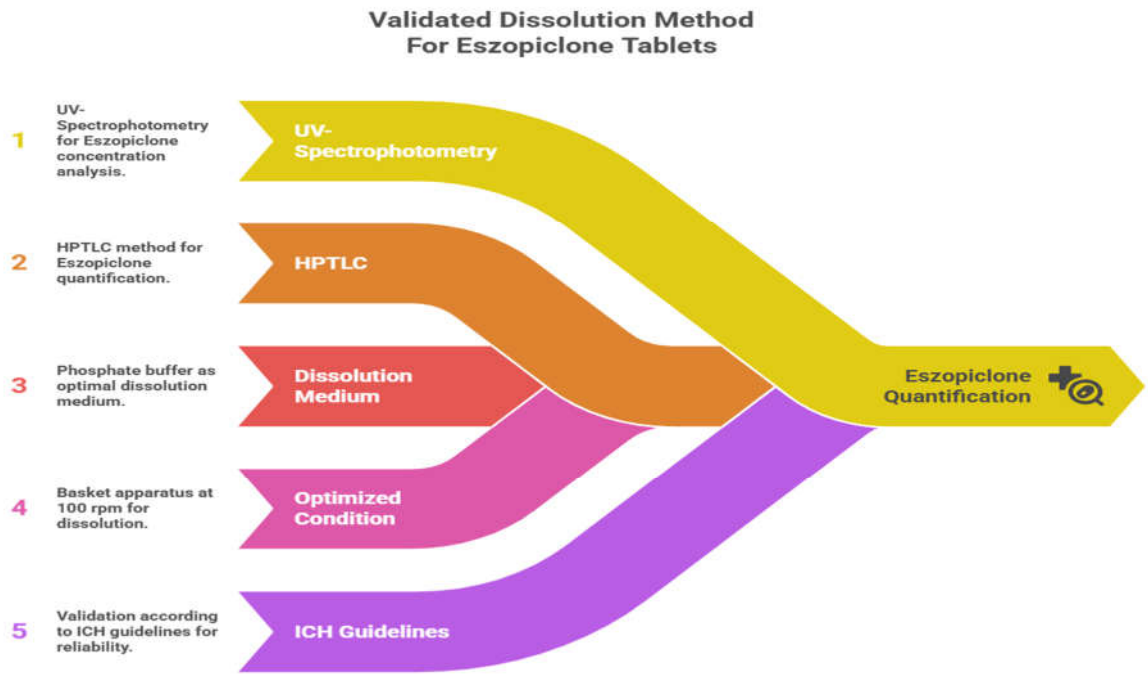
DEVELOPMENT AND VALIDATION OF DISSOLUTION METHOD USING UV-SPECTROPHOTOMETRY AND HPTLC FOR ESZOPICLONE TABLETS

Dr. Mrinalini C. Damle , Shruti S. Gaikwad
Department of Quality Assurance, All India Shri Shivaji Memorial Society's College of Pharmacy, Pune, Maharashtra, India 411001.

ABSTRACT

Eszopiclone is employed in the treatment of insomnia. A dissolution method for Eszopiclone tablets was developed and validated using UV-Spectrophotometry and High-Performance Thin-Layer Chromatography. The HPTLC method employed a CAMAG system with silica plates, utilizing a mobile phase of chloroform : methanol (9.5:0.5V/V). Phosphate buffer pH 6.8 was selected as the dissolution medium, and the basket apparatus at 100 rpm was found to be the optimal condition. The method was validated according to ICH guidelines . The results showed that the method is specific, with no interference from placebo or excipients, and linear over the concentration range of 20-100 ng/band for HPTLC and 2-10 µg/mL for UV-Spectrophotometry. The method was precise, with % RSD values less than 2, and accurate, with % recovery values between 98-102%. The robustness of the method was also established, with minimal variations in dissolution conditions. The developed method was successfully applied to quantify Eszopiclone in tablets, demonstrating its suitability for quality control and formulation development.

Keywords: Dissolution, Eszopiclone, ICH guidelines, HPTLC, UV Spectrophotometric method.



