Int. J. Pharm. Investigation, 2025; 15(2):583-591.
https://www.jpionline.org

Original Article

Development and Validation of a UV Spectrophotometric Method for Almotriptan Malate Estimation in Bulk and Niosomal Formulation

Shweta Sulakude, Nisha Shirkoli*

Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, INDIA.

ABSTRACT

Background: Almotriptan Malate is a selective serotonin receptor agonist developed for migraine management in adults. Its bioavailability is 69.1%, with an elimination half-life of 3 hours and has low aqueous solubility. This study aims to develop a UV spectrophotometric method for Almotriptan Malate and Niosomal formulations, for determining concentration of Active Pharmaceutical Ingredient (API) in drug products. **Materials and Methods:** A combination of 50% ethanol and phosphate buffer pH 6.8 was used as a solvent system. A UV spectrophotometric method was developed, identifying Almotriptan Malate's absorption peak (λ_{max}) at 227 nm. The method was validated for precision, accuracy, and linearity using calibration curves. The limit of detection and quantification were used to determine sensitivity of the method. Precision, ruggedness and robustness were assessed using Relative Standard Deviation (RSD). Niosomes were prepared using ethanol injection, and particle size and zeta potential were measured to evaluate their self-assembly properties. **Results and Discussion:** The UV spectrophotometric method displayed a strong linear calibration curve, with regression coefficient 0.9974 confirming its reliability for Almotriptan Malate estimation. The LOD and LOQ were satisfactory and the study indicated a percentage RSD below 2% for precision analysis. Robustness tests showed that the method remained stable under different conditions. Niosomal formulations had a particle size of 148.3 nm and a zeta potential indicating strong stability. The percentage yield that was obtained by drug entrapment efficiency was 82.80 % therefore endorsing the Niosomal system as quite effective for Almotriptan Malate drug delivery. **Conclusion:** The developed UV spectrophotometric method is accurate, precise, and reproducible for the estimation of Almotriptan Malate in bulk and Niosomal formulations.

Keywords: Almotriptan Malate, UV-spectroscopic method validation, ICH guidelines.

Correspondence:
Ms. Nisha Shirkoli
Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi-590010, Karnataka, INDIA.
Email: nishashirkoli@klepharm.edu

Received: 16-10-2024;
Revised: 23-11-2024;
Accepted: 18-01-2025.

INTRODUCTION

Migraine is considered one of the most frequent and most severe types of primary headaches with significant impairment of the patient's functioning. A significant fraction of the world population is affected by the Migraine syndrome due to a stressful lifestyle. This disorder occurs in approximately 11.6% populations of the world. As compared to men, women are mostly affected by this disease (Lade *et al*., 2022). Almotriptan Malate is one of the many Triptans-a group of drugs that are usually recommended for the acute management of migraines. Migraine is thought to be a disease of the serotonin receptors in the brain and these drugs affect these receptors to help provide relief (Kalra and Elliott, 2007).

Almotriptan Malate (Figure 1) is a selective 5-Hydroxytryptamine (5-HT) receptor agonist used in the acute treatment of migraine. Several analytical methods have been described for the estimation of Almotriptan in pharmaceutical dosage forms and biological fluids employing LC-MS/MS (Ravikumar *et al*., 2012). Almotriptan is the first FDA-approved drug to treat migraine in adults with or without an aura. Almotriptan Malate is characterized by a short half-life and up to 3 hr; The bioavailability of the oral form amounted to 69.1%. The currently available oral delivery system of Almotriptan Malate has limited bioavailability because of poor aqueous solubility (Lade *et al*., 2022).

