Research Article

TLC-Densitometric Method for Estimation of Eletriptan Hydrobromide in Bulk and Pharmaceutical Dosage Forms

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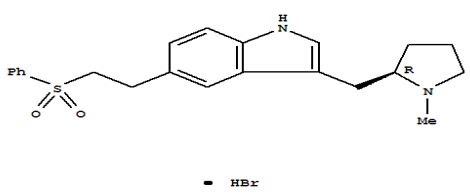
ABSTRACT

A new, simple, precise and accurate high performance thin layer chromatographic method has been proposed for the determination of eletriptan hydrobromide in a tablet dosage form. The drug was separated on aluminum plates precoated with silica gel 60 F_{254} with dichloromethane: acetone: aceticacid (3:2:0.2 v/v/v) was used as mobilephase. Quantitative analysis was performed by densitometric scanning at 230 nm. The method was validated for linearity, accuracy, precision and robustness. The calibration plot was linear over the ranges 200-700 ng/band for eletriptan hydrobromide. The method was successfully applied to the analysis of drug in a pharmaceutical dosage form.

Keywords: High-performance thin-layer chromatography, eletriptan hydrobromide, tablets.

INTRODUCTION

Eletriptan hydrobromide[1], a selective 5-hydroxytryptamine $1_b/1_d$ (5-ht_{1b}) receptor agonist, is chemically designated as (*R*)-3-[(-1-methylpyrrolidin-2-yl)methyl]-5-(2-phenyl sulfonylethyl)-1H-indole, whose empirical formula is $C_{22}H_{26}N_2O_2S.HBr$, with a molecular weight of 463.40. Eletriptan hydrobromide is a white to light pale colored powder which is soluble in water. Eletriptan hydrobromide is used to treat severe migraine headaches. Eletriptan hydrobromide is available in market as conventional tablets (Relpax and Relert). Till now, a few procedures based on liquid chromatography have been reported for the quantitative determination and forced degradative studies of eletriptan in biologicalfluids and pharmaceutical dosage forms and by LC-MS[2], HPLC[3,4] and Spectrophotometric methods[5,6] respectively. Literature survey reveals that no HPTLC method for determination of eletriptan hydrobromide in bulk and pharmaceutical dosage forms is reported. So it is felt worthwhile to develop a high performance thin layer chromatography method.



Structure of Eletriptan HBr

This paper describes a simple, accurate, precise, and sensitive HPTLC method for determination of eletriptan hydrobromide in bulk and pharmaceutical dosage forms. The proposed method was optimized and validated in accordance with guidelines suggested through International Conference on Harmonization[7].

EXPERIMENTAL

Materials and instruments

Methanol, butanol, toluene, ethyl acetate, acetic acid, hexane, acetone and dichloromethane (all are of Analytical Reagent grade) were obtained from Sisco Research Laboratories, Mumbai, India. Standard bulk drug sample of eletriptan hydrobromide (99.98% pure) was obtained as a gift sample from SMS Pharmaceuticals, Hyderabad, India. The pharmaceutical dosage form used in this study was relpax tablets with a declared content of 20 mg eletriptan hydrobromide (Relpax, USA)

Mobile phase

Mobile phase was prepared by taking dichloromethane-acetone-acetic acid in the ratio of 3:2:0.2 (v/v) then filters through 0.45µ nylon membrane filter.

Standard solution of eletriptan hydrobromide

Accurately weighed 10mg of Eletriptan HBr pure drug and transferred to 100ml volumetric flask. It was dissolved in sufficient quantity of methanol and the volume was made up with

the same solvent (Working Standard). This solution contains 0.1mg/ml of Eletriptan hydrobromide.

Procedure for recording chromatograms

A standard stock solution of eletriptan hydrobromide was prepared by dissolving 10 mg drug in 100 ml methanol to furnish a concentration of 100 µg/ml. Chromatography was performed on 10 cm × 10 cm aluminum plates precoated with 250-µm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Before use the plates were prewashed with methanol and activated at 110^{o} C for 5 min. Samples were applied to the plates as bands 6 mm wide and 10mm apart by means of a Camag (Switzerland) Linomat V sample applicator equipped with a 100 µl syringe (Hamilton, Bonaduz, Switzerland). Linear ascending development was performed in a 10 cm × 10 cm twin trough glass chamber (Camag), with dichloromethane-acetone-acetic acid in the ratio of 3:2:0.2 (v/v/v) as mobile phase and the chamber was presaturated with mobile phase vapour for 10 min. The development distance was 8.5 cm and the development time approximately 60 min.

