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Development and Validation of UV-Spectrophotometric and RP-HPLC Methods for the Determination of Eszopiclone in Bulk Drug and Tablet Dosage Forms

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Abstract

A simple, accurate, precise, and economical UV-spectrophotometric method and a rapid reverse-phase high-performance liquid chromatographic (RP-HPLC) method were developed and validated for the determination of Eszopiclone in bulk drug and tablet dosage forms. In the UV method, Eszopiclone exhibited maximum absorbance at 305 nm using 50% v/v methanol as solvent. Beer's law was obeyed in the concentration range of 4–32 µg/mL with a correlation coefficient of 0.999. The RP-HPLC method was developed using a Phenomenex Gemini C18 column (250 × 4.6 mm, 5 µm) with methanol: water (80:20, v/v) as mobile phase at a flow rate of 1.0 mL/min and detection at 305 nm. The retention time of Eszopiclone was found to be 5.38 min. Both methods were validated according to ICH guidelines with respect to linearity, accuracy, precision, ruggedness, robustness, specificity, LOD, and LOQ. The percentage recovery ranged between 99.54–100.24%, indicating good accuracy. The proposed methods were successfully applied for routine analysis of Eszopiclone in bulk drug and tablet formulations.

Keywords: Eszopiclone, UV spectroscopy, RP-HPLC, Method validation, ICH guidelines, Tablet analysis.

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1. Introduction

Sleep disorders represent one of the most prevalent health concerns worldwide and significantly affect the quality of life, productivity, and overall well-being of affected individuals. Among these disorders, insomnia is characterized by difficulty in initiating sleep, maintaining sleep, or experiencing restorative sleep despite adequate opportunity for sleep [1]. Chronic insomnia has been associated with impaired cognitive function, increased risk of cardiovascular diseases, depression, anxiety, metabolic disorders, and reduced work performance. The increasing prevalence of insomnia has resulted in a growing demand for effective pharmacological therapies that provide rapid onset of action while minimizing adverse effects [2].

Eszopiclone is a cyclopyrrolone derivative belonging to the class of non-benzodiazepine hypnotic agents commonly referred to as "Z-drugs." Chemically, Eszopiclone is designated as (S)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-5-yl-4-methylpiperazine-1-carboxylate [3]. Unlike conventional benzodiazepines, Eszopiclone selectively binds to the benzodiazepine-binding site of the γ-aminobutyric acid type A (GABA-A) receptor complex, thereby enhancing inhibitory neurotransmission within the central nervous system [4]. This pharmacological action promotes sleep induction, prolongs total sleep duration, and improves sleep quality without causing significant alterations in normal sleep architecture [5].

Eszopiclone is widely prescribed for the short-term and long-term management of insomnia because of its favourable pharmacokinetic profile, rapid onset of action, and comparatively lower incidence of residual daytime sedation [6]. Following oral administration, the drug is rapidly absorbed, achieving peak plasma concentrations within approximately one hour, with an elimination half-life of nearly six hours in healthy adults [7]. The therapeutic efficacy and safety of Eszopiclone depend largely on maintaining the appropriate drug concentration in pharmaceutical formulations, emphasizing the necessity for reliable analytical methods for its quantitative estimation [8].

Analytical method development plays a pivotal role throughout the pharmaceutical product life cycle, including drug discovery, formulation development, process optimization, quality control, stability studies, and regulatory compliance. Every pharmaceutical dosage form must undergo rigorous analytical evaluation to ensure that it meets predefined specifications for identity, purity, potency, and content uniformity [9]. Consequently, the development of analytical methods that are simple, accurate, precise, sensitive, reproducible, and economical remains an essential requirement for pharmaceutical industries and quality control laboratories.

Among the various analytical techniques available for pharmaceutical analysis, ultraviolet-visible (UV–Vis) spectrophotometry remains one of the most widely employed methods because of its operational simplicity, rapid analysis, minimal sample preparation, and relatively low cost. The technique is particularly suitable for routine assay of pharmaceutical compounds possessing chromophoric functional groups capable of absorbing ultraviolet radiation. Quantitative determination is based on the Beer–Lambert law, which establishes a linear relationship between absorbance and analyte concentration within a specified range. Owing to its affordability and ease of operation, UV spectrophotometry is extensively utilized in routine quality control laboratories, academic research institutions, and pharmaceutical manufacturing facilities [10].

