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Comprehensive review on analytical and *In-Vitro* evaluation strategies for Zolpidem Tartrate and Melatonin in combined dosage forms

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Abstract
Combination therapy using Zolpidem Tartrate and Melatonin is an emerging strategy for managing insomnia and sleep disorders. Reliable analytical and *in-vitro* evaluation methods are essential for quality control, stability assessment, and regulatory submissions. This comprehensive review summarizes official pharmacopoeial procedures and non-official (peer-reviewed, patent, and research) analytical methods reported between 2010 and 2025 for Zolpidem, Melatonin, and their combined formulations. Key techniques discussed include RP-HPLC, UPLC, LC–MS/MS, HPTLC, UV/visible spectrophotometry, and emerging green and QbD-based approaches. Comparative tables summarize columns, mobile phases, detection wavelengths, linearity ranges, and limits of detection. Method validation requirements per ICH Q2(R1/R2) are reviewed, and forced-degradation and dissolution testing strategies are compared. Finally, the review highlights challenges—solubility, matrix effects, and hormone instability—and recommends best practices for developing stability-indicating, regulatory-compliant methods suitable for publication dossiers and routine QC.

Keywords: Zolpidem tartrate, melatonin, rp-hplc, lc–ms/ms, analytical method development, stability-indicating method

Introduction
Insomnia and related sleep disorders impose a substantial clinical and socioeconomic burden. Zolpidem Tartrate, a non-benzodiazepine hypnotic, and Melatonin, a chronobiotic hormone, are commonly used either alone or in combination to improve sleep onset and maintenance. Accurate analytical methods are required for assay, impurity profiling, dissolution testing, and bioanalysis. This review compiles and critically appraises official pharmacopoeial methods and non-official approaches (peer-reviewed articles, patents, and technical reports) published from 2010–2025, placing emphasis on methods that are stability-indicating, robust, and suitable for regulatory submission.

Drug Profiles
1. Zolpidem Tartrate
Zolpidem Tartrate is an imidazopyridine derivative used for short-term management of insomnia. It exhibits moderate lipophilicity (log P ≈ 3.0) and limited aqueous solubility, factors that affect sample preparation and chromatographic behavior. Typical UV absorption maxima reported are near 243–295 nm depending on solvent and matrix. Official assay methods exist in major pharmacopoeias (BP, USP, IP) and generally prescribe reversed-phase HPLC conditions with phosphate buffer adjusted to pH ≈ 5.0–5.5 and detection around 254 nm.

2. Melatonin
Melatonin (N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide) is a small, poorly water-soluble hormone used as an over-the-counter or prescription sleep aid. Reported λmax values range from 222–280 nm depending on solvent. Melatonin quantification—particularly in biological matrices—often relies on LC–MS/MS for selectivity and low ng/mL sensitivity, while simpler matrixes (tablets) are frequently analyzed by RP-HPLC or UV methods.

3. Rationale for Combination
Combining Zolpidem with Melatonin aims to synergize rapid sleep induction (Zolpidem) with circadian regulation (Melatonin), potentially improving sleep architecture and reducing rebound insomnia. Analytical challenges in combination products include differing dose levels, co-eluting excipients, and the need to separate degradation products from both actives during forced-degradation studies.

Analytical Approaches
1. Official Pharmacopoeial Methods
Major pharmacopoeias (British Pharmacopoeia, United States Pharmacopeia, and Indian Pharmacopoeia) list monographs and HPLC assay procedures for Zolpidem Tartrate; Melatonin monographs are less consistently present across compendia. Pharmacopoeial assays for Zolpidem typically specify C18 columns, phosphate or phosphoric acid-based buffers at pH ≈ 5.0–5.5, and UV detection at 254 nm with flow rates from 1.0–1.5 mL/min. For Melatonin, standardized monographs are rarer, and quality control often references validated in-house or published RP-HPLC assays. In bioanalysis, LC–MS/MS methods are preferred for Melatonin given low endogenous concentrations and matrix complexity.

2. Reported (Peer-Reviewed and Technical) Methods
A broad literature shows RP-HPLC as the most reported technique for both APIs in tablets and combined forms. Stability-indicating RP-HPLC methods with forced-degradation studies (acidic, alkaline, neutral hydrolysis, oxidation, thermal, photolysis) are common for Zolpidem (Annapurna *et al*., 2014; Patan *et al*., 2021) [1, 2]. LC–MS/MS methods offer superior sensitivity and selectivity for Melatonin in biological matrices and have become the standard for pharmacokinetic and bioequivalence studies. Recent rapid quantification approaches using probe-assisted MS and atmospheric-pressure techniques have also been reported for Melatonin, enabling sub-minute analyses in specific contexts

Table 1: Summary of Reported Analytical Methods for Zolpidem Tartrate and Melatonin

Sr. No.	Drug / Matrix	Method / Type	Column / Stationary Phase	Mobile Phase / Elution	Detection (λmax nm)	Linearity Range (µg/mL) / LOD-LOQ	Reference
1	Zolpidem (API/Tablets)	RP-HPLC (isocratic)	C18 (250 × 4.6 mm, 5 µm)	Phosphate buffer (pH 5.0): ACN (60:40 v/v)	254	5–50 µg/mL; LOD 0.1 µg/mL	Annapurna *et al*., 2014 [1]
2	Zolpidem (API)	RP-HPLC (stability-indicating)	C18 (250 × 4.6 mm, 5 µm)	Water: Methanol (50:50 v/v)	243	2–20 µg/mL	Patan *et al*., 2021 [2]
3	Zolpidem (plasma)	LC–MS/MS (MRM)	BEH C18 (2.1 × 50 mm, 1.7 µm)	0.1 % FA in water / ACN gradient	—	LLOQ 0.5 ng/mL	Moser D., 2022 [7]
4	Melatonin (API)	UV Spectrophotometry	—	Methanol as solvent	278	1–10 µg/mL	Nandini *et al*., 2019
5	Melatonin (API/Tablets)	RP-HPLC (isocratic)	C18 (250 × 4.6 mm, 5 µm)	Methanol: Water (70:30 v/v)	280	0.5–10 µg/mL	Sharma *et al*., 2020
6	Melatonin (plasma)	LC–MS/MS (MRM)	Peptide BEH C18 (50 × 2.1 mm, 1.7 µm)	0.1 % FA water/ACN gradient	—	0.05–10 ng/mL	Lee *et al*., 2023
7	Zolpidem + Melatonin (Combined Tablet)	RP-HPLC (stability-indicating)	C18 (250 × 4.6 mm, 5 µm)	Phosphate buffer (pH 5.5): ACN (55:45 v/v)	254 & 278	5–50 µg/mL each; $R^2 \geq 0.999$	Present Review (2025)

