Melatonin: chemical identity and published assays

26 November 2026

Melatonin (N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide, C13H16N2O2, 232.28 Da) is a tryptophan-derived indoleamine supplied exclusively as Melatonin for laboratory research. This briefing details its chemical struc

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Chemical Identity and Catalog Context

Melatonin is catalogued as a high-purity small-molecule research chemical distinct from the peptide portfolio. Its CAS number is 73-31-4 (PubChem CID 896). In laboratory settings it is typically supplied as a crystalline solid or in solution vehicles compatible with in vitro and in vivo experimentation. Certificates of analysis for catalog material routinely document identity by retention time, UV spectrum or mass spectrum, purity by HPLC area normalization (commonly 98–99.5%), and limits on residual solvents or related substances. Users are reminded that lot-specific verification using orthogonal methods is standard laboratory practice prior to experimental use.

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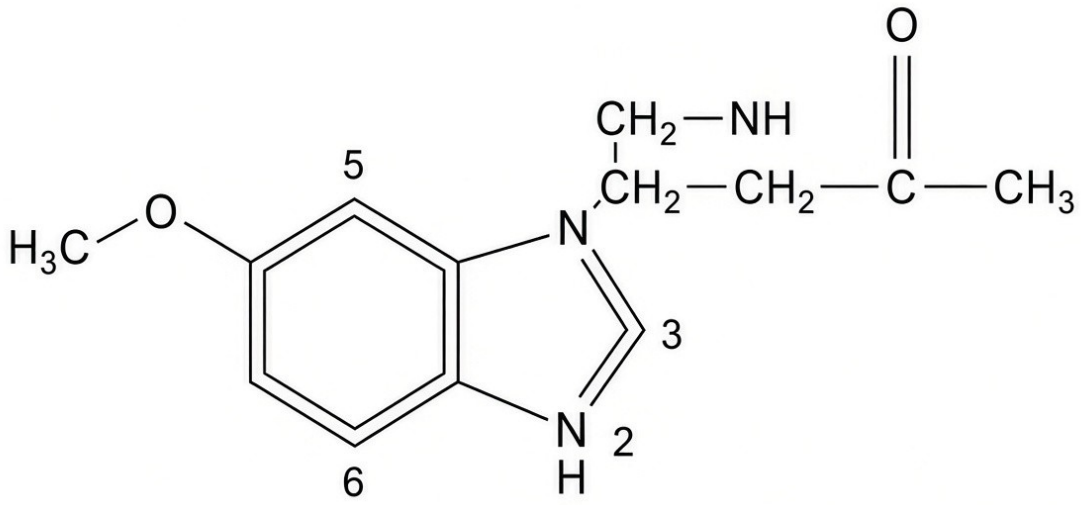
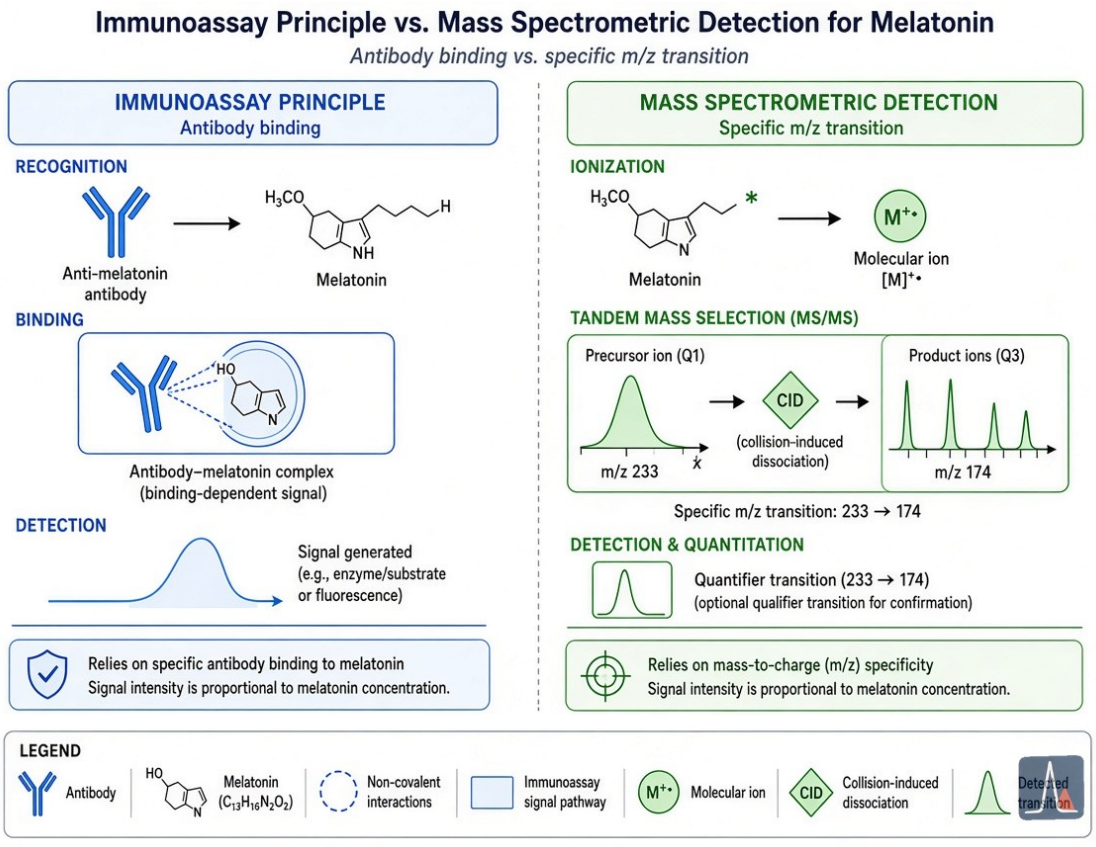