After chromatography the plates were dried in a current of air by using air blowing drier. Densitometric scanning was performed with a Camag TLC Scanner 3 at 230 nm for all measurements. The scanner was operated by Wincats software version 1.2.3. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 20 mm/s.

After chromatographic development, bands were scanned over the range 200-400 nm (spectrum scan speed 20 nm/s) so that the drug could be estimated at 230 nm, which is ascertained by taking the spectrum at different concentrations between 200–600 ng with 100 ng increment. Further it is also observed that spectra are similar in their behavior.

The standard stock solution of eletriptan hydrobromide (100 µg/ml) was applied on a TLC plate, in the range 2–7µl, by use of the Linomat V sample applicator and 100 µl syringe. The plate was developed and scanned under the conditions described above. Each amount was analyzed five times and peak areas were recorded. A calibration plot of peak area against respective amount was established for eletriptan hydrobromide.

Sample solution of eletriptan hydrobromide

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg eletriptan hydrobromide was weighed and transferred to a standard 100 ml volumetric flask containing approximately 50 ml methanol. The mixture was ultra sonicated for 5 min then diluted to volume with methanol. The solution was filtered using Whatmann 41 filter paper. 2-7µl of the filtrate was applied to a TLC plate. After development of chromatogram the peak area of the bands were measured at 230 nm and the amount of drug in each tablet was determined from the calibration plot. The analytical procedure was repeated six times for the homogenous powder sample.

VALIDATION

The described methods has been validated for the assay of eletriptan hydrobromide

LOD and LOQ

The limit of detection of a compound is defined as the smallest level of analyte that gives a measurable response. LOD was determined by using the equation

$$\mathbf{LOD = 3.3 \times \sigma/S}$$

The limit of quantitation is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ was determined by employing the relationship

$$\mathbf{LOQ = 10 \times \sigma/S}$$

where σ = the standard deviation of the response and S = the slope of the calibration curve.

Precision

To study intra-day and inter-day precision, three different concentrations of sample solutions were prepared and applied to the TLC plates. All the solutions were analyzed in triplicate on the same day and on three different days to record intra-day and inter-day variations in the results, respectively.

Accuracy

To check the accuracy of the method, recovery was measured by the addition of standard drug solution at three different levels (50, 100, and 150%) to pre-analyzed sample solution (200 ng/band for eletriptan hydrobromide was selected so that after standard addition sample would be in the linear range). Three replicate estimations were carried out for each concentration level.

Robustness

The effect of small, deliberate variation of the analytical conditions on the peak areas of the drugs was examined in order to assess the

robustness of the method. The robustness of the method was checked for 200 and 700 ng/band for eletriptan hydrobromide.

RESULTS AND DISCUSSIONS

For optimization of method, different mobile phase compositions were employed to achieve good separation. The method development was initiated by using different proportions of mobile phase consisting variety of solvents like: toluene, methanol, ethylacetate, dichloromethane, methanol, butanol, Aceticacid, water, toluene, methanol, acetone, toluene, methanol, ethylacetate, aceticacid, hexane, ethylacetate. Of these the mixture dichloromethane-acetone-acetic acid in the ratio of 3:2:0.2 (v/v) was found to be suitable for the studies. The R_F value of eletriptan hydrobromide was **0.76 ± 0.01**. The proposed HPTLC method was validated in terms of linearity, precision, accuracy, specificity and robustness. The calibration plot was found to be linear over the range 200–700 ng/band for eletriptan hydrobromide, with a correlation coefficient of 0.9998. The LOD and LOQ were found to be 50 and 200 ng/band, respectively. The values of percent relative standard deviations were found to be **0.24** and **0.30** for Intraday and inter day precision studies, respectively which indicate that the method is precise. The method was also evaluated by assay of commercially available tablets (relpax) containing eletriptan hydrobromide. The resulting densitogram for a standard and sample solution of eletriptan hydrobromide is presented under Fig. 1 and 2 indicated a concentration of 600 ng/band. Six replicate analyses were performed on accurately weighed amount of the tablets and the percent assay was found to be 99.46 ± 0.45 for eletriptan hydrobromide (Table 1). To study the accuracy of the method, recovery studies were performed. For eletriptan hydrobromide, recovery ranged from 97.96 to 100.05% with values of percent RSD ranging from 0.25 to 0.51 indicating that the proposed HPTLC method is highly accurate (Table 2). To confirm the specificity of the proposed method, the solution of formulation was spotted on TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak. Study of the robustness of the method revealed that the peak areas were unaffected (RSD < 2%) by small changes in composition and volume of mobile phase indicating appreciable robustness of the method. The method validation parameters are presented in (Table 3). The three dimensional pictogram of eletriptan hydrobromide was shown in Fig. 3.

