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Development Of Validated Hptlc Method for Determination of Eletriptan Hydrobromide as Bulk Drug and In Tablet Dosage Form

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ABSTRACT

The aim of the present study was to develop a highly precise and accurate HPTLC method for the estimation of eletriptan hydrobromide in pharmaceutical formulations. The method was systematically validated according to International Council for Harmonisation (ICH) guidelines, and various validation parameters, including accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), recovery studies, and linearity range, were thoroughly evaluated. The developed HPTLC method demonstrated simplicity, rapid execution, robustness, and high precision, making it suitable for routine quality control analysis of eletriptan hydrobromide in both bulk drug and pharmaceutical combinations. The method exhibited excellent linearity in the concentration range of 100–500 µg/mL, with a correlation coefficient (r^2) of 0.995, indicating a strong linear relationship between peak area and drug concentration. Accuracy studies showed satisfactory percent recoveries, confirming the method's reliability. Precision was demonstrated through repeatability and intermediate precision studies, with low relative standard deviation values. The LOD and LOQ were found to be within acceptable limits, signifying the method's sensitivity for detecting and quantifying low levels of the drug. Recovery studies further confirmed the absence of interference from excipients present in the pharmaceutical formulations. Overall, the proposed HPTLC method is simple, fast, cost-effective, and highly reliable. It can be successfully applied for the routine analysis and quality assessment of eletriptan hydrobromide in various dosage forms, ensuring the drug's consistency, stability, and compliance with regulatory standards over its shelf life.

Keywords: *Eletriptan HBr, HPTLC Method, Validation, Accuracy, Precision, LOD, LOQ*

1. INTRODUCTION

Eletriptan hydrobromide 1 is a 5- Hydroxytryptamine1B/1D receptor agonist. Eletriptan binds with high affinity to 5-HT1B, 5-HT1D and 5-HT1F receptors, has modest affinity for 5-HT1A, 5-HT1E, 5-HT2B and 5-HT7 receptors. Eletriptan is chemically designated as (R)-3-[(l-Methyl-2- pyrrolidinyl)methyl]-5-[2- (phenylsulfonyl)ethyl]

lHindolemonohydrobromide. Literature survey has revealed the availability of few spectrophotometric and HPLC methods but no spectrophotometric methods were proposed by using the combination of ethanol and distilled water as solvent. Hence the present work has been carried out.

Although a HPLC method is reported for the simultaneous estimation of the above components, it is either tedious or time consuming method. Nowadays HPTLC is becoming a routine analytical technique because of its advantages of low operating cost, high sample throughput, simplicity, and speed, the need for minimum sample clean up, reproducibility, accuracy, reliability, and robustness. The literature survey revealed that there is no method available yet to simultaneously estimate psoralen, bakuchicin and bakuchiol by HPTLC. In light of these all observations it was decided to develop a validated HPTLC method for simultaneous estimation of psoralen, bakuchicin and bakuchiol. The method was applied to study the effect of

pH and GI enzymes on these three components in simulated gastro- intestinal fluids. In addition the method was successfully applied for the standardization of mono and polyherbal formulations containing *Psoralea corylifolia* Linn.

2. MATERIALS & METHODS

2.1. Reagents & chemicals

Analytically pure standard eletriptan hydrobromide was obtained as a gift sample from SMS Pharmaceuticals, Hyderabad, India. The pharmaceutical dosage form used in this study was relpax tablets labelled to contain 20 mg eletriptan hydrobromide was procured from local pharmacy. Toluene, methanol and acetic acid (all AR grade) were obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

2.2. Instrumentation

A HPTLC (Camag) system including Camag Linomat V sample applicator, Hamilton syringe (100 µL), Camag TLC Scanner-3 with win CAT software version 1.4.2, Camag twin- trough chamber (10×10 cm) (CAMAG, Muttenz, Switzerland) were used for the present work.

2.3 Method of estimation

2.3.1 Selection of solvent

The solubility was checked by dissolving the drug in different organic solvents and drug was found to be freely soluble in methanol. Methanol of analytical grade was used.

2.3.2 Selection of detection wavelength

It was selected by recording UV spectra of stock solution of drug prepared in 10 mL of methanol and Eletriptan showed maximum absorbance at 224 nm which is selected for the detection.

2.3.3 Preparation of standard stock solution

Accurately weighed 20 mg drug was dissolved in 10 mL solvent to have 2 mg mL^{-1} concentration which was properly diluted with methanol to obtain 100 ng μL^{-1} final concentration.

2.3.4 Sample solution preparation

Twenty tablets were accurately weighed and then finely powdered. Powder quantity equivalent to 20 mg was taken and shifted to a 10 mL flask consisting 5 mL methanol. The content was sonicated for 15 min and filtered. The volume was adjusted up to the mark with methanol to attain the finishing concentration 2000 ng μL^{-1} which was further diluted by methanol to obtain 100 ng μL^{-1} as final concentration. Two µL volume of this solution was applied on TLC plate to get final sample concentration of 200 ng $band^{-1}$. Peak areas of the bands were measured at 224 nm after chromatographic development.