Chemical structure of melatonin (N-2-(5-methoxy-1H-indol-3-yl)ethylacetamide).



Conceptual comparison of immunoassay and LC-MS/MS approaches for melatonin analysis.

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Molecular Structure and Physicochemical Properties

The structure consists of an indole heterocycle substituted with a methoxy group at position 5 and a 2-acetamidoethyl chain at position 3. This arrangement confers characteristic spectroscopic signatures: UV absorption maxima near 223 nm and 278 nm arising from the indole π-system, and strong native fluorescence (excitation 280nm, emission 340–350nm). The molecule exhibits limited aqueous solubility but dissolves readily in ethanol, methanol, DMSO, or dilute alkaline solutions. It is sensitive to light and oxidative conditions, necessitating protected handling and appropriate storage to prevent formation of degradation products such as AFMK or AMK. These physicochemical features directly inform the design of chromatographic, spectrometric, and spectroscopic assays used to characterize research batches.

Published Chromatographic Assays for Purity, Related Substances, and Stability

Reversed-phase high-performance liquid chromatography (RP-HPLC) on C18 stationary phases is the primary method for chemical characterization and purity assessment. The USP melatonin monograph employs isocratic elution (acetonitrile–phosphate buffer, pH 3.5)with UV or PDA detection for the assay (typical run time 5 min on modernized 2.5 µm particle columns) and gradient elution for related compounds and impurities (e.g., 5-methoxytryptamine, 5-methoxytryptophol). System suitability parameters include resolution >2.0 between critical pairs, RSD <2.0 % for replicate injections, and appropriate relative retention times. Modernized protocols using sub-3 µm or 2.5 µm C18 columns achieve significantly increased throughput, improved efficiency, reduced solvent consumption, and lower environmental impact while fully meeting monograph criteria for linearity, accuracy, precision, and specificity. 1 2

Fluorescence detection (HPLC-FLD) and electrochemical detection (HPLC-ECD) provide enhanced sensitivity for trace-level work. These methods are stability-indicating and resolve melatonin from photo-oxidation or hydrolysis products. Validation parameters reported across studies routinely demonstrate linearity (r > 0.999), recoveries 99–103 %, and limits of quantification suitable for both high-purity material and low-concentration research samples.