INTRODUCTION

Eszopiclone is a sedative and hypnotic agent. It is used for the treatment of insomnia. It is characterized by a chemical structure that is differ from benzodiazepines, barbiturates, and hypnotic substances[1]. It received FDA approval on December 15, 2004, and is official in USP 2024[2]. It exerts its effects through interaction with GABA-A receptor complex. Eszopiclone is the active dextrorotatory stereoisomer of zopiclone, and its structure is classified within the group of medications referred to as cyclopyrrolones[3] Fig.1. It is reported that Eszopiclone is slightly soluble in ethanol, soluble in phosphate buffer and sparingly soluble in methanol. It is available as tablet dosage form with label claim of 1mg, 2mg and 3 mg per tablet[4].

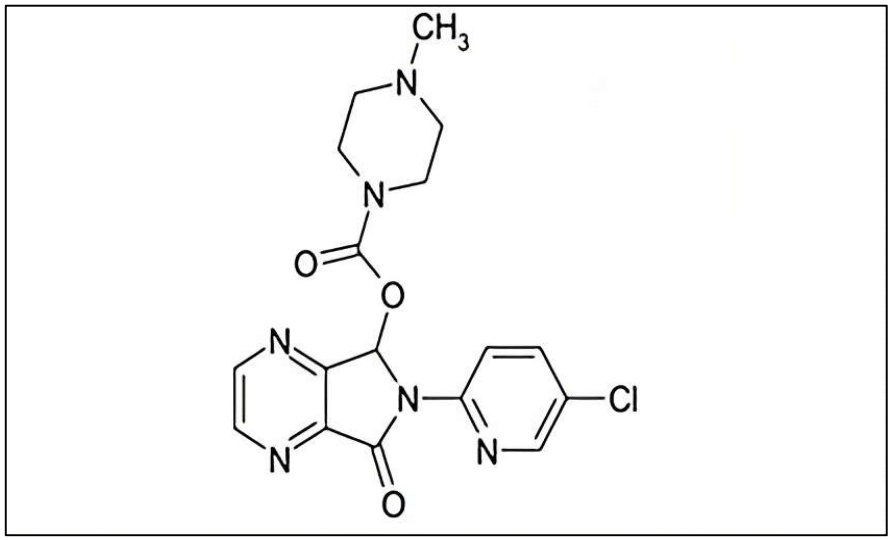


Fig. 1: Chemical structure of Eszopiclone

Dissolution refers to the rate and extent to which a drug substance is dissolved over a specified period. It is quantified as the percentage release of drug substances found in dosage forms such as tablets, capsules, ointments, and oral suspensions. In this study, a dissolution method was developed for an immediate release tablet containing 2 mg of Eszopiclone[5].

The most effective method for evaluating the therapeutic efficacy of a drug is through in vivo determination of bioavailability, which occurs when a new formulation is launched in the market. Nevertheless, to ensure consistency from batch to batch, this approach can become expensive, labor-Intensive, and time-consuming; therefore, the in vitro dissolution test has emerged as the optimal quality control tool to quantitatively verify the biological availability of the drug from its formulation[6].

For products designated for immediate release, the basket (Apparatus 1, typically operating at 100 rpm) and paddle (Apparatus 2, generally functioning at 50 to 75 rpm) are standard[7,8]. For developmental objectives, it is advisable to generate dissolution patterns at brief intervals, specifically at 10, 15, 20, 30, and 45 minutes[9,10].

The literature survey indicates that there is no official dissolution method present in pharmacopoeias, and there is not a singular method established for dissolution testing for eszopiclone tablets using UV and HPTLC techniques.

A high-performance thin-layer chromatography (HPTLC) method and a UV spectrophotometric method were developed for quantifying Eszopiclone in dissolution tests. The HPTLC method offered the advantage of high-throughput analysis, while the UV method provided simplicity and cost-effectiveness. Both methods were validated according to International Council for Harmonization (ICH) guidelines, evaluating parameters such as specificity, linearity, precision, accuracy, and robustness to ensure their reliability and suitability for routine analysis[11-13].

MATERIALS AND METHOD

Instruments

Dissolution samples were analyzed using HPTLC system (CAMAG with winCATS 1.4.2) and UV–Visible spectrophotometer (Shimadzu UV 1780), and other equipment used were Analytical Balance (Shimadzu-ATX224R), Dissolution apparatus USP (type I) (Electrolab Inspire-08) and Auto Digital pH meter (Labtronics LT 11).