Niosomes are also known as Non-ionic Surfactant Vesicles (NSV) is nanoscale-sized vesicles that have an aqueous-filled core and one or several lipid layers (Makeshwar and Wasankar, 2013). The advantages of niosomes include very low toxicity, stable chemical

DOI: 10.5530/ijpi.20250153

structures, biocompatibility, ability to encapsulate hydrophilic or lipophilic drugs, and low cost of this formulation (Patel *et al.*, 2014). Poloxamer 407 is commonly used to prepare thermosensitive *in situ* gel to increase the viscosity and mucoadhesiveness of the formulations. In some investigations, temperature-sensitive gelling systems have been employed for the nasal administration of drugs. This *in situ* gel converts gel at systemic temperature (31-33ºC) and remains in liquid condition at room temperature (25±0.56) (Ravikrishna and Krishnaveni, 2019).

The International Council on Harmonization (ICH) requirements for analytical method validation are followed by this method, which seeks to be dependable, quick, and economical. In the present study, we report the development and validation of a UV-visible spectrophotometric method for the estimation of Almotriptan malate in bulk and AM-loaded Niosomes (Suneetha *et al.*, 2012). The comparison studies were done with the previous studies, and some limitations were found and reported in Table 1.

The validation parameters as per ICH guidelines are Accuracy, Precision, Repeatability, Intermediate Precision, Reproducibility, Limit of Detection (LOD), Limit of Quantitation (LOQ), Range and Linearity, and Solvent Stability (Sharma *et al.*, 2018).

MATERIALS AND METHODS

Drug sample

Almotriptan Malate (pure Drug) was procured from MSN Laboratories Ltd., Pvt. Hyderabad.

Reagents and chemicals

The experiment's analytical-grade chemicals and reagents were obtained from the KLE College of Pharmacy's storage facility in Belagavi. Potassium dihydrogen phosphate, sodium dihydrogen phosphate, and ethanol of HPLC grade were used.

Instruments and Apparatus

The Shimadzu UV/visible spectrophotometer, model 1800, equipped with UV-Probe software, was used in conjunction with 10 mm Quartz cuvettes and automated wavelength adjustment. Before use, pipettes, volumetric flasks, and measuring cylinders used in the laboratory were calibrated.

Method Development

The first step considered while developing the UV spectrophotometric technique included: the selection of the appropriate solvent system and determination of the maximum absorbance wavelength (λ_{max}). After a literature review in the area of solubility, Almotriptan malate solubility studies were conducted in a real-life environment. Numerous experiments in a range of solvents (methanol, water, ethanol, Phosphate buffer pH 6.4, and Phosphate buffer 6.8) were conducted to choose the mobile phase. Finally, a 50:50% v/v mixture of solvents including ethanol and phosphate buffer pH 6.8 was determined. Solutions containing analytes had their UV spectra examined in the 200-400 nm range.

Method Validation

The analytical technique was tested for performance validation by assessing its linearity, accuracy, precision, robustness solvent stability, specificity, and ruggedness under the ICH criteria for analytical method validation. The method's appropriateness was confirmed by using ICH guidelines Q2A and Q2B 12 (ICH guidance Q2a; ICH guidance Q2b).

Preparation of Solvent System

Mobile Phase

50 mL of precisely measured ethanol was dissolved in 50 mL of freshly prepared phosphate buffer pH 6.8.

Standard Stock Solution

The 10 mL volumetric flask containing 10 mg of Almotriptan malate was used to prepare the standard stock solution. Almotriptan Malate was weighed and combined with 50% v/v ethanol: 50%v/v phosphate buffer pH 6.8 to develop a stock solution that contained 1000 µg/ mL.

Working stock solutions

From the 0.1 mL Almotriptan Malate standard stock solution, 1 mL was pipette into 10 mLvolumetric flask, the volume was made up with mobile phase before the analysis. Almotriptan Malate concentrations ranging from 0.2 to 1 mL were taken out and pipetted into identical 10 mL volumetric flasks. Using the same mobile phase, the volume was adjusted to the appropriate level.

Selection of wavelength

Almotriptan Malate was tested at 400-200 nm using 10 µg/mL of the corresponding stock solution as ethanol: pH 6.8 phosphate buffer blank solutions (50:50% v/v).