2.3.5 Chromatographic conditions:

Eletriptan hydrobromide solutions were applied to the plates by means of a Camag (Muttenz, Switzerland) Linomat V automated spray-on band applicator equipped with a 100.00-µL syringe (Hamilton) and operated with the settings of band length 6 mm, application rate 10 s µL−1, distance between bands 12 mm, distance from the plate edge 10 mm, and distance from the bottom of the plate 10 mm. Plates were developed to 8 cm beyond the origin with Toluene: methanol: acetic acid (8.5: 1: 0.5, v/v/v) as mobile phase in twin trough glass chambers after saturation of the chamber with mobile phase vapour for 30 min (the optimum chamber-saturation time) at room temperature. After development, mobile phase was evaporated from the plate by use of an air-dryer for 10 min. Densitometric scanning was then performed in absorbance mode at 224 nm using the deuterium lamp as the source of radiation. The drug was resolved adequately with Rf 0.62 ± 0.003. A Camag model III TLC scanner linked with CATS (V 3.5, Camag) integration software was used. The slit dimensions were 5 mm × 0.45 mm and the scanning speed 10 mm s^{-1}. Peak area and concentration data were treated by linear least-squares regression analysis. The amount of Eletriptan was determined from the respective calibration plots obtained by plotting the concentration of standard against peak area.

2.4 Validation of the method

The proposed method was validated for specificity, linearity, precision, accuracy and robustness following the ICH guidelines [30, 31, 32].

2.4.1 Linearity, LOD and LOQ

Linearity was determined by construction of calibration plots and linear least-squares regression analysis as described above. The limit of detection (LOD) and limit of quantification (LOQ) were determined by diluting known concentrations of Eletriptan solution until the average responses were approximately three (For LOD) or ten times (for LOQ) the responses of the blank. Six replicate determinations were performed using methanol alone as blank.

2.4.2 Precision

Precision study was carried out for the repeatability of sample application and measurement and the result was expressed as % RSD of peak areas. Variability of the method was studied by analysing aliquots of standard solutions of Eletriptan (10, 20, 30, 40, 50 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision), and the results were expressed as % RSD.

2.4.3. Robustness

Robustness of the method was checked by making intentional changes in the parameters. Small change in the mobile phase composition was tried (± 1 ml methanol). The amount of mobile phase and temperature were varied in the range of ± 5%. The plates were prewashed with methanol and activated at 60°C ± 5 for 5, 10, 15 min respectively prior to chromatography.

2.4.4. Accuracy

The accuracy of the method was tested by performing recovery studies at three levels (80, 100, and 120% standard addition). The amount of eletriptan present in the formulation was determined from the regression equation. Known amount of the standard was added at three levels and recovery was found. The percent recovery as well as the average percent recovery was calculated.

3. RESULTS AND DISCUSSION

3.1. Optimization of chromatographic conditions

A solvent system that would give dense and compact spot with appropriate and significant Rf value was desired for quantification of Eletriptan in pharmaceutical formulations. A variety of solvent systems like chloroform-methanol, chloroform-ethanol, chloroform-toluene, chloroform-benzene-toluene, and ethyl acetate-methanol were attempted for separating and resolving spot of Eletriptan. Eventually, combination of Toluene: methanol: acetic acid (8.5: 1: 0.5, v/v/v) probably resolved spot of drug with better peak shape. The drug was resolved adequately with Rf 0.62 ± 0.003. (Fig. 1)