However, UV spectrophotometric methods may exhibit limitations in terms of specificity when pharmaceutical formulations contain multiple components, degradation products, or excipients that absorb at similar wavelengths. Therefore, chromatographic techniques are frequently employed to overcome these limitations and provide improved analytical selectivity [11].

Reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as one of the most reliable and versatile analytical techniques for quantitative pharmaceutical analysis. RP-HPLC offers several advantages, including high resolution, excellent sensitivity, superior specificity, reproducibility, rapid separation, and the capability to analyze complex pharmaceutical matrices. The technique is particularly valuable for assay determination, impurity profiling, dissolution testing, stability studies, pharmacokinetic investigations, and stability-indicating method development. The combination of a nonpolar stationary phase with an appropriately optimized mobile phase enables efficient separation of analytes based on their hydrophobic interactions, resulting in accurate and reproducible chromatographic performance [12].

Numerous analytical methods for the determination of Eszopiclone have been reported in the literature, including spectrophotometric methods, HPLC methods, liquid chromatography–mass spectrometry (LC–MS), ultra-performance liquid chromatography (UPLC), capillary electrophoresis, and bioanalytical methods for plasma estimation. Although these techniques provide satisfactory analytical performance, many require sophisticated instrumentation, complex mobile phase compositions, lengthy analysis times, expensive solvents, or extensive sample preparation procedures. Such limitations reduce their suitability for routine quality control applications, particularly in laboratories with limited analytical resources [13].

Consequently, there remains a continuing need for analytical methods that combine simplicity with high analytical performance. The ideal pharmaceutical analytical method should require minimal sample preparation, use readily available reagents, provide rapid analysis, demonstrate excellent accuracy and precision, and comply with international regulatory requirements [14].

Method validation constitutes an indispensable component of analytical method development. Validation establishes documented evidence that an analytical procedure consistently produces reliable results suitable for its intended purpose. According to the International Council for Harmonisation (ICH) guideline Q2(R1), analytical methods should be validated for parameters including specificity, linearity, analytical range, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability, as applicable. Proper validation ensures the reliability, reproducibility, and regulatory acceptance of analytical methods used in pharmaceutical quality assurance [15].

In the present investigation, both UV-spectrophotometric and RP-HPLC methods were developed and validated for the quantitative estimation of Eszopiclone in bulk drug and tablet dosage forms. For the UV method,

methanol was selected as the solvent because of the excellent solubility and spectral stability of Eszopiclone. The absorption spectrum of the drug was recorded within the ultraviolet region to determine the wavelength of maximum absorbance (λmax), which was subsequently employed for quantitative analysis. Beer–Lambert's law was evaluated over an appropriate concentration range to establish linearity, and the method was optimized to achieve maximum sensitivity and reproducibility [16].

Simultaneously, an RP-HPLC method was developed using a C18 reversed-phase column with an optimized methanol–water mobile phase and ultraviolet detection. Various chromatographic parameters, including mobile phase composition, flow rate, detection wavelength, and injection volume, were systematically optimized to obtain sharp, symmetrical peaks with acceptable retention time and high theoretical plate count. The optimized chromatographic conditions were subsequently validated according to ICH recommendations [17].

Both analytical methods were evaluated for linearity, accuracy, precision, ruggedness, robustness (for the HPLC method), specificity, and applicability to commercial tablet formulations. Statistical evaluation of the analytical results was performed to assess the reliability and reproducibility of the developed methods. Comparative assessment of the UV spectrophotometric and RP-HPLC methods was also undertaken to establish their suitability for routine quality control analysis [18].

The successful development and validation of these analytical procedures are expected to provide pharmaceutical industries, academic laboratories, and quality control facilities with rapid, reliable, economical, and regulatory-compliant methods for the routine determination of Eszopiclone in bulk drug substances and finished pharmaceutical dosage forms [19].

2. Materials and methods

2.1 Materials

Eszopiclone was obtained as a gift sample from Sun Pharmaceuticals Ltd., Vapi, Gujarat, India. HPLC-grade methanol and double RO water were used throughout the study.

