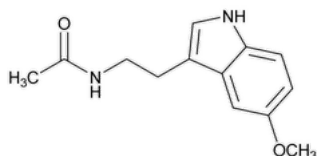


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Melatonin



$C_{13}H_{16}N_2O_2$ 232.28

N-Acetyl-5-methoxytryptamine;

N-(2-(5-Methoxy-1*H*-indol-3-yl)ethyl) acetamide CAS RN®: 73-31-4.

DEFINITION

Melatonin contains NLT 98.5% and NMT 101.5% of melatonin ($C_{13}H_{16}N_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** [▲ SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Infrared Spectroscopy: 197K ▲](#) (CN 1-MAY-2020)

Change to read:

- **B.** [▲ SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Ultraviolet-Visible Spectroscopy: 197U ▲](#) (CN 1-MAY-2020)

Analytical wavelength: 277 nm

Sample solution: 10 µg/mL of Melatonin in isopropyl alcohol

Acceptance criteria: Meets the requirements. Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

- **C. HPLC IDENTIFICATION TEST**

Analysis: Proceed as directed in the Assay.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

- **PROCEDURE**

Buffer: 0.5 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5, and filter.

Mobile phase: Acetonitrile and *Buffer* (25:75)

System suitability solution: 0.1 mg/mL of [USP Melatonin RS](#) and 0.02 mg/mL [USP Melatonin Related Compound A RS](#) in *Mobile phase*

Standard solution: 0.1 mg/mL of [USP Melatonin RS](#) in *Mobile phase*

Sample solution: 0.1 mg/mL of Melatonin in *Mobile phase*

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 222 nm

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

Flow rate: 1.0 mL/min

Injection size: 10 µL

System suitability

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

[NOTE—The relative retention times for melatonin related compound A and melatonin are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 between melatonin and melatonin related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of melatonin in the portion of Melatonin taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of [USP Melatonin RS](#) in the *Standard solution* (mg/mL)

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

Acceptance criteria: 98.5–101.5% on the dried basis

IMPURITIES

• [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

• [CHLORIDE AND SULFATE, Chloride \(221\)](#)

Standard: 0.10 mL of 0.020 N hydrochloric acid

Sample: 0.36 g of Melatonin

Acceptance criteria: NMT 0.02%

• **RELATED COMPOUNDS**

Solution A: Acetonitrile

Solution B: Use *Buffer*, prepared as directed in the Assay.

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	25	75
7	25	75
15	80	20
18	25	75
25	25	75

Diluent: Mixture of *Solution A* and *Solution B* (25:75)

System suitability solution: 0.1 mg/mL of [USP Melatonin RS](#) and 0.02 mg/mL of [USP Melatonin Related Compound A RS](#) in *Diluent*

Standard solution: 5 µg/mL of [USP Melatonin RS](#) in *Diluent*

Sample solution: 1 mg/mL of Melatonin in *Diluent*

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 222 nm

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

Flow rate: 1.0 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for melatonin related compound A and melatonin are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between melatonin and melatonin related compound A

Relative standard deviation: NMT 2.0% for the melatonin peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Melatonin taken:

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

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

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

C_S = concentration of [USP Melatonin RS](#) in the *Standard solution* (mg/mL)