World Journal of Chemistry 6 (1): 49-52, 2011
ISSN 1817-3128

Densitometric Application for Rizatriptan Benzoate in Bulk and Dosage Forms

[1]S. Sharma and [2]M.C. Sharma

[1]Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya, Bhind (M.P) 477001, India
[2]School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India

Abstract: A simple, fast, specific and precise High Performance Thin Layer Chromatographic method has been developed for estimation of Rizatriptan benzoate in bulk and pharmaceutical formulations. The chromatographic separation was carried out on precoated silica gel $60F_{254}$ aluminium plates using a mixture of toluene-methanol-triethylamine 9:3:1 (v/v/v) as mobile phase and densitometric evaluation of spots was carried out at 287 nm using Camag TLC scanner III with CATS 1.3.4 version software. The experimental parameters like band size of the spot application, chamber saturation time, solvent front migration, slit width etc. were critically studied and optimum conditions were evolved. The drug was satisfactorily resolved with R_f value 0.48±0.01. The accuracy and repeatability of the proposed method was ascertained by evaluating various validation parameters like linearity (200 to 800 ng/spot), precision (intra-day RSD 0.76-1.84% inter-day RSD 0.89-1.79% accuracy (99.89%±0.54), and specificity according to ICH guidelines. The limits of detection and quantification were 46 and 88ng/spot, respectively. HPTLC method provides a faster and cost effective quantitative control for routine analysis of Rizatriptan benzoate in its formulation.

Key words: Rizatriptan benzoate · HPTLC · Densitometric estimation

INTRODUCTION

Rizatriptan Benzoate (RZB), (N, N-dimethyl-2-[5-(1,2, 4-triazole-1-ylmethyl)-1H-indol-3-yl] ethanamine monobenzoate) is a triptan drug and it is a selective 5-Hydroxy Triptamine1B/1D (5-HT 1B/1D) receptor agonist [1]. Rizatriptan binds with high affinity to human cloned 5-HT1B and 5-HT1D Receptors. Rizatriptan has weak affinity for other 5-HT1 receptor subtypes (5-HT1A, 5-HT1E, and 5-HT1F) and the 5-HT7 receptor, but has no significant activity at 5-HT2, 5-HT3, á- and ß-adrenergic, dopaminergic, histaminergic, muscarinic or benzodiazepine receptors. Current theories on the etiology of headache suggest that symptoms are due to local cranial vasodilatation and/or to the release of vasoactive and pro-inflammatory peptides from sensory nerve ending in an activated trigeminal system [2-4]. Hence a RP – LC method was developed and validated as per ICH guidelines. The literature reveals that various methods for the determination of Rizatriptan benzoate and pharmaceutical validations among these methods are HPLC method for Rizatriptan benzoate [5-8]. Author and his research team have developed Method development for different pharmaceutical dosage form [9-16]. The primary goal was to develop and validate a HPTLC method for the rapid quantization of the drug. The present study illustrates development and validation of a simple, accurate, economical and reproducible procedure for determination of Rizatriptan benzoate by HPTLC as bulk and tablet dosage forms.

Experimental

Chromatographic Conditions: All other chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals Corporation ltd. Mumbai, India. Deionized and ultrapure water used in all experiments was obtained from Milli – Q system (Millipore).Silica gel $60F_{254}$ TLC plates (20×10 cm & 10×10 cm, layer thickness 0.2mm, Merck, Germany) were used as stationary phase. The instrument used in the present study was Camag HPTLC system comprising Camag Linomat V automatic sample applicator, Hamilton syringe (100µl), Camag TLC scanner III with Wincats software. The HPTLC system consisted of Linomat V auto sprayer connected to a nitrogen cylinder, a twin trough glass chamber (10×10 cm), saturated with filter paper for 30 minutes. The solvent system consisted of toluene-methanol-triethylamine 9:3:1 (v/v/v). Scanning wavelengths for Rizatriptan benzoate was 287 nm. HPTLC was performed on 10×10cm precoated silica gel $60F_{254}$

Corresponding author: S. Sharma, Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya, Bhind (M.P) India.

precoated plates from E.Merck. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried, and activated for 30 minutes at 135°C with the plates being placed between two sheets of glass to prevent deformation of the aluminium during heating.

Preparation of Stock Solution and Sample Application: Standard stock solution of Rizatriptan benzoate was prepared by dissolving 10 mg of drug in 10 mL of methanol to get the concentration of 1 mg/mL from which 1 mL was further diluted to 10 mL with methanol to obtain a working standard having a concentration of 100 ng/µL. The standard and formulation samples of Rizatriptan were spotted on precoated TLC plates in the form of narrow bands of lengths 8 mm, with 15 mm from the bottom and left margin and with 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 100 ng^{-1}.

Validation of Method: The method was validated as per the ICH guidelines [17] in terms of linearity, accuracy and specificity, intra-day and inter-day precision, repeatability of measurement of peak area as well as repeatability of sample application. Preparation of a calibration curve, aliquots 2, 4, 6, 8, 10, 12 µl of standard stock solution of Rizatriptan (100 ng/µL) were applied on the TLC plate under nitrogen stream. TLC plates were developed under above established conditions. Area under peak was recorded and plotted against concentration. The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug was confirmed by comparing the R_f and spectra of the sample spots with that of standard drug. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150%. Chromatogram was obtained and the peak areas were noted. At each level of the amount, three determinations were carried out.The intra-day precision was determined by analyzing standard solutions of Rizatriptan in range 200-800 ng/band for three times on the same day while inter-day precision was determined by analyzing corresponding standards on three different days over a period of one week (Table 1). Robustness studies were carried out by examining the effect of small, deliberate variation of the analytical conditions on the peak areas of the drug. Factors varied were volume of mobile phase (± 0.5%), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to study the effect. The robustness of the method was checked at amount of 400 ng/band. The limit of detection (LOD) and limit of quantitation (LOQ) was determined on the basis of signal to noise ratio. LOD was the amount of the applied sample producing a peak area that is equal to the sum of the mean blank area and three times the standard deviation. LOQ was the amount of the applied sample producing a peak area that is equal to the sum of the mean blank area and ten times its standard deviation. Stock solution of Rizatriptan (0.1 mg mL^{-1}) was prepared and different volume of stock solution in the range 200 to 800 ng were spotted in triplicate. The amount Rizatriptan by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3 × A.S.D.) / b and quantification limit was calculated by (10×A.S.D.) / b, where "b" corresponds to the slope obtained in the linearity study of method.

Table 1: Intra-day and Inter-day precision of RP-TLC method