2.2.1 Instruments

- Shimadzu UV-2450 UV-visible spectrophotometer
- Shimadzu HPLC system with PDA detector
- Shimadzu AUX-120 analytical balance
- Systronics pH meter
- Enertech ultrasonicator

2.2.2 UV-Spectrophotometric Method

Preparation of Standard Solution

10 milligrams of Eszopiclone were dissolved in methanol and diluted to 100 mL to obtain a stock solution containing 100 µg/mL.

Determination of λ_{max}

From the stock solutions, 1.0 ml of ESZ was transferred to 10 ml volumetric flask and the volume was adjusted to the mark with same solvent to obtain Strength 10µg/ml. The solution was scanned in the UV range 200-400 nm. The solution was scanned between 200 and 400 nm. Maximum absorbance was observed at 305 nm.

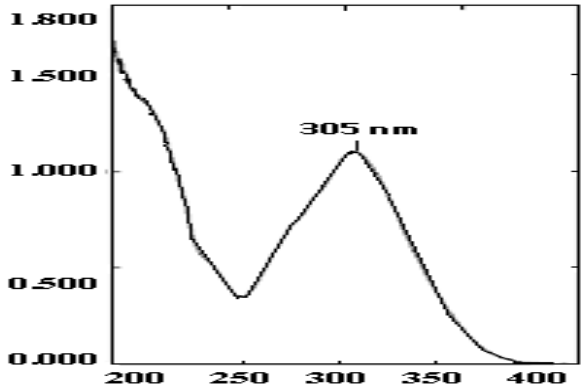

Figure 1. Spectrum of Eszopiclone in 50% v/v methanol

Linearity

Appropriate aliquot portions of stock standard solution of ESZ were transferred in to five separate 10 ml volumetric flasks, volume was the volume was adjusted to the mark with 50 %(v/v) methanol to obtain concentrations of 4, 8, 12, 16, 20, 24, 28 and 32µg/ml. Absorbances of these solutions were recorded at 305.0 nm. The method showed linearity over the concentration range of 4–32 µg/mL.

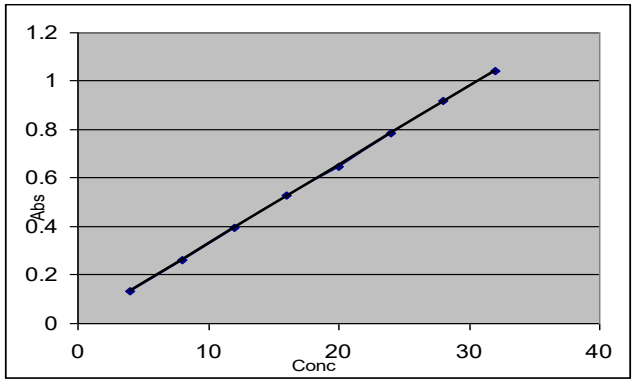

Figure 2: Calibration Curve of ESZ at 305.0 nm

2.2.3 RP-HPLC Method

Chromatographic Conditions

- Column: Phenomenex Gemini C18 (250 × 4.6 mm, 5 µm)
- Mobile phase: Methanol: Water (80:20 v/v)
- Flow rate: 1.0 mL/min
- Detection wavelength: 305 nm
- Injection volume: 20 µL
- Column temperature: 25°C

Preparation of Standard Stock Solution:

Standard stock solution was prepared by dissolving 10 mg of ESZ in 100 ml methanol that gives concentration of 100 µg/ml of ESZ.

Optimized Chromatographic Conditions:

Table 1: Optimized Chromatographic Conditions

Chromatographic Mode	Chromatographic Condition
Standard solution	100 µg/ml of ESZ in methanol
HPLC System	Shimadzu HPLC system
Pump	LC-10 AT VP solvent delivery system
Detector	SPD M-10AVP photo diode array detector
Data processor	Class-M 10 data station
Stationary phase	Phenomenex Gemini C18 column (250 mm x 4.6.0 mm, 5 µ)
Mobile phase	Methanol: Water (80:20, v/v)
Detection wavelength	305 nm
Flow rate	1 ml/min
Sample size	20 µl
Column temperature	25 ^{0}C