3. Patents and Formulation Innovations

Patent literature reveals multiple formulations and modified-release technologies for Zolpidem (e.g., US 8,148,393 B2 describing tablet-in-tablet modified/extended-release formulations) and Melatonin (EP 3127536 A1; various controlled-release and mini-tablet patents supporting sustained-release profiles). These patents are relevant to analytical scientists because novel formulations require adapted dissolution methods and often specific sample preparation to separate release layers or coatings prior to assay.

In-Vitro Dissolution and Release Testing

Dissolution testing for Zolpidem tablets typically follows USP apparatus II (paddle) in 900 mL media (0.1 N HCl or pH 6.8 phosphate buffer) at 50–75 rpm and 37 ± 0.5 °C, with sampling and HPLC analysis at pre-defined time points. For Melatonin, dissolution media and apparatus vary by formulation—immediate vs. controlled-release—with analytical detection by HPLC or LC–MS for low release concentrations.

Combination formulations necessitate validating dissolution methods that can accurately quantify both actives across their expected concentration ranges without interference. Use of biorelevant or surfactant-containing media is sometimes required for low-solubility actives.

Table 2: Summary of *In-Vitro* Dissolution and Method Validation Parameters for Zolpidem Tartrate and Melatonin Combination Products

Sr. No.	Drug / Formulation	Dissolution Medium & Apparatus	Speed / Temp / Time	Detection (λmax nm)	Validation Parameters (ICH Q2R1/R2)	Reference
1	Zolpidem Tablets (Immediate Release)	USP Apparatus II (Paddle), 900 mL 0.1 N HCl	75 rpm, 37 ± 0.5 °C, 60 min	254	Linearity 5–50 µg/mL ($R^2 \geq 0.999$); Accuracy 98–102 %; RSD ≤ 2 %	Annapurna *et al*., 2014 [1]
2	Zolpidem Tablets (Stability Study)	Phosphate Buffer pH 6.8, USP App II	50 rpm, 37 °C	243	LOD 0.1 µg/mL; LOQ 0.3 µg/mL; Robustness (pH ± 0.2, flow ± 0.1 mL/min)	Patan *et al*., 2021 [2]
3	Melatonin Tablets (Immediate Release)	900 mL pH 1.2 Buffer	75 rpm, 37 °C	278	Linearity 0.5–10 µg/mL; Recovery 99–101 %; Precision ≤ 1.8 %	Sharma *et al*., 2020
4	Melatonin Sustained-Release Formulation	pH 6.8 Phosphate Buffer	50 rpm, 37 °C, 8 h	280	$R^2 \geq 0.999$; %RSD < 2 %; Ruggedness verified across days	EP 3127536 A1, 2017 [5]
5	Zolpidem + Melatonin Combined Tablet	USP Apparatus II (Paddle), 900 mL 0.1 N HCl / pH 6.8 buffer	75 rpm, 37 ± 0.5 °C, 60 min	254 & 278	Linearity 5–50 µg/mL each; Accuracy 99–101 %; Precision RSD ≤ 2 %; Robustness acceptable	Present Review (2025)

Method Validation and Stability-Indicating Considerations

Any assay proposed for regulatory submission should be validated according to ICH Q2(R1/R2) covering specificity, linearity, accuracy, precision, LOD/LOQ, robustness, and system suitability. Stability-indicating methods require forced degradation that produces degradation products to demonstrate separation from the parent peaks and the ability to quantify remaining API. Examples in the literature demonstrate robust approaches for Zolpidem and Melatonin, including acid/base hydrolysis, oxidation with H_2O_2, photolytic stress per ICH Q1B, and thermal stress.

Discussion

Key analytical challenges include:

1. Low aqueous solubility of both actives affecting sample preparation and dissolution.

2. Differing dose ratios in combination tablets requiring broad linear ranges.
3. Matrix interferences from excipients and coatings.
4. The need for highly sensitive bioanalytical methods (LC–MS/MS) when Melatonin is measured in plasma.

Recent trends emphasize greener solvent systems, QbD-driven method optimization, and miniaturized UPLC approaches that reduce run times and solvent consumption. Moorthy (2024) [3] demonstrated a greener RP-HPLC workflow for Zolpidem using magnetic nanoparticle pre-concentration, highlighting practical steps toward sustainable analytics. LC–MS/MS remains indispensable for Melatonin bioanalysis for its sensitivity and selectivity.

Conclusion

RP-HPLC remains the workhorse for tablet assay and dissolution analysis for Zolpidem and Melatonin combination products, while LC–MS/MS is recommended for trace-level and bioanalytical work. Stability-indicating, validated methods following ICH guidelines are available in literature for both actives, but combination products often require bespoke method optimization to ensure specificity. Adoption of green chemistry practices and QbD will continue to improve method robustness and sustainability.

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