CONCLUSION

The validated HPTLC method for eletriptan hydrobromide is simple, rapid, accurate, precise, sensitive, specific and robust and can thus be used for routine analysis of eletriptan hydrobromide in a tablet dosage form.

Table 1: Analysis of Marketed Formulation of Eletriptan Hydrobromide by HPTLC

Tablet sample	Label claim (mg)	Amount found*	% Label Claim	%RSD
Eletriptan hydrobromide	20	19.99	99.90	0.25

*Average of six determinations

Table 2: Result From Recovery Studies of Eletriptan Hydrobromide

S. No.	Spiked level	%Recovery*	% RSD
1.	50%	100.05	0.25
2.	100%	99.98	0.34
3.	150%	97.96	0.51

*Average of three determinations

Table 3: Method Validation Parameters

Parameters	Results of eletriptan hydrobromide
Linearity(ng/band)	200-700
Correlation coefficient	0.9998
LOD(ng/band)	50
LOQ(ng/band)	200
precision	
Interday (%RSD)	0.23
Intraday (%RSD)	0.24

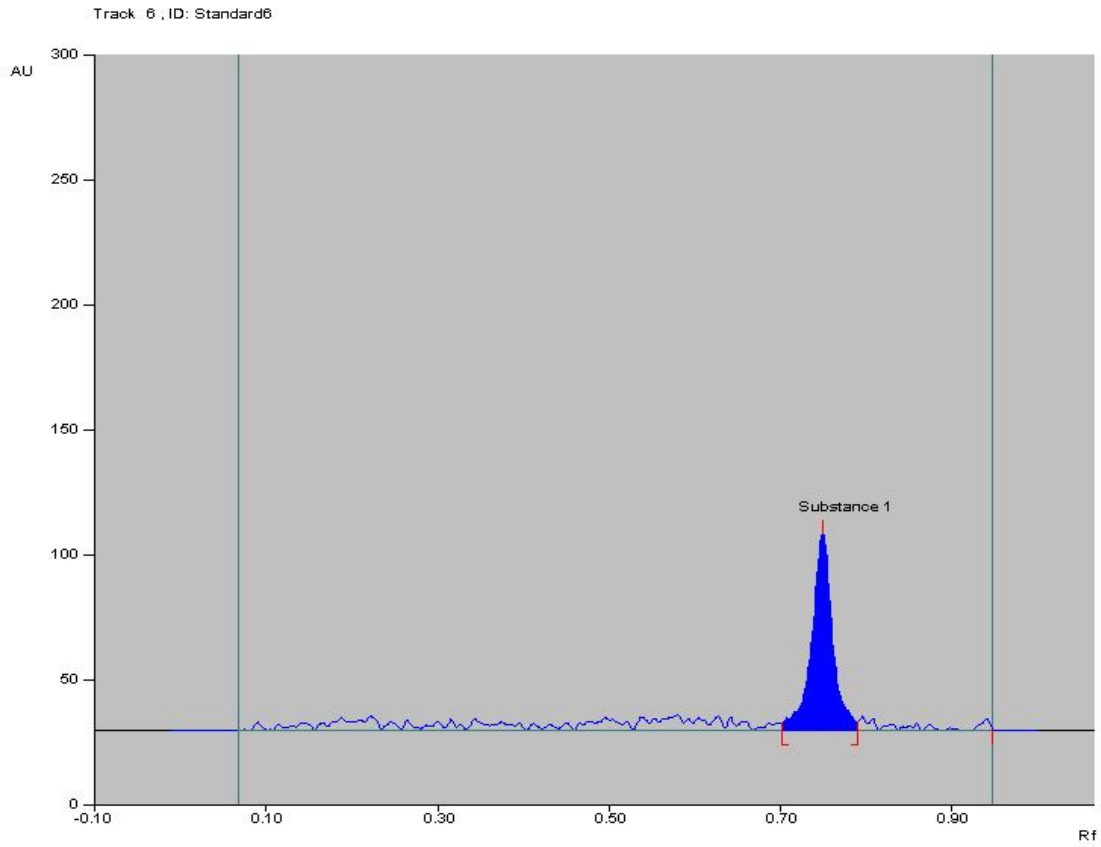


Fig. 1: Densitogram obtained from a standard solution of Eletriptan (600 ng/band; Rf = 0.76 ± 0.01)