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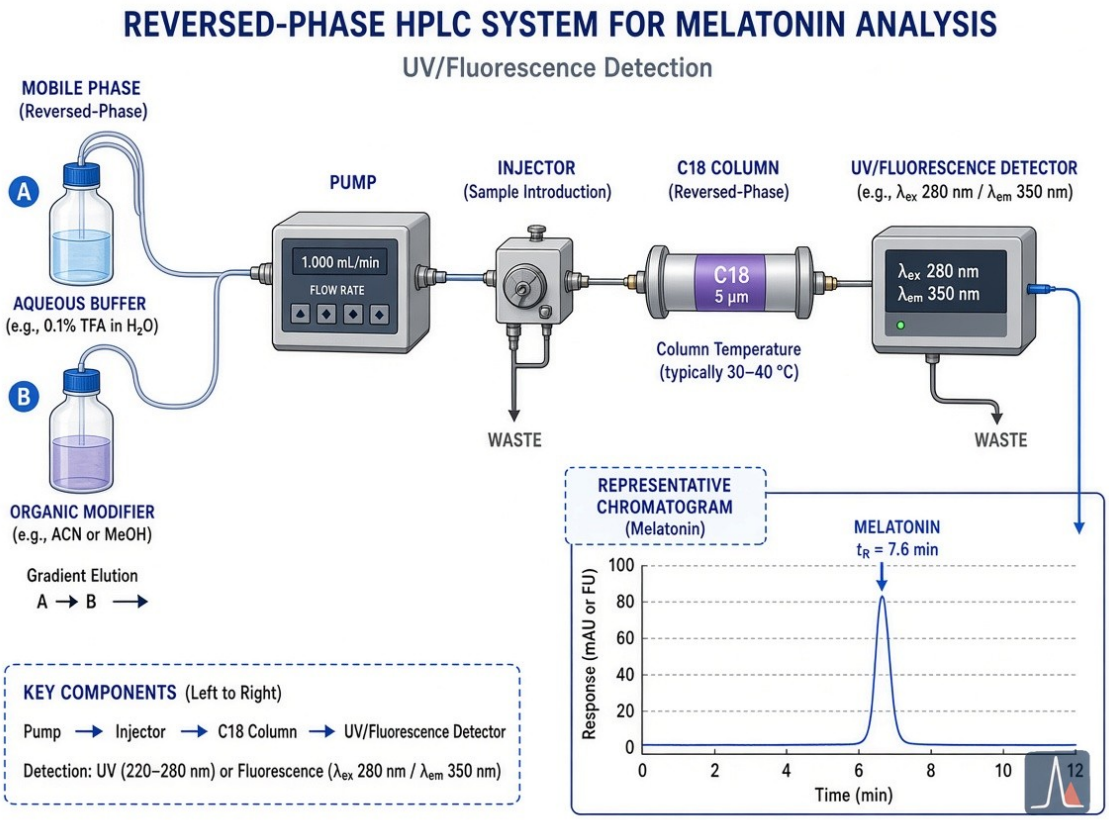

Schematic representation of RP-HPLC assay commonly used for melatonin purity and impurity profiling.

Mass Spectrometric, NMR, and Spectroscopic Characterization

Liquid chromatography–tandem mass spectrometry (LC-MS/MS or UHPLC-MS/MS) is widely regarded as the reference method for unequivocal identity confirmation and high-sensitivity quantification. The protonated molecular ion $[M+H]^+$ at m/z 233 is monitored with characteristic fragments; deuterated internal standards (e.g., melatonin-d4) correct for matrix effects. Reported limits of quantification reach 0.018 pg/µL or lower in biological matrices, with excellent precision (CV <10 %) and specificity. Supercritical fluid chromatography–MS/MS (SFC-MS/MS) and GC-MS (after derivatization) have also been validated for melatonin and precursors such as N-acetylserotonin. 3

Nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR) provides definitive structural assignment, with characteristic signals for the indole NH, methoxy singlet, acetamide methyl, and methylene groups. Quantitative NMR (qNMR) is increasingly used for absolute purity determination of reference standards. Complementary techniques include Fourier-transform infrared (FTIR) spectroscopy for functional group confirmation (amide I/II, indole NH, C–O–C methoxy), UV-Vis spectrophotometry, and native fluorescence spectroscopy. These orthogonal methods are routinely combined in pharmacopeial and research characterizations to establish batch identity and rule out synthetic by-products or degradants. 4