Linearity and range

The Almotriptan Malate was mixed in varied amounts (0.2-1 mL for Almotriptan Malate) to achieve different concentrations (1-5 µg/mL) and then scanned. To accommodate for variability, this process was carried out three times, statistical analysis (regression) was used to see whether changes in Almotriptan Malate concentration could be used to predict changes in absorbance (Mishra and Mundada, 2021).

Limit of Detection and Limit of Quantification

The ICH defines the Quantitation Limit (QL), also referred to as the limit of quantification, as LOQ=10σ/S, as well as the detection limit (DL or LOD) as LOQ=3.3σ/S. S stands for the calibration

Table 1: Comparative study of published articles on the UV method.

Journal name	Title	Limitations
International Journal of Medicinal Chemistry and Analysis.	Spectrophotometric estimation of Almotriptan malate in bulk and pharmaceutical formulations by multivariate technique (Prabhavthi *et al*., 2016).	The paper mentions that there is no interference observed from excipients of the formulation, but it is not clear about the excipients that have been tested or what conditions the validation of this section of the method has been done. The phenomenon of long-term stability of the analyzed samples is not considered in the study.
World Journal of Pharmaceutical Research.	A simple spectrophotometric assay of Almotriptan malate in bulk and pharmaceutical formulations (Prasad *et al*., 2012).	The authors do not specify what range of concentrations the method is effective and could therefore be an issue for medicines that have varying levels of Almotriptan malate. However, the employing of chloroform in the method may take some precautionary issues for safety and environmental concerns hence not applicable to some laboratories.
International Journal of Scientific and Technology Research	Visible spectrophotometric analysis of Almotriptan Malate in bulk and formulations (Kumari *et al*.,2012).	Previous methods were found to be both tedious and less specific, highlighting the necessity for improvement in the analytical techniques employed. Conventional visible spectrophotometric methods used in the determination of Almotriptan malate may still cause problems in terms of specificity.
International Journal Chemical Sciences.	Spectrophotometric determination of a Almotriptan Malate in pharmaceutical dosage forms (Jesus *et al*., 2020).	The research did not provide adequate information on the conditions studied for the formation of colored complexes and might cause some difficulties in the reproduction of the results. The research did not attempt to establish the efficiency of the method or the stability of the coloured complex.

curve's slope, and σ for the response's standard deviation (Sanchez, 2018; Srinivas *et al*., 2021).

Precision

The study developed the interday and intraday precision of the Almotriptan Malate by executing 3 replicates of concentrations: 0.2 mL, 0.6 mL, and 1 mL of Almotriptan Malate at the 227 λ_{max}. Almotriptan Malate percentage RSD values were developed (Patel *et al*., 2023).

Ruggedness

Ruggedness is the ability of the approach to tolerate both intentional and inadvertent changes. Triplicate measurements were taken at three distinct concentrations using two separate UV equipment (Gaikwad *et al*., 2023).

Robustness

To evaluate the robustness (reliability) of the UV spectrophotometry approach, two distinct wavelengths of 227 nm±2 nm were used to analyze the drug. This produced equations for multiple regression and their associated coefficients, which were useful in assessing how well the procedure worked in slightly different circumstances (Friedrich *et al*., 2009).

Accuracy (recovery studies)

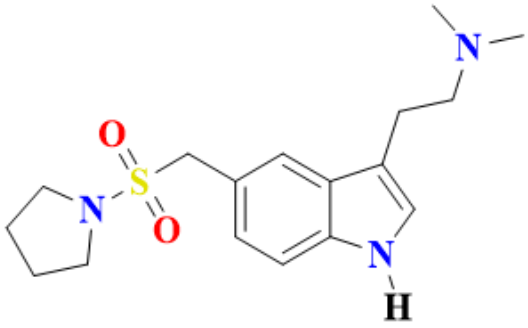
A recovery study was carried out to evaluate the accuracy of the method. This was developed by pre-analysing a sample and then adding standard Almotriptan Malate solution at three distinct concentration levels (50%, 100%, and 150%) (Jain *et al*., 2011).