Chemicals and reagents

The Eszopiclone reference standard was received as a gift sample. The chemicals utilized included methanol and water, chloroform, Potassium dihydrogen phosphate Disodium dihydrogen phosphate . For the purpose of dissolution, commercially available tablets with a label claim of 2 mg were obtained from a local pharmacy.

Preparation of Stock Solution of Eszopiclone

A precise measurement of 10 mg of Eszopiclone was placed into a clean and calibrated 10 ml volumetric flask. It was then dissolved in the dissolution medium, and the volume was adjusted to the mark with the phosphate buffer pH 6.8. From this stock solution, further dilutions were performed using the dissolution medium to achieve a final concentration of 4 µg/ml.

Selection of analytical wavelength

A solution of 4 µg/ml (Transferred the 0.4 ml of the solution of 100 µg/ml into a 10 ml volumetric flask and made up the volume up to the mark with phosphate buffer pH 6.8) was prepared and scanned over 200-400 nm using a UV – Spectrophotometer. As illustrated in Fig.2, the wavelength identified for further research was 303 nm.

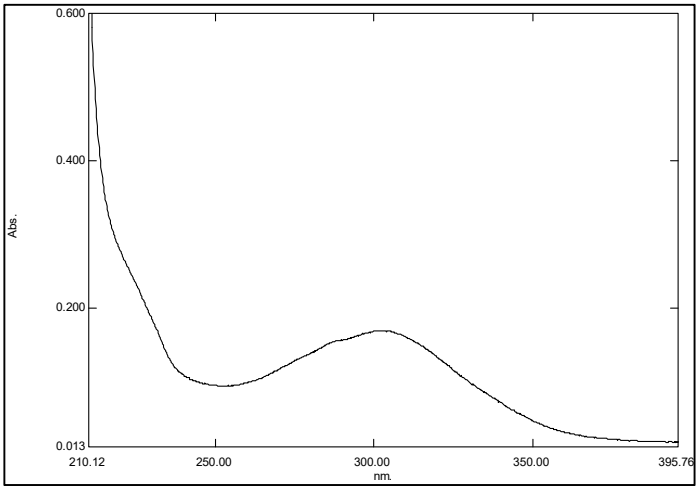


Fig. 2: UV Spectrum of Eszopiclone in phosphate buffer pH 6.8 (4 µg/ml)

SELECTION OF DISSOLUTION CONDITION

The choice of dissolution medium was based on Eszopiclone's physical characteristics and solubility. Given its solubility in phosphate buffer, this medium was selected for dissolution studies. Considering its pKa of 9.2, a moderately weak base, it's better absorbed in the intestine where the pH range is 5-7.5. Although Eszopiclone is more soluble in acidic conditions, its absorption is enhanced in more alkaline conditions (pH 5-7.5) where it remains unionized. Therefore, phosphate buffer with a pH range of 5 to 7.5 was tried as the dissolution medium to simulate the intestinal environment and evaluate Eszopiclone's dissolution profile[14,15]. To develop a suitable dissolution method for Eszopiclone Tablets, various conditions were tested. Initially, USP Type II apparatus (Paddle) was used with different dissolution media such as phosphate buffer at pH 5, 5.5, and 6.8 having 500 ml dissolution volume, and 37°C temperature, at speeds of 50 and 75 rpm. Samples were analyzed at 30, 45, and 60 minutes.

METHOD VALIDATION

The develop HPTLC and UV method was validate as per ICH Q2R2 guidelines for various parameter like specificity, linearity, precision, Accuracy and solution stability.

specificity

The method's specificity was assessed to ensure that the formulation excipients and the dissolution medium did not affect the analysis of Eszopiclone tablets. A placebo sample containing the standard blend of excipients, was prepared to validate the method's reliability. Specificity of method is assessed by analyzing the blank dissolution medium, placebo solution, standard solution, and dissolution sample.

Linearity

The linearity of the analytical procedure refers to the capability to perform a test response that is proportional to the concentration of the drug solution within a specified range. The linearity was assessed for a concentration range of 20–100 ng/band for the HPTLC method, while for UV, the range established was 2–10 µg/ml. A calibration curve was created, and the regression coefficient was calculated.