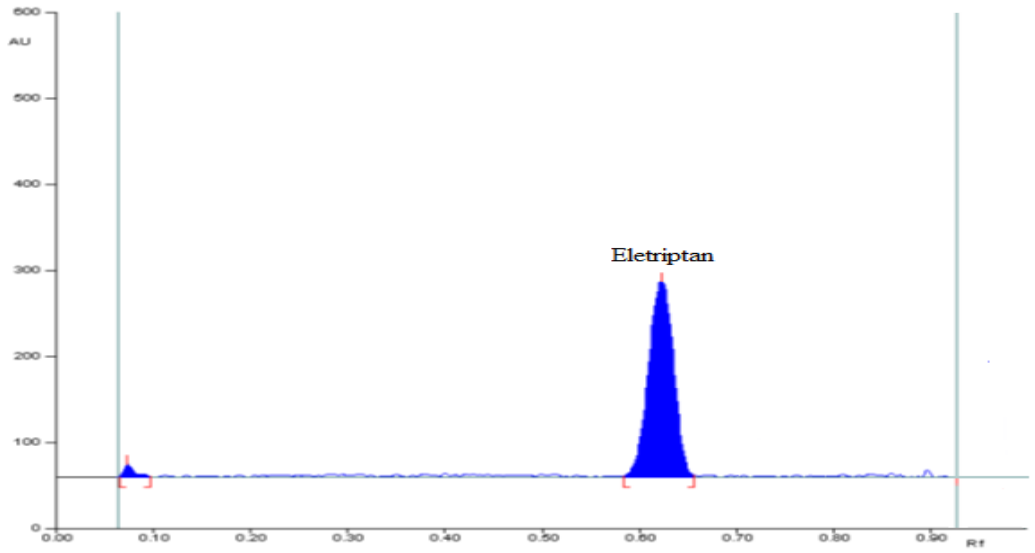

Fig. 1 Densitogram for Eletriptan reference standard (300 ng band^{-1}, Rf = 0.62)

3.2. Validation of the method

Preparation of calibration curve

For preparation of a calibration plot, volumes 1, 2, 3, 4 and 5 µL of standard solution of Eletriptan (100 ng µL-1) were spotted onto the TLC plates. The developed method was found to be linear in the concentration range 100-500 ng band-1 with high correlation coefficient. The linear regression equation was found to be y = 17.283x + 134.77 having correlation coefficient 0.995. The 3D spectra obtained for linearity in the concentration range 100-500 ng/band is represented in following figure 2.

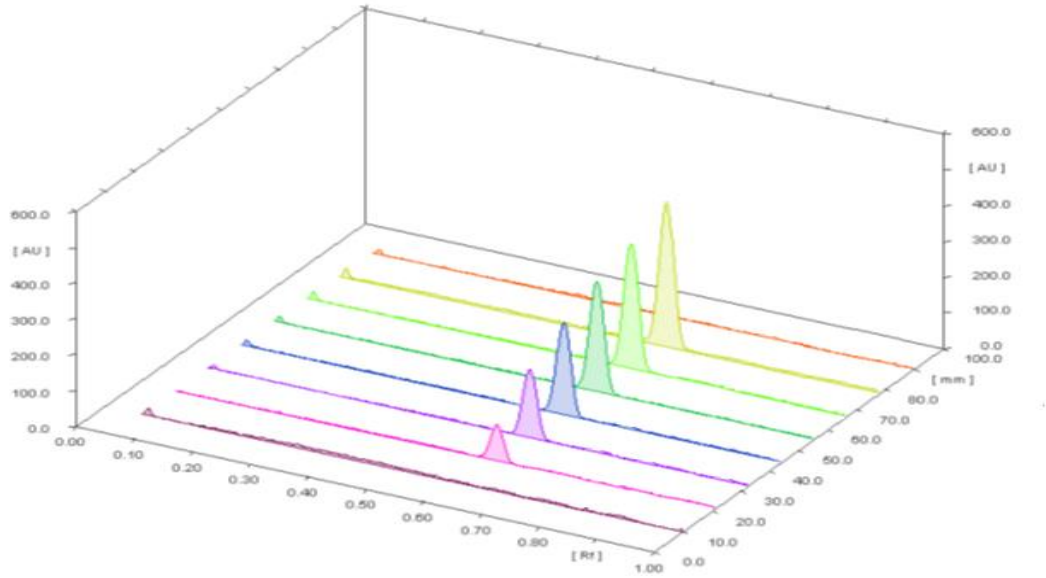

Fig. 2 3D spectra obtained for linearity in the concentration range 100-500 ng/band

Precision

Set of three different concentrations in three replicates of standard solutions of Eletriptan were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.82 to 1.04. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.80 to 1.09. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 11.45 ng band^{-1} and 34.70 ng band^{-1}, respectively.

Recovery studies

Recovery studies were carried out by adding standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 200 ng band^{-1} from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drug in capsule dosage form. The results obtained are shown in below Table 1.

Table 1: Recovery study of Eletriptan

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Amount found (ng band^{-1})	% Recovery±R.S.D.
Eletriptan	200	160	360.67	100.18±0.81
	200	200	399.69	99.91±0.67
	200	240	443.58	100.81±0.70

*Average of three determinations

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition (± methanol), wavelength (± 1 nm) was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

The overall summary of validation parameters is given below:

Table 2: Summary of validation parameters

Sr. No.	Validation parameters	Results
1.	Linearity range (ng band^{-1})	100-500
2.	Correlation coefficient (r)	0.995
3.	Limit of detection (ng band^{-1})	11.45
4.	Limit of quantitation (ng band^{-1})	34.70
5.	Accuracy	100.30 ± 0.72
6.	Precision (% R.S.D.)	
	Intraday precision	0.82-1.04
	Inter day precision	0.80-1.09
7.	Robustness	Robust
8.	Specificity	Specific

4. CONCLUSIONS

We established a HPTLC method for the estimation of Eletriptan. The proposed method was found to be suitable for estimation of eletriptan in formulations as it is proved to be precise, reproducible, reliable, accurate and robust..

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