Solvent and standard stock solution stability

For three days (72 hr), the solutions were stored in unstable circumstances to test the experiment's stability. Following this time, they assessed if the solutions had not changed (Uday *et al*., 2021).

Formulation of Niosomes

Almotriptan Malate-loaded Niosomes were prepared by an ethanol injection method. Emulsifier, Lipid, and Almotriptan Malate were accurately weighed and dissolved into 10mL of solvent using bath sonication at 60°C. At the same temperature, the clear organic solution was very rapidly poured into the solution of the aqueous phase and the mixture was vigorously stirred at 500-650 rpm by the Teflon-coated bead in a Remi magnetic stirrer. The liquids turned into a milky solution as a confirmation that Niosomes were being formed within the solution. To evaporate the solvent the solution was allowed to evaporate while stirring for 30 min at room temperature, and the samples were placed in vacuum for 10-15 min. Last the



N,N-dimethyl-2-(5-((pyrrolidin-1-ylsulfonyl)methyl)-1*H*-indol-3-yl)ethan-1-amine

Figure 1: Structure of Almotriptan Malate.

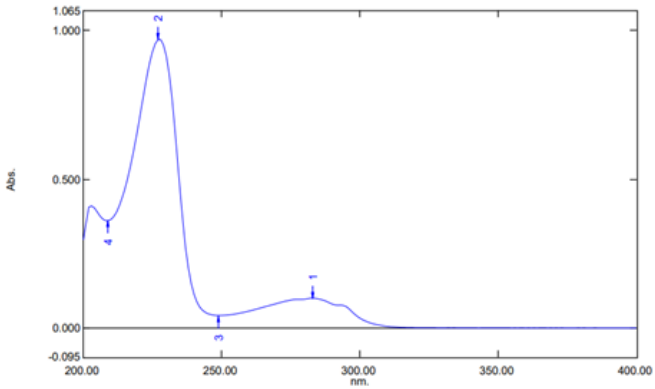


Figure 2: UV-spectrum of Almotriptan Malate.

volume of the Niosomal dispersion was, further diluted up to 30 mL by using water. The obtained Niosomal dispersion was then sonicated under a probe sonicator and filtered through 2-20 m filter out to have the homogenized size distribution and proper vesicle closure (Katrolia *et al*., 2019; Estupiñan *et al*., 2020).

Characterization of Niosomal Dispersion

Drug-loaded Niosomal Dispersion was diluted with distilled water to achieve appropriate concentrations for analysis. Dynamic Light Scattering (DLS) using a Malvern Zeta Sizer instrument was employed to determine the Polydispersity Index (PDI) and zeta potential of the drug-loaded Niosomal Dispersion. All measurements were made in triplicate to minimize the probability of data variability.

Separation of Unentrapped Drug from Niosomes

A volume of 2.5 mL of drug-loaded Niosomal dispersion was subjected to centrifugation to isolate the unencapsulated drug fraction. This process was performed using a KUBOTA-7000 high-capacity refrigerated centrifuge (Japan) at 15,000 rpm for 30 m at 4°C. Following centrifugation, 0.1 mL of the supernatant, containing the free (unentrapped) drug, was collected, and subsequently diluted in 10 mL of Milli-Q water. The diluted supernatant was filtered through a Millex® Syringe Filter (PTFE) to eliminate Niosomal particles.