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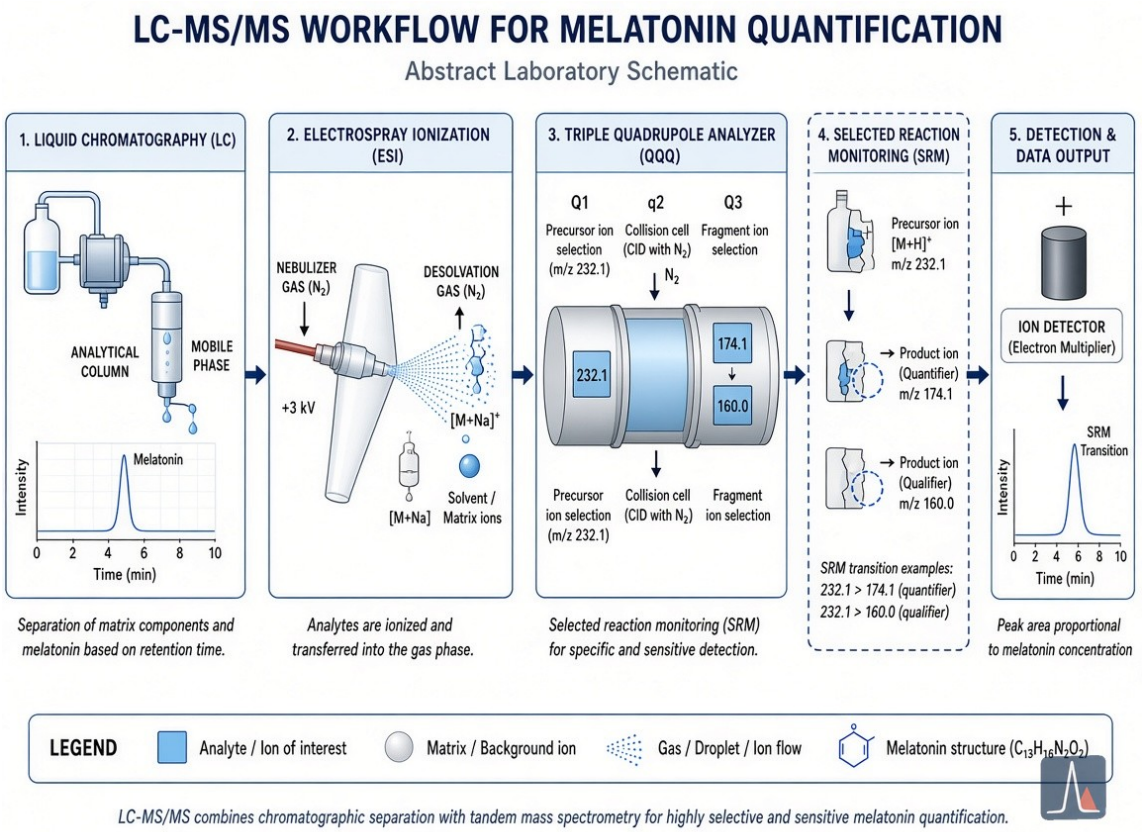

Schematic of LC-MS/MS method used as reference for melatonin identity and low-level quantification.

Immunoassays, Biosensors, and Functional Characterization

Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) have been extensively published for high-throughput measurement in plasma, saliva, urine, pineal extracts, and plant tissues. Their sensitivity allows detection below 1–2 pg/mL; however, cross-reactivity with structurally related indoles (serotonin metabolites, N-acetylserotonin) is well documented (15–40 % in some commercial kits). Direct (non-extracted) assays are particularly susceptible to overestimation of basal/daytime levels. Comparative studies using mass spectrometry as the reference demonstrate that true daytime plasma melatonin is typically <1 pg/mL, a benchmark that many early immunoassays failed to achieve. Extraction steps (C18 cartridges) improve specificity. A 2019 critical review concluded that while immunoassays remain useful for circadian phase marking when properly validated, LC-MS/MS provides the most accurate reference values. [5]

Emerging biosensors utilizing molecularly imprinted polymers, nanomaterials, or electrochemical transducers offer promising sensitivity and portability but are not yet primary for chemical identity confirmation of research material. Functional characterization in laboratory models commonly employs radioligand binding assays (using 2-[^{125}I]-iodomelatonin or [^{3}H]-melatonin) on MT1/MT2 receptors and downstream cAMP inhibition assays to confirm agonist activity, although these evaluate biological rather than chemical identity.