Accuracy

The accuracy of an analytical method indicates how closely the measured value aligns with the true value. This is quantified as a percentage recovery. The percent recovery of Eszopiclone was evaluated using the standard addition method, with concentration levels of 80%, 100%, and 120% applied to the dissolution sample.

Precision

Repeatability: The ability to accurately apply a method over a brief period of time under the same operational conditions. It is carried out by calculating the percentage RSD of six distinct sample solutions at 100% concentration.

Intermediate precision: It was carried out on various days using a sample solution at 100% concentration, and the percentage RSD was calculated.

LOQ

The LOQ for the developed HPTLC and UV methods were determined in accordance with ICH guidelines, utilizing the slope of the calibration line and standard deviation of the peak area

and absorbance at lowest concentration.

Robustness:

The robustness was examined by evaluating minor variations in the dissolution conditions, including the composition of the dissolution media, the agitation rate, and the volume of the dissolution medium. The % RSD was calculated.

Solution Stability

The stability of the sample solution was assessed by storing it at 37°C for 4 hours. A freshly prepared standard solution was used for comparison to determine the sample's stability. The acceptance criteria for stability were set within 98% to 102% of the initial value, ensuring the sample remained stable throughout the testing period.

RESULTS

OPTIMIZATION OF DISSOLUTION METHOD

The dissolution experiments were carried out using a eight-station bath dissolution apparatus, where Eszopiclone tablets (n=6) were added to 500 mL vessels of each dissolution medium, utilizing a basket dissolution apparatus and stirring at a speed of 100. The temperature was maintained at 37 ± 0.5 °C. Aliquots of 10 mL were manually withdrawn at 30 and 45 minutes. An equal volume of medium at 37 ± 0.5 °C was replenished to ensure a constant volume. The standard solution utilized in all dissolution tests was prepared with eszopiclone having a concentration of 4 µg/ml. The optimized dissolution condition shown in Table 1.

Table 1 Optimized dissolution conditions

Sr No.	PARAMETER	OPTIMIZED CONDITION
1.	Medium	Phosphate Buffer pH 6.8
2.	Volume	500 ml
3.	Apparatus	USP Type-I (Basket)
4.	Temperature	37°C
5.	RPM	100
6.	Time Point	30 min

Specificity

This placebo sample was placed in vessels with 500 mL of dissolution medium and stirred at 37 °C for 30 minutes at 100 RPM using a Basket apparatus. Aliquots were taken and analyzed using UV and HPTLC. Fig.3, 4,.5 and 6 shows a densitogram of blank solution, placebo solution, standard solution and dissolution sample solution respectively and Fig 7 shows the overlay of UV spectrum of Dissolution sample and placebo sample. The results demonstrate that there is no interference from the placebo solution, excipients, or dissolution media, indicating that they are unlikely to impact the quantitation of the main analyte, thereby confirming the specificity of the analytical method.