Table 2: **Method development parameters.**

Sl. No.	Parameters	Specifications
1	Analytes	Almotriptan Malate
2	Solvent	Ethanol: Phosphate buffer pH 6.8 (50:50% v/v)
3	Maximum absorbance of Almotriptan Malate	227nm

Table 3: **LOD and LOQ Data.**

LOD and LOQ Data	
Analytes	**Almotriptan Malate Concentration (µg/mL)**
LOD	1.12 µg/mL
LOQ	3.42 µg/mL

Quantification of Encapsulated Drug UV Spectrophotometrically

The Almotriptan Malate-loaded Niosomal dispersion sample was again centrifuged using a High-Speed Refrigerated Centrifuge (Floor Model, 7000 Kubota, Japan) at 19,000 rpm for 60 min. Almotriptan Malate content in the Niosomes was calculated based on the absorbance of the appropriately diluted supernatant at 227 nm using UV/visible spectroscopy.

The % EE was calculated to be;

$$\%EE = \frac{\text{Total entrapped drug} - \text{Amount of the drug in supernatant}}{\text{Total entrapped drug}} X100$$

RESULTS

Method development

To perform estimation, the analyte must dissolve in a solvent. Method development parameters are illustrated in Table 2.

Method Validation

Almotriptan Malate showed maximum absorption at 227 nm, respectively shown in Figure 2.

Linearity

The experiment showed a linear relationship between Almotriptan Malate concentration and absorbance of 0.2-1 µg/mL (Figure 3). This was valid at the examined wavelength of 227 nm. Strong correlation coefficients (R^2) were found to support the outcome, with Almotriptan malate having an R^2 of 0.9974.

LOD and LOQ

The study assessed the Limit of Detection (LOD) and Limit of Quantitation (LOQ), two crucial criteria for our analytical approach. The lowest amount of drug (analyte) that we can accurately measure but still reliably detect is known as the limit of detection, or LOD. On the other hand, LOQ is defined as the limit below which the presence of the medication cannot be confirmed accurately and measured. Table 3 contains the precise LOD and LOQ values.

Table 4: System precision data for Almotriptan Malate.

Concentration (µg/mL)	Absorbance (Average of three replicates)
0.6	0.611
0.6	0.619
0.6	0.612
0.6	0.612
0.6	0.613
0.6	0.611
STD Dev	0.003
Average	0.613
%RSD	0.494

Precision

The percent Relative Standard Deviation (%RSD), estimated for three replicates at three distinct concentrations for Almotriptan Malate, consistently was found below 2%, indicating that the analysis procedure was accurate. Tables 4-6 give the detailed results.

Ruggedness

The %RSD for Almotriptan Malate was measured using various analysts and instruments to evaluate the robustness of the method. All replicates' %RSD values stayed below 2%, demonstrating the method's reliability and repeatability. Table 7 provides more information on these results.

Robustness

The analysis procedure was slightly altered to evaluate robustness. All of the %RSD values that were determined using the regression coefficients (the resultant data) were less than 2%. These outcomes, shown in Tables 8 and 9 show that little modifications do not affect the procedure.

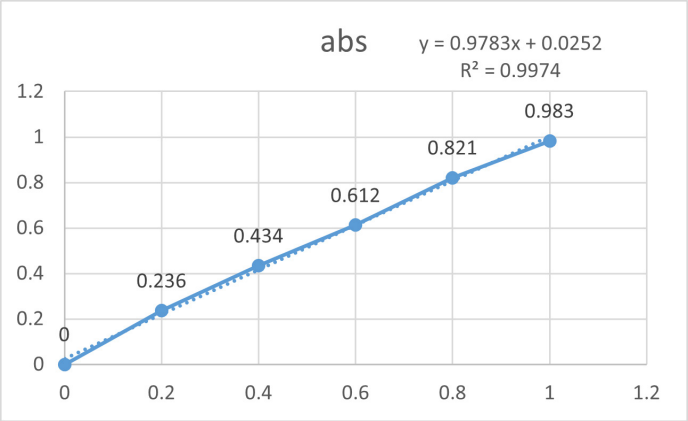


Figure 3: Calibration Curve.

