21 mg of $C_6H_6N_2O$.

01/2009:1452
corrected 6.6

NICOTINE

Nicotinum



$C_{10}H_{14}N_2$ M_r 162.2
[54-11-5]

DEFINITION

3-[(2*S*)-1-Methylpyrrolidin-2-yl]pyridine.

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

CHARACTERS

Appearance: colourless or brownish viscous liquid, volatile, hygroscopic.

Solubility: soluble in water, miscible with anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *Ph. Eur. reference spectrum of nicotine.*

TESTS

Appearance of solution. Dissolve 1.0 g in *water R* and dilute to 10 mL with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5, BY_5 or R_5 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*): – 140 to – 152.

Dissolve 1.00 g in *anhydrous ethanol R* and dilute to 50.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (a). Dissolve the contents of a vial of *nicotine for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in 1.0 mL of *water R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 10.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase:

– *mobile phase A*: to 900 mL of *water R*, add 25 mL of a 60 g/L solution of *acetic acid R*, then add 6 mL of *concentrated ammonia R1*. Adjust to pH 10.0 with *dilute ammonia R2* or *dilute acetic acid R* and dilute to 1000 mL with *water R*;
– *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 3.01	100 → 95	0 → 5
3.01 - 28	95 → 74	5 → 26
28 - 32	74 → 60	26 → 40

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *nicotine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F and G.

Relative retention with reference to nicotine (retention time = about 17.8 min): impurity E = about 0.3; impurity C = about 0.55; impurity F = about 0.7; impurity A = about 0.8; impurity D = about 0.86; impurity G = about 0.9; impurity B = about 1.6.

System suitability: reference solution (a):

– *resolution*: minimum 2.5 between the peaks due to impurity G and nicotine.

Limits:

– *impurities A, B, C, D, E, F, G*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
– *total*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*): maximum 0.5 per cent, determined on 1.00 g.

ASSAY

Dissolve 60.0 mg in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

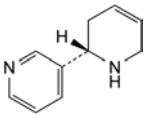
1 mL of *0.1 M perchloric acid* is equivalent to 8.11 mg of $C_{10}H_{14}N_2$.

STORAGE

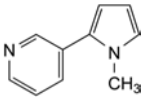
Under nitrogen, in an airtight container, protected from light.

IMPURITIES

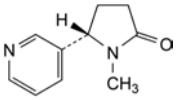
Specified impurities: *A, B, C, D, E, F, G.*



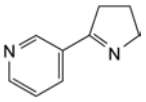
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridyl (anatabine),



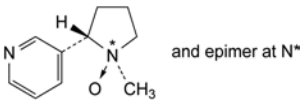
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



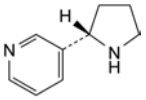
C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



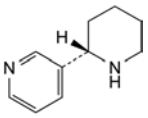
D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



E. (1*RS*,2*S*)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (nicotine *N*′-oxide),



F. 3-[(2*S*)-pyrrolidin-2-yl]pyridine (nornicotine),



G. 3-[(2*S*)-piperidin-2-yl]pyridine (anabasine).

01/2009:1792
corrected 6.6

NICOTINE RESINATE

Nicotini resinas

DEFINITION

Complex of nicotine (3-[(2*S*)-1-methylpyrrolidin-2-yl]pyridine) with a weak cationic exchange resin.

Content: 95.0 per cent to 115.0 per cent of the declared content of nicotine ($C_{10}H_{14}N_2$) stated on the label (anhydrous susbtance).

It may contain glycerol.

CHARACTERS

Appearance: white or slightly yellowish powder.

Solubility: practically insoluble in water.