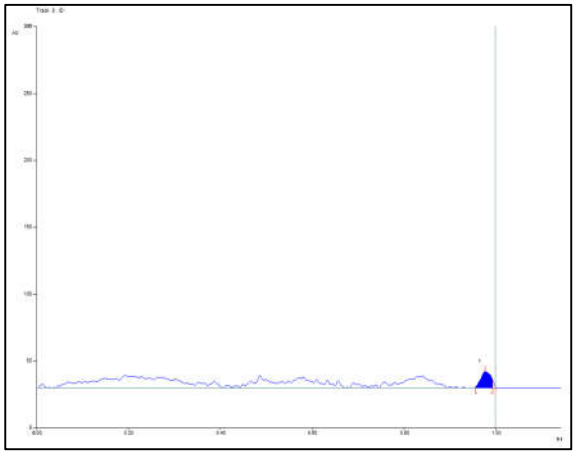


Fig 3 Representative densitogram Placebo solution

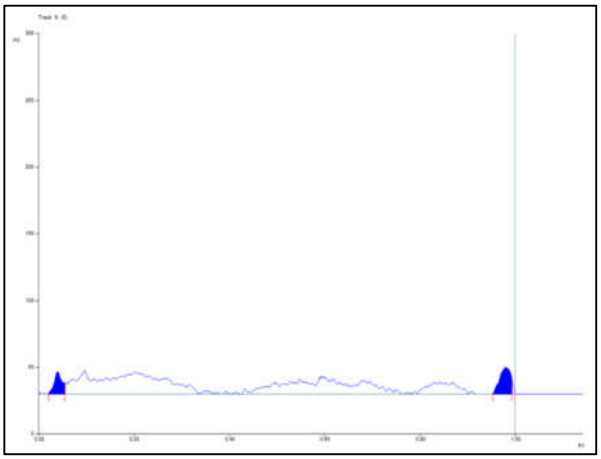


Fig 4 Representative densitogram of of Blank Solution

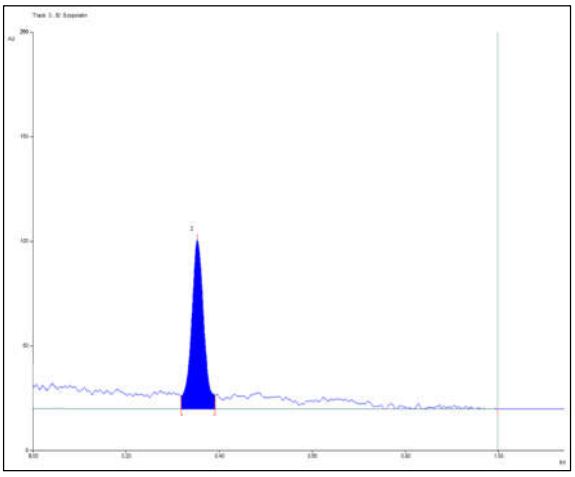


Fig 5 Densitogram of Standard dissolution sample(40 ng/band,Rf=0.37)

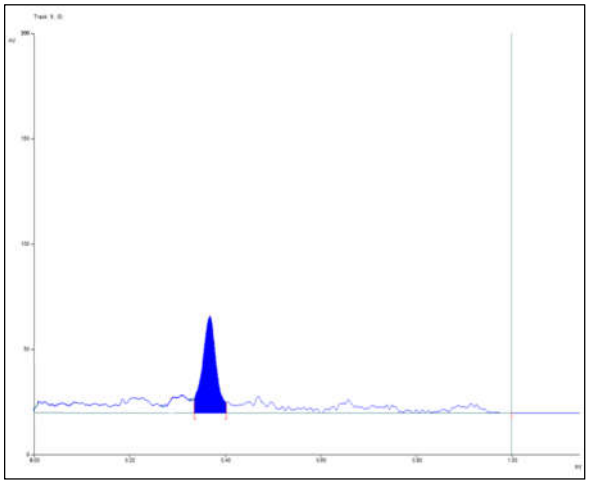


Fig 6 densitogram of Eszopiclone dissolution sample (40 ng/band, Rf=0.37)

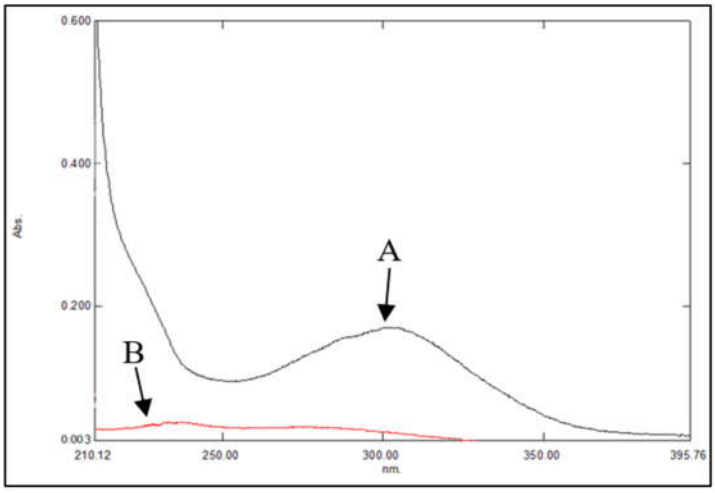


Fig 7 Overlay of UV spectrum A) Eszopiclone B) Placebo solution

Linearity:

The linearity assessment was conducted by preparing a final concentration of 4 µg/ml. From this solution, volumes of 5, 10, 15, 20, and 25 µl were applied to the TLC plate to achieve a concentration range of 20-100 ng/band and solution evaluated by scanning six distinct solutions across a concentration range of 2-10 µg/ml in UV. The value of R^2 was found to be 0.9975 and 0.9936, respectively Densitogram that illustrates the linearity is displayed in Fig 8 and the calibration curve of HPTLC and UV shown in Fig 9 and Fig 10 respectively.