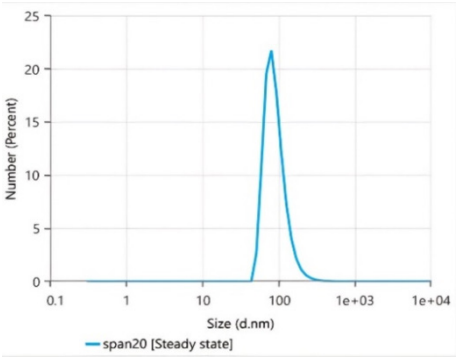


Figure 4A: Particle size.

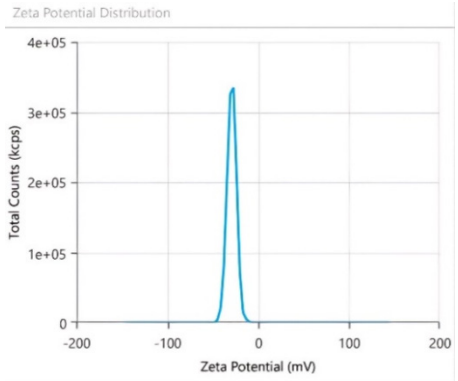


Figure 4B: Zeta potential.

Table 5: Almotriptan Malate Interday Precision data.

Concentration (µg/mL)	Almotriptan Malate (227 nm)		
	0^{th} hr	1^{st} hr	5^{th} hr
0.2	0.235	0.236	0.232
%RSD	0.888	0.651	0.863
0.6	0.612	0.619	0.615
%RSD	0.570	0.161	0.324
1	0.983	0.98	0.985
%RSD	0.256	0.410	0.203

Table 6: Intraday Precision data for Almotriptan Malate.

Concentration(µg/mL)	Almotriptan Malate (227 nm)		
	Day 1	Day 2	Day 3
Almotriptan Malate	Absorbance	Absorbance	Absorbance
0.2	0.236	0.235	0.232
%RSD	0.888	0.843	0.888
0.6	0.612	0.615	0.619
%RSD	0.570	0.337	0.487
1	0.988	0.985	0.990
%RSD	0.254	0.309	0.266

Table 7: Ruggedness data of Almotriptan Malate.

Concentration (µg/mL)	Almotriptan Malate (227 nm)	
	Change in Analyst	Change in Instrument
Almotriptan Malate	Absorbance	Absorbance
0.2	0.232	0.233
%RSD	0.893	0.851
0.6	0.62	0.619
%RSD	0.817	0.404
1	0.99	0.989
%RSD	0.254	0.154

Accuracy (recovery studies)

Almotriptan Malate Samples were spiked and recovery was measured to test accuracy. Almotriptan Malate recovered exceptionally well (93.3-96.7%); see Table 10.

Solvent and standard stock solution stability

To prove that the working solution remains stable during analysis and does not deteriorate in the diluent media, utilizing regression coefficients, the %RSD values were all less than 2%. Table 11 results show that the stock solutions have undergone only minor alterations, indicating that they have remained stable throughout the study.

Characterization of Niosomal Dispersion

The particle size distribution was found at 148.3 (Figure 4A) nm indicating a desirable size range for the Niosomes. A Polydispersity Index (PDI) of 0.207, close to 1, suggested a uniform particle size distribution. The zeta potential of -29.07 mV (Figure 4B) showed good stability.

Spectrophotometric Analysis of Niosomes

Analysis of the final Niosomal dispersion revealed high Entrapment Efficiencies (EE) of 82.8% for Almotriptan Malate respectively. These values suggest the desirable entrapment efficiency of a major portion of the drug within the Niosomes.

DISCUSSION

The absorptivity of the drugs remained consistent across a concentration range of 0.2-1 µg/mL for Almotriptan Malate. This behaviour complies with beer-lambert's law where there should be a linear response which means that the process used can be trusted to analyze the drug within the ranges being tested. Very high R^2 values specifically indicate that there exists a very good linear relationship between concentration and absorbance spot for the drug. From the results, it can be concluded, that LOQ is higher than LOD because it demands greater sensitivity and precision from the analysis. The test was good in terms of accuracy since the results were similar for all degrees of the drug being analysed. The robustness of the method can be observed

Table 8: Robustness data of Almotriptan Malate.

