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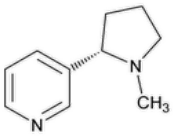
Nicotine

Change to read:



$C_{10}H_{14}N_2$ ▲162.24▲ (USP 1-Aug-2022)

3-(1-Methyl-2-pyrrolidinyl)pyridine;

β-Pyridyl-α-*N*-methyl pyrrolidine CAS RN®: 54-11-5; UNII: 6M3C89ZY6R.

Change to read:

DEFINITION

Nicotine contains NLT ▲98.0%▲ (USP 1-Aug-2022) and NMT ▲102.0 %▲ (USP 1-Aug-2022) of nicotine ($C_{10}H_{14}N_2$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197F

Standard: Transfer an amount of USP Nicotine Bitartrate Dihydrate RS equivalent to 100 mg of nicotine to a 100-mL glass-stoppered tube. Add 20 mL of 1 M ammonium hydroxide, 5 mL of 10 M sodium hydroxide, and 20 mL of *n*-hexane. Shake for 5 min, and allow the phases to separate. Transfer the upper hexane phase to an evaporating dish, and evaporate on a steam bath.

Sample: Nicotine

Acceptance criteria: Meets the requirements

Change to read:

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the ▲*Assay.*▲ *(USP 1-Aug-2022)*

ASSAY

Change to read:

• **Procedure**

▲**Solution A:** Add 25 mL of 1 M glacial acetic acid to 900 mL of water, and then add 6 mL of ammonium hydroxide. Adjust with either 2 M glacial acetic acid or 2 M ammonium hydroxide to a pH of 10.0, and dilute with water to 1000 mL.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.01	95	5
28	74	26

Time (min)	Solution A (%)	Solution B (%)
32	60	40
34	100	0
40	100	0

System suitability solution: 1.5 mg/mL of USP Nicotine Bitartrate Dihydrate RS and 6 µg/mL each of USP Nicotine Related Compound A RS, USP Nicotine Related Compound B RS, USP Nicotine Related Compound C RS, USP Nicotine Related Compound D RS, USP Nicotine Related Compound E RS, USP Nicotine Related Compound F RS, and USP Nicotine Related Compound G RS in water. [NOTE—The concentration of each related compound is in terms of the free base.]

Standard solution: 2.4 mg/mL of USP Nicotine Bitartrate Dihydrate RS in water

Sample solution: 0.8 mg/mL of Nicotine in water

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for nicotine, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nicotine ($C_{10}H_{14}N_2$) in the portion of Nicotine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of nicotine from the *Sample solution*

r_S = peak response of nicotine from the *Standard solution*

C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = concentration of Nicotine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nicotine, 162.24

M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

▲ (USP 1-Aug-2022)

Acceptance criteria: ▲98.0%–102.0%▲ (USP 1-Aug-2022) on the anhydrous basis

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

▲**Solution A, Solution B, Mobile phase, System suitability solution, Sample solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.▲ (USP 1-Aug-2022)

Standard solution: 2.5 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water

Sensitivity solution: 1.2 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water, from the *Standard solution*

▲▲ (USP 1-Aug-2022)

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for nicotine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Nicotine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nicotine from the *Standard solution*

C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = concentration of Nicotine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nicotine, ▲162.24▲ (USP 1-Aug-2022)

M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria: See *Table 2*. ▲The reporting threshold is 0.05%.▲ (USP 1-Aug-2022)

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nicotine related compound E	0.3	0.3
Nicotine related compound C	0.55	0.3
Nicotine related compound F	0.7	0.3
Nicotine related compound A	0.8	0.3
Nicotine related compound D	0.86	0.3
Nicotine related compound G	0.9	0.3
Nicotine	1.00	—
Nicotine related compound B	1.6	0.3
Any other unspecified impurity	—	0.10
Total impurities	—	0.8

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 20 mg/mL in alcohol

Acceptance criteria: −130° to −143°

• **Water Determination ⟨921⟩, *Method I*:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Store under nitrogen in well-closed containers below 25°, protected from light and moisture.

Change to read:

• **USP Reference Standards ⟨11⟩**

USP Nicotine Bitartrate Dihydrate RS

USP Nicotine Related Compound A RS

Anatabine;
1,2,3,6-Tetrahydro-2,3'-bipyridine.
$C_{10}H_{12}N_2$ 160.22

USP Nicotine Related Compound B RS

Nicotyrine;
3-(1-Methyl-1*H*-pyrrol-2-yl)pyridine.
$C_{10}H_{10}N_2$ 158.20

USP Nicotine Related Compound C RS

Cotinine;
(*S*)-1-Methyl-5-(pyridin-3-yl)pyrrolidin-2-one.
$C_{10}H_{12}N_2O$ 176.22

USP Nicotine Related Compound D RS

Myosmine;
3-(4,5-Dihydro-3*H*-pyrrol-2-yl)pyridine fumarate.
$C_9H_{10}N_2 \cdot C_4H_4O_4$ 262.26

USP Nicotine Related Compound E RS

Nicotine *N*-oxide;
(1*RS*,2*S*)-1-Methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide oxalate.
$C_{10}H_{14}N_2O \cdot C_2H_2O_4$ 268.27

USP Nicotine Related Compound F RS

Nornicotine;
3-(Pyrrolidin-2-yl)pyridine.
$C_9H_{12}N_2$ ▲148.21▲ (USP 1-Aug-2022)

USP Nicotine Related Compound G RS

Anabasine;
(*S*)-3-(Piperidin-2-yl)pyridine.
$C_{10}H_{14}N_2$ ▲162.24▲ (USP 1-Aug-2022)

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
NICOTINE	Documentary Standards Support	SM42020 Small Molecules 4
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM42020 Small Molecules 4

Chromatographic Database Information: Chromatographic Database

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