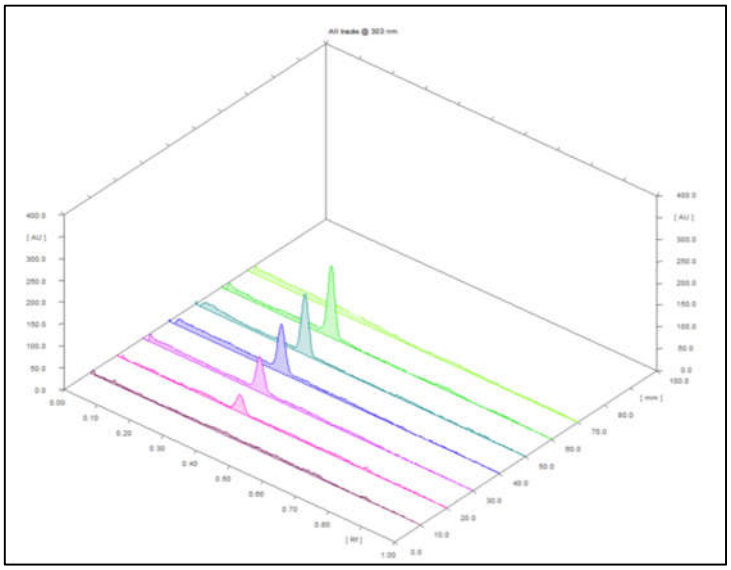


Fig 8 3D Densitogram of Eszopiclone Linearity (20-100 ng/band)

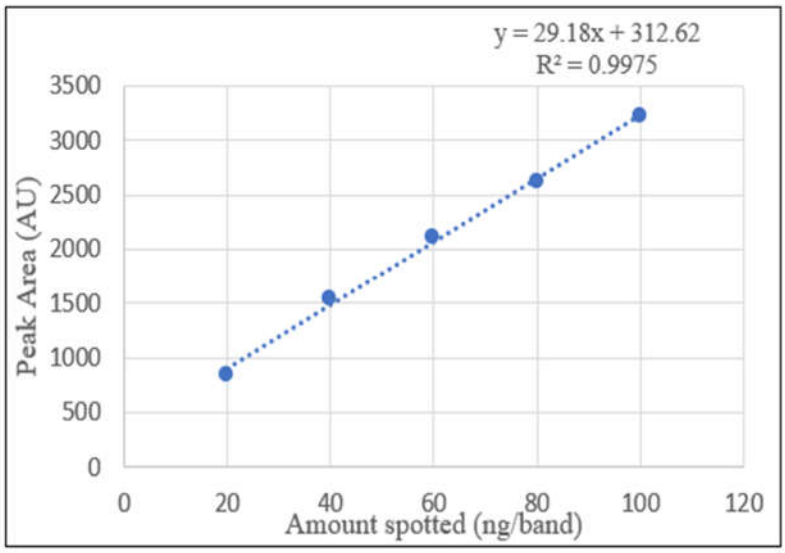


Fig 9 Calibration Curve for Eszopiclone (20-100 ng/band)