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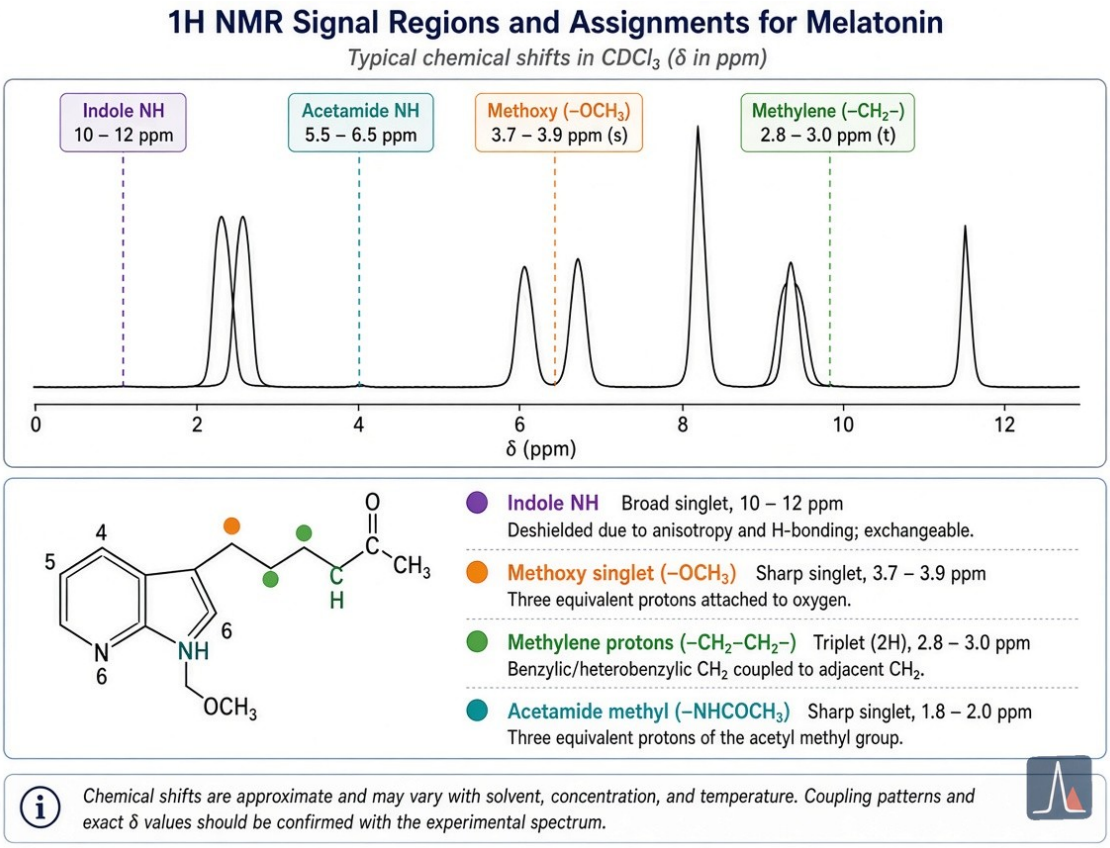

Representative NMR features used for structural confirmation of melatonin.

Evidence Context, Recurrent Limitations, and Catalog Implications

The published literature on melatonin assays spans pharmacopeial monographs, analytical chemistry journals, and pineal-research reviews from the 1970s to the present. Primary sources include USP modernization studies demonstrating faster, greener HPLC methods, LC-MS/MS validation papers achieving sub-picogram sensitivity, and comparative reviews that establish mass spectrometry as the accuracy benchmark. Both favorable (high precision, low LOD) and unfavorable or null findings (immunoassay cross-reactivity, overestimation, matrix effects) are documented. No single method is universally superior; choice depends on the research matrix and required specificity.

Recurrent limitations include photodegradation during sample handling, matrix interferences in complex biological samples, inter-laboratory variability when immunoassays are used without MS cross-validation, and the need for certified reference materials. Early RIA methods often produced higher basal values than later MS-confirmed ranges. For catalog users, these data support reliance on orthogonal confirmation—typically RP-HPLC-PDA for purity and retention-time matching, ESI-MS for molecular ion confirmation, and NMR or FTIR for structural identity—when verifying research material. Lot-specific COAs should document compliance with these techniques. Researchers studying circadian, antioxidant, or receptor-mediated effects benefit from selecting matrix-appropriate, fully validated assays and reporting complete method details.

Melatonin complements other catalog entries such as Glutathione and NAD in redox and neurobiology research. Laboratory investigators are encouraged to apply the most specific

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available methods to ensure reproducible characterization of the supplied research compound.

Sources

Primary literature from PubMed, USP monographs, and analytical journals (Kennaway 2019 review; USP modernization studies 2024; LC-MS/MS and HPLC validation papers). All citations correspond to peer-reviewed method development, validation, or comparative studies. Full DOIs and PMIDs are available in the referenced publications.

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