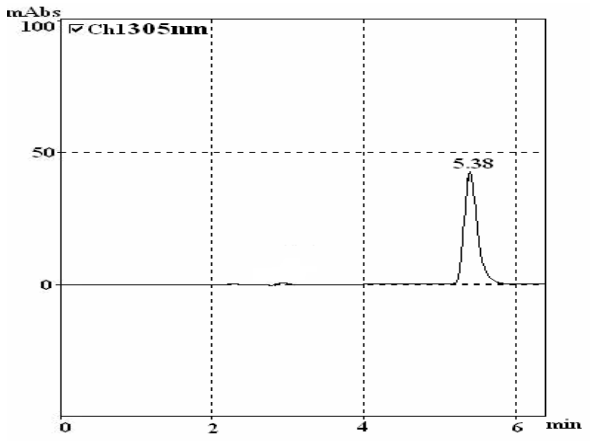

Figure 3: Chromatogram of ESZ standard

The retention time of Eszopiclone was found to be 5.38 minutes.

2.2.4 Application of proposed method to tablet formulations:

To determine the content of ESZ in conventional tablets (Label claim 1 mg ESZ per tablet); the twenty tablets were weighed, their mean weight determined and they were finely powered and powder equivalent 1.0 mg ESZ was transferred into a 100 mL volumetric flask containing 40 mL methanol, sonicated for 30 min and diluted to 100 mL with methanol (10 µg/mL). The resulting solution was filtered, using 0.22 µm filter (Millifilter, Milford, MA) and an injected into system. The amount of ESZ was determined.

2.2.5 Validation of Proposed Method:

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy

Accuracy of the method was assessed at three different concentration levels i.e. 80%, 100, 120%. To the preanalysed sample solution (10 µg/ml of ESZ) a known quantity of drug standard was added at three different levels and reanalyzed by proposed method. The percentage recovery ranged from 99.54% to 100.24%, demonstrating excellent accuracy.

Precision

The repeatability, intraday, and interday studies showed %RSD values below 2%, indicating good precision.

Robustness

Minor changes in flow rate and mobile phase composition did not significantly affect chromatographic performance.

Sensitivity

LOD and LOQ were found to be:

LOD: 0.31 µg/mL

LOQ: 0.54 µg/mL

Ruggedness

The methods demonstrated excellent reproducibility between analysts.

Table 2: Results of Validation Parameters

Parameters	Observation
Linearity range (µg/mL)	4-28
Regression equation	Y= 29274 X + 4427.8
LOD (µg)	0.31
LOQ (µg)	0.54
Recovery (%)	99.92
Precision (% RSD)	
Intra- day (n = 3)	1.37 – 1.85
Inter-day (n = 3)	0.43 – 1.51
Repeatability (n = 5)	0.65
Ruggedness (% RSD)	
Analyst I (n = 6)	0.34
Analyst II (n = 6)	0.40
Robustness	Robust
Specificity	Specific

3. Results and discussion

The HPLC analysis was performed on the Phenomenex Gemini C_{18} (250 mm × 4.60 mm), 5µm particle size in isocratic mode, at 25 ^{0}C temperature using a mobile phase consisting of methanol: water (80:20*v/v*) at a flow rate of 1.0 mL/min. The detection was carried out at 305 nm. The average retention time for ESZ was found to be 5.38 min. Linearity was observed in the concentration range from 5-30 µg/mL (r^2 = 0.9993). The limit of detection and quantitation of ESZ was 0.31 µg and 0.54 µg, respectively. The method has been successively applied for the determination of ESZ in capsule. There was no interference from the excipients commonly present in the tablet. The drug content was found to be 100.24 % for ESZ. Accuracy of the method was studied by the recovery studies at three different levels 80 %, 100 % and 120 % level. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.75– 100.24. The % RSD below 2.0 shows the high precision of proposed method. According to USP (621), system suitability tests are an integral part of chromatographic methods. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions

4. Conclusion

The developed UV-spectrophotometric and RP-HPLC methods for Eszopiclone are simple, rapid, economical, precise, accurate, and validated according to ICH guidelines. Both methods can be successfully employed for routine quality control analysis of Eszopiclone in bulk drug and pharmaceutical dosage forms.

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