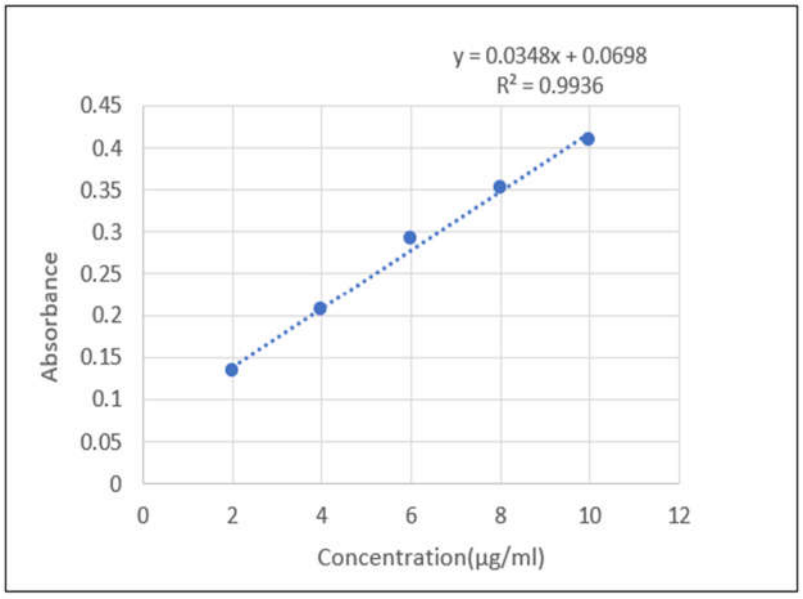


Fig 10 Calibration curve of Eszopiclone (linearity 2-10 µg/ml)

Accuracy

The accuracy estimated by determining the % recovery at three levels 80, 100, and 120%. The % recovery at each level was calculated and is shown in Tables 2 and 3.

Table 2 Accuracy studies (HPTLC)

Sr. No.	Level	Amount spotted (ng/band)	Amount spotted after standard addition (ng/band)	Total amount (ng/band)	Amount recovered	% Recovery
1.	80%	40	32	72	72.75	101%
2.	100%	40	40	80	79.95	99.9%
3.	120%	40	48	88	100.92	100.9%

Table 3 Accuracy studies (UV)

Level	Concentration of Amount of Tablet (µg/ml)	Concentration of Amount of API and excipient mixture (µg/ml)	Total concentration (µg/ml)	Amount Recovered	% Recovery
80%	4	3.2	7.2	7.27	101.05 %
100%	4	4	8	7.90	98.85 %
120%	4	4.8	8.8	8.68	98.68 %

Precision

The precision of method was expressed by repeatability and intermediate precision which was found to be as follows Tables 4.

Tables 4 Precision Studies

Sr. No.	Precision	Amount spotted (HPTLC)	Concentration (UV)	% RSD	
				HPTLC	UV
1.	Repeatability	40 ng/band	4 µg/ml	1.54	1.51
2.	Intermediate precision	40 ng/band	4 µg/ml	1.96	1.65

LOQ

The LOQ value for HPTLC and UV method was found to be 5.4 ng/band and 0.71 µg/ml respectively . The low LOD and LOQ values indicate sensitivity of the developed methods.

Robustness

The method was found to be robust as % RSD obtained from calculation of average of peak area for HPTLC and absorbance for UV was found to be within limit. Hence, method was proven to be robust see in Table 5

Table 5 Robustness

Parameter	Condition		%RSD	
			HPTLC	UV
Buffer Composition • **Potassium dihydrogen phosphate – 11.45 g** • **Disodium dihydrogen phosphate-28.8 g (± 1g)**	Potassium dihydrogen phosphate	Disodium dihydrogen phosphate	1.09	0.96
	10.45 g	27.8 g		
	12.45 g	29.8 g	1.13	1.05
Agitation Rate (100 RPM ; ±2 RPM)	98 RPM		1.86	1.45
	102 RPM		1.54	1.71
Volume of Dissolution medium (500 ml ;±5ml)	495 ml		1.02	1.06
	105 ml		1.17	1.81

Solution Stability

The samples were found to be stable and the results found were as follows Table 6**.**

Table 6 Solution Stability

Sr no	Condition	% Recovery	
		HPTLC	UV
1	Standard Solution	101.9 %	97.8 %
2	Sample solution	99.8 %	101.4 %

DISCUSSION

The development and validation of a dissolution method for Eszopiclone tablets (2mg) were successfully carried out using a systematic approach. Various dissolution media, apparatus, and rotation speeds were evaluated to establish the most discriminating conditions. Phosphate buffer pH 6.8 was chosen as the dissolution medium, considering Eszopiclone's pKa of 9.2 and its solubility characteristics. The basket apparatus at a stirring speed of 100 rpm and a collection time of 30 minutes were found to be the optimal conditions, ensuring adequate mixing and representation of the drug's release profile. The dissolution method was validated according to ICH guidelines. The validated dissolution method, coupled with HPTLC and UV spectrophotometric quantification, proved to be suitable for the analysis of Eszopiclone tablets. The method's discriminatory power allows for the evaluation of batch-to-batch quality and stability of the medicinal product. In light of the absence of an official dissolution method in pharmacopoeias and the unavailability of any documented dissolution method for Eszopiclone tablets utilizing HPLC and UV spectrophotometric techniques, this developed method fills a significant gap.

