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Application Note

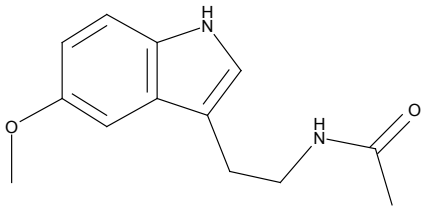
As published in "The Handbook of Analytical Methods for Dietary Supplements"

0056 - Melatonin by HPLC

Chemical Name: *N-[2-(5-Methoxy-1H-indole-3-yl)ethyl]acetamide; N-acetyl-5-methoxytryptamine*

Molcular Weight: 232.28

Chemical Formula: $C_{13}H_{16}N_2O_2$



Melatonin

Solubility: Soluble in aqueous solutions, ethanol, and other polar organic solvents.

Other Physical/Chemical Data: UV max = 223 nm (ε = 27,550), 278 nm (ε= 6300)
Melting point = 116°C to 118°C

Uses: Soluble in aqueous solutions, ethanol, and other polar organic solvents.

Modes of Action:

Melatonin is a naturally occurring hormone that is secreted at night by the pineal gland to help regulate the sleep cycle.

Chemical Markers:

Although many methods have been published for the analysis of melatonin in biological samples, there is little in the literature regarding the analysis of melatonin in dietary supplements. Nevertheless, published methods often can be adapted easily for use with these products. Most methods utilize reversed-phase HPLC with UV, native fluorescence, derivatization with fluorescence, or electrochemical detection. For the very low levels of melatonin found in biological samples, fluorescence or electrochemical detection is required to obtain the needed sensitivity; however, for dietary supplements, which generally contain between 1 and 20 mg of melatonin per dosage unit, UV detection is sufficiently sensitive. Surprisingly, no method has been published on the analysis of melatonin dietary supplements using HPLC with UV detection.

Melatonin can be extracted from the sample matrix with acidic aqueous buffer, methanol, or other polar organic solvents. It is light sensitive, therefore solutions should be protected from light.

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Methods of Analysis

Xie et al.[4] utilized HPLC with electrochemical (EC) detection for the quantitation of melatonin in tablets and capsules. The results were confirmed by MS detection.

Method 1:

The method found at www.nsfina.org was used.

Sample Preparation:

Extract melatonin from tablet and capsule samples with a solution of 20% acetonitrile in 0.1 M perchloric acid using sonication. Filter, and dilute with water to a concentration of between 0.1 and 1 mcg/mL. Inject and analyze by HPLC.

Chromatography:

Column: BAS Unijet C18, 5 µm, 150 × 1 mm.
Mobile phase: 20% Acetonitrile–80% buffer [15 mM sodium perchlorate, 40.6 mM sodium citrate, 2.15 mM sodium octylsulfonate, 10 mM diethylamine hydrochloride, and 27 mM disodium edetate (EDTA)].
Flow rate: 100 µL/minute
Column temperature: 35°C
Injection volume: 5 µL
Detection: Electrochemical with a glassy carbon working electrode (3 mm) at an applied voltage of +850 mV versus Ag/AgCl.

Validation Data:

Linearity: 0.1 to 1 ng on column (r = 0.9999)
Accuracy: Not specified
Precision: Not specified
Selectivity: Not specified
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: LOD = 3 pg on column

Method 2:

Cartoni et al.[5] utilized capillary electrophoresis (CE) with UV detection for the quantitation of melatonin in pharmaceutical tablets.

Sample Preparation:

Dissolve the tablets in water, filter, and add tryptophan internal standard. Inject this solution on the CE system.

Chromatography:

Column: Silica capillary, 44 cm (37 cm to detector) × 50 µm ID, coated with polysiloxane-bonded phase.
Running buffer: 30 mM tetraborate, pH 9.2.
Voltage: 20 kV (44 µA current).
Temperature: 25°C
Injection: Pressure, 10,340 Pa for 0.5 second
Detection: UV at 215 nm

Validation Data:

Linearity: 0.01 to 0.10 mg/mL (r^2 = 0.9983).
Accuracy: 95.3% to 107% recovery of spiked samples.
Precision: Not specified
Selectivity: Not specified
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: LOD = 0.002 mg/mL.

References:

1. Zhdanova IV, Wurtman RJ, Lynch HJ, et al. Sleep-inducing effects of low doses of melatonin ingested in the evening . *Clin Pharmacol Ther.* 1995;57:552-8.
2. Petrie K, Dawson AG, Thompson L, Brook R. A double-blind trial of melatonin as a treatment in international cabin crew. *Biol Psychiatry.* 1993;33:526-30.
3. Webb SM, Puig-Domingo M. Role of melatonin in health and disease. *Endocrinology.* 1995;42:221-34.
4. Xie F, Wong P, Yoshioka K, et al. Determination of melatonin in commercially available products by LCEC and LC/MS/MS. *J Liq Chromatogr Relat Technol.* 1998;21:1273–82.
5. Cartoni GP, Coccioli F, Jasionowska R, et al. Rapid analysis of melatonin in pharmaceutical tablets by capillary electrophoresis with UV detection. *Chromatographia*. 2000;52:603.

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