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*).

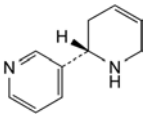
1 mL of *0.1 M perchloric acid* is equivalent to 8.11 mg of $C_{10}H_{14}N_2$.

STORAGE

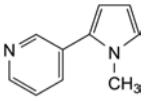
Under nitrogen, in an airtight container, protected from light.

IMPURITIES

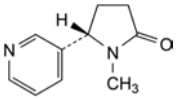
Specified impurities: *A, B, C, D, E, F, G.*



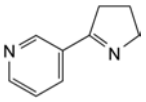
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridyl (anatabine),



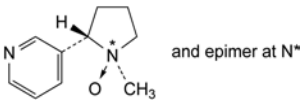
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



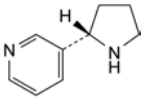
C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



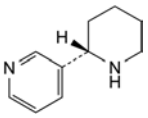
D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



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).

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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.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: shake a quantity of the substance to be examined equivalent to 100 mg of nicotine with a mixture of 10 mL of *dilute ammonia R2*, 10 mL of *water R*, 5 mL of *strong sodium hydroxide solution R* and 20 mL of *hexane R* for 5 min. Transfer the upper layer to a beaker and evaporate to produce an oily residue. Record the spectrum of the oily residue as a thin film between *sodium chloride R* plates.

Comparison: *Ph. Eur. reference spectrum of nicotine.*

B. Nicotine release (see Tests).

TESTS

Nicotine release: minimum 70 per cent of the content determined under Assay in 10 min.

Transfer an accurately weighed quantity of the substance to be examined equivalent to about 4 mg of nicotine, to a glass-stoppered test-tube, add 10.0 mL of a 9 g/L solution of *sodium chloride R* previously heated to 37 °C and shake vigorously for 10 min. Immediately filter the liquid through a dry filter paper discarding the 1st millilitre of filtrate. Transfer 1.0 mL of the filtrate to a 20 mL volumetric flask, dilute to 20 ml with *0.1 M hydrochloric acid* and mix. Determine the absorbance (*2.2.25*) at the minima at about 236 nm and 282 nm and at the maximum at 259 nm using 1.0 mL of a 9 g/L solution of *sodium chloride R* diluted to 20 mL with *0.1 M hydrochloric acid* as compensation liquid.

Calculate the percentage of nicotine release using the following expression:

$$\frac{20 \times 10^6 \times \left(A_{259} - 0.5A_{236} - 0.5A_{282}\right)}{323 \times C \times m}$$

323	=	specific absorbance of nicotine at 259 nm;
C	=	percentage of nicotine in the substance to be examined determined in the assay;
m	=	mass of the substance to be examined, in milligrams;
A_{236}, A_{259}, A_{282}	=	absorbances of the solution at the wavelength indicated by the subscript.

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

Test solution. Weigh a quantity of the substance to be examined equivalent to 30.0 mg of nicotine into a glass-stoppered test-tube, add 10.0 mL of *dilute ammonia R2* solution and shake vigorously for 10 min. Centrifuge for 20 min at about 3000 r/min. To 5.0 mL of the clear solution, add 5 mL of a 60 g/L solution of *acetic acid R* and dilute to 25.0 mL with *water R*.

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*.

Reference solution (c). Dissolve 46.0 mg of *nicotine ditartrate CRS* in *water R* and dilute to 25.0 mL with the same solvent.

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 5.0 per cent.

Suspend 1.0 g in 20.0 mL of *methanol R*, shake for 30 min and allow to stand for 30 min. Use 10 mL of the methanol layer for the titration. Carry out a blank titration.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of nicotine ($C_{10}H_{14}N_2$) (anhydrous substance) from the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate CRS*.

STORAGE

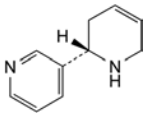
In an airtight container, protected from light.

LABELLING

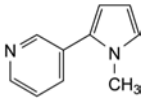
The label states the content of nicotine.

IMPURITIES

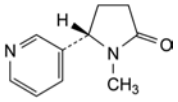
Specified impurities: *A, B, C, D, E, F, G.*



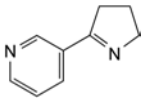
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridyl (anatabine),



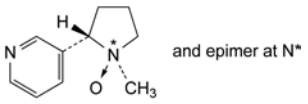
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



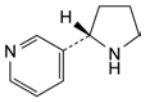
C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



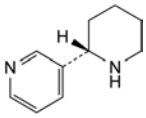
D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



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).

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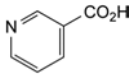
NICOTINIC ACID

Acidum nicotinicum



$C_6H_5NO_2$ M_r 123.1

[59-67-6]

DEFINITION

Pyridine-3-carboxylic acid.

Content: 99.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water, soluble in boiling water and in boiling ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification: *A, B.*

Second identification: *A, C.*

A. Melting point (*2.2.14*): 234 °C to 240 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *nicotinic acid CRS.*

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

Solvent mixture. Dissolve 6.8 g of *potassium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 7.0 with *dilute sodium hydroxide solution R* and dilute to 1000 mL with *water R*.

Test solution. Dissolve 50 mg in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 25.0 mL with the solvent mixture.

Spectral range: 237-262 nm.

Absorption maximum: at 262 nm.

Absorption minimum: at 237 nm.

Absorbance ratio: A_{237}/A_{262} = 0.46 to 0.50.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.120 g of the substance to be examined in 200 µL of *dilute ammonia R1* and dilute to 10.0 mL with mobile phase A.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b). Dissolve the contents of a vial of *nicotinic acid impurity mixture CRS* (impurities A and B) in 1.0 mL of mobile phase A.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped silica gel for chromatography, alkyl-bonded for use with highly aqueous mobile phase R* (4 µm);
– *temperature*: 15 °C.

Mobile phase:

– *mobile phase A*: dilute 2 mL of *acetic acid R* in 950 mL of *water R*, adjust to pH 5.6 with *dilute ammonia R1* and dilute to 1000 mL with *water R*;
– *mobile phase B*: *acetonitrile R, methanol R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 30	100→20	0→80
30 - 35	20	80

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 250 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with *nicotinic acid impurity mixture CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention with reference to nicotinic acid (retention time = about 6 min): impurity A = about 2.7; impurity B = about 2.8.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to impurities A and B.

Limits:

– *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
– *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
– *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).