The use of the same dissolution method for the 1 mg strength Eszopiclone tablet is scientifically justified and supported by LOQ value of both methods. Both the 1 mg and 2 mg tablets are formulation-equivalent, meaning they share the same qualitative and quantitative composition and manufacturing process, differing only in drug strength. The validated dissolution method demonstrates adequate sensitivity and linearity across a range that includes the expected drug concentrations from a 1 mg dose. Furthermore, the dissolution medium (phosphate buffer pH 6.8, 500 mL) provides sufficient sink conditions for both strengths, ensuring consistent dissolution behavior. According to ICH guideline Q2(R2) and USP General Chapter <1092>,

a dissolution method may be applied across multiple strengths when the dosage forms are proportional and the method's analytical range encompasses the expected concentration levels. Therefore, no changes to the method are required when applying it to the 1 mg tablet, and only verification may be necessary to confirm performance.

The successful application of this method can ensure the quality control of Eszopiclone marketed tablets, ultimately contributing to the assurance of their efficacy and safety. . Furthermore, the developed method can be utilized for formulation development, optimization, and comparison of different Eszopiclone tablet formulations, facilitating the development of high-quality products.

STATEMENT AND DECLARATIONS:

Ethical Approval

Ethical approval is not required for this study.

Competing interests

Nil

Funding

Nil

ACKNOWLEDGEMENTS

Authors are grateful to the Principal as well as the management of the institute for providing well maintained facilities for research work. We thank Emcure Pharmaceuticals, Pune for providing the bulk drug as gift sample.

REFERENCE

1. https://go.drugbank.com/drugs/DB00402 (Accessed on: 13 January 2025)
2. United States Pharmacopeia (2024). USP Monographs, Eszopiclone. USP-NF. Rockville, MD: United States Pharmacopeia. DOI: https://doi.org/10.31003/USPNF_M5095_07_01
3. https://pubchem.ncbi.nlm.nih.gov/compound/Eszopiclone (Accessed on: 13 January 2025)
4. https://www.webmd.com/bio/kimberly-rath Lunesta on Aug 22, 2024.

5. Pasupuleti Sunitha, Shaik Muneer Pasha, Siliveru Anil kumar, Souvik Samanta, S. Soumya, Shaistha Tarannum. A Review on Dissolution Method Development and Validation. IJPAR [Internet]. 2024 Nov. 23 [cited 2025 Jun. 22];13(4):709-15.
6. Prachi Barsagade, Roshan Khetade, Kalyani Nirwan, Tikesh Agrawal, Santosh Gotafode, Review Article of Dissolution Test Method Development and Validation of Dosage Form by Using RP-HPLC, Int. J. Pharm. Sci. Rev. Res., 70(1), September - October 2021, Pages: 28-38.
7. Dissolution Testing and Acceptance Criteria for Immediate Release Solid Oral Dosage Form Drug Products Containing High Solubility Drug Substances; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2018.
8. https://www.drugs.com/eszopiclone.html.(Accessed on: 2 June 2025)
9. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=fd047b2b-05a6-4d99-95cb955f14bf329f.(Accessed on: 2 June 2025)
10. Dressman J, Kramer J. Pharmaceutical Dissolution Testing. United Kingdom: Taylor and Francis Group; 2005. p. 419-32.
11. ICH Guideline Q2(R2), Revised the ICH Q2(R2) Guideline on Validation of Analytical Procedures, 2024.
12. The Dissolution Procedure: Development and Validation. In USP 43–NF 38. The United States Pharmacopeial Convention, Inc., 2022.
13. Nissankararao S, Kallam V, Bhimavarapu R. Dissolution method development and validation: a review. Int. J. Pharm. Res. Develop. 2013;5:106-12.
14. "Eszopiclone Monograph for Professionals". Drugs.com. American Society of Health-System Pharmacists. Retrieved 6 April 2019.
15. Brahmankar, D.M. and Jaiswal, S.B. (2009) Biopharmaceutics and Pharmacokinetics. 2nd Edition, Vallabh Prakashan, Delhi, 5-75.