Change in wavelength			
Concentration (µg/mL)	Almotriptan Malate (225 nm)	Almotriptan Malate(227 nm)	Almotriptan Malate(229 nm)
Almotriptan Malate	Absorbance	Absorbance	Absorbance
0.2	0.221	0.236	0.231
%RSD	0.896	0.888	0.858
0.6	0.609	0.612	0.619
%RSD	0.510	0.570	0.570
1	0.978	0.988	0.989
%RSD	0.256	0.254	0.210

Table 9: Robustness (Change in Mobile phase).

Concentration	Methanol: Buffer (52:48)	Methanol: Buffer (48:52)
0.6	0.622	0.615
%RSD	0.729	0.53106

Table 10: Accuracy data of Almotriptan Malate.

Level	Concentration (mg/L)	Absorbance	Mean Absorbance	Measured Concentration (mg/L)	%Recovery
50%	0.3	0.562	0.559	0.28	93.3%
		0.554			
		0.561			
100%	0.6	0.745	0.740	0.58	96.7%
		0.735			
		0.740			
150%	0.9	0.910	0.909	0.85	94.4%
		0.915			
		0.901			

Table 11: Solution stability (*n=3).

Concentration (µg/mL)	Almotriptan Malate (227 nm)	
	Old	Fresh
	Absorbance	Absorbance
0.2	0.235	0.231
%RSD	0.888	0.893
0.6	0.612	0.61
%RSD	0.570	0.817
1	0.983	0.985
%RSD	0.256	0.254

from the fact that it gives fairly comparable results for somewhat different conditions in the laboratory or analyses. The Results at different Almotriptan Malate concentration levels showed %RSD values below 2% showing that the present method is precise and reproducible. The robustness of a method is indicated by its reliability in routine use. This entailed consciously using variation in the analysis method and comparing the resultant effect. The method's resistance to minor changes (as shown in the results) makes it reliable for routine analysis. The accuracy of the method, or how closely it reflects the true amount of drug present, was evaluated through a recovery study. Solution stability studies ensured that the working solution was not degraded hence the results of the final assay were not tainted by any false results. This stability is so important in order not to affect the outcome. Niosomes efficiently captured a large portion of the drugs: 59.8 for Almotriptan Malate (high entrapment efficiency). This finding suggests a promising approach for targeted delivery applications, where the drugs can be delivered to specific sites within the body.

CONCLUSION

The method for the simultaneous estimation of Almotriptan Malate was developed and validated according to ICH guidelines. The statistical analysis results confirmed the simplicity, specificity, selectivity, linearity, precision, ruggedness, robustness, Accuracy (recovery studies), Solvent and standard stock solution stability, Characterization of Niosomal Dispersion, and reproducibility of the developed method for simultaneous estimating Almotriptan Malate in bulk and AM-loaded Niosomes.

The ICH recommendations were followed in the development and validation of a technique for the simultaneous determination of Almotriptan Malate. The statistical analysis results demonstrated that the existing approach is straightforward, robust, reproducible, specific, linear, and selective for the simultaneous quantification of Almotriptan malate in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and management of KLE College of Pharmacy, Belagavi for providing the necessary facilities to conduct the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

API: Active Pharmaceutical Ingredient; **UV:** Ultra-violet; **ICH:** International Council on Harmonisation; **LC:** Liquid Chromatography; **MS:** Mass Spectrometry; **MS/MS:** Tandem Mass Spectrometry; **HPLC:** High-Performance Liquid Chromatography; **NSV:** Non-ionic surfactant vesicles.

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Cite this article: Sulakude S,Shirkoli N. Development and Validation of a UV Spectrophotometric Method for Almotriptan Malate Estimation in Bulk and Niosomal Formulation. Int. J. Pharm. Investigation. 2025;15(2):583-91.