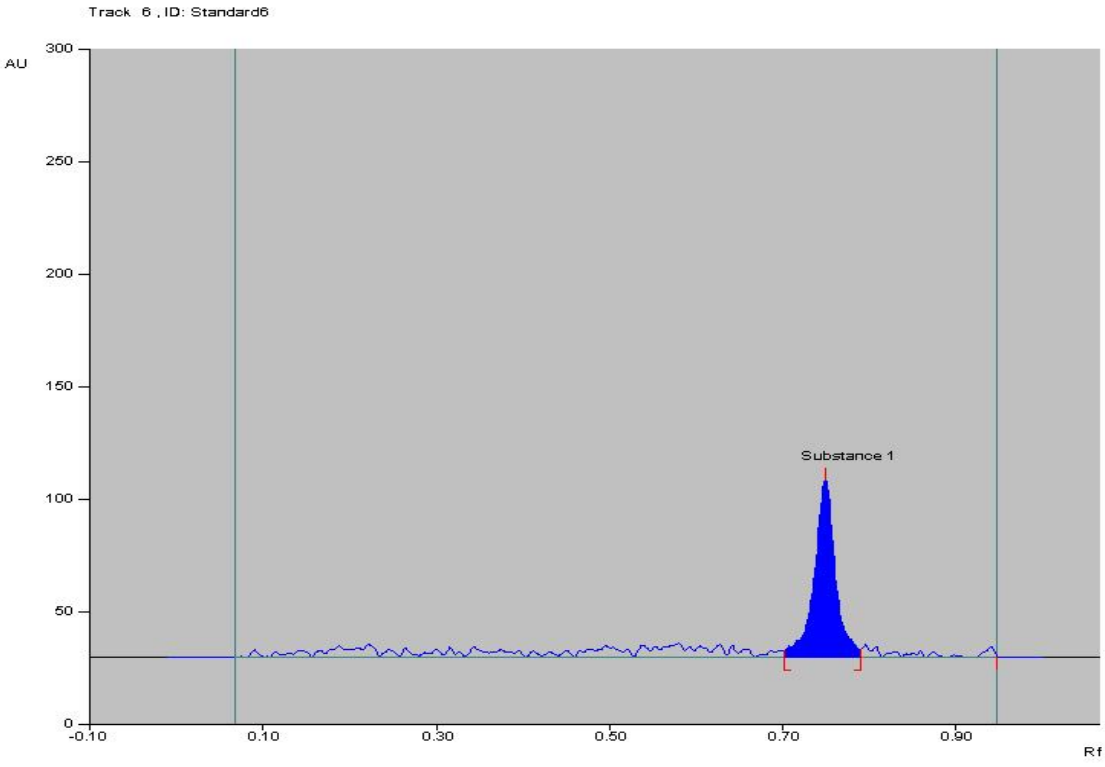


Fig. 2: Densitogram obtained from sample solution of Eletriptan (600 ng/band; Rf = 0.76 ± 0.01)

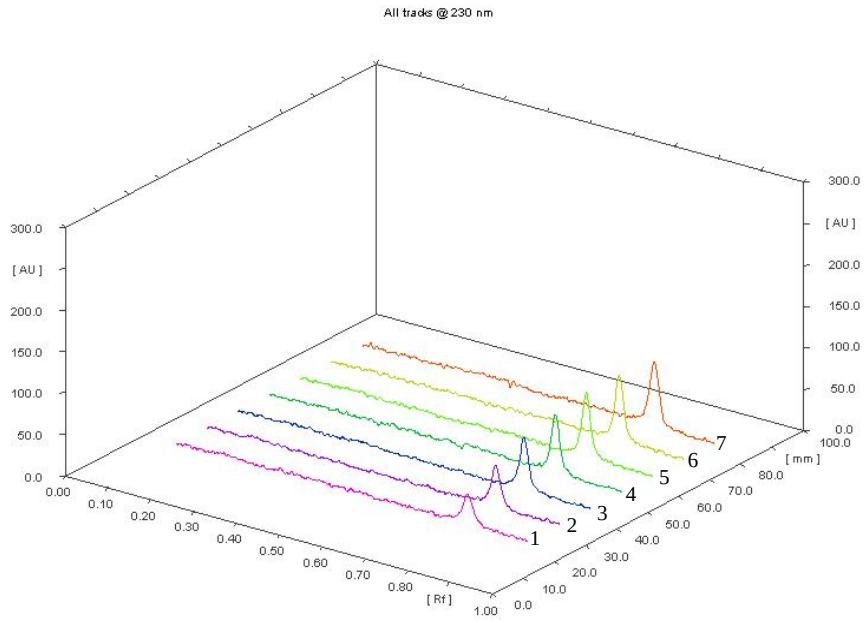


Fig. 3: Three dimensional Chromatogram of Eletriptan (Peaks 1-6,(200-700ng/spot) of Standard eletriptan & Peak-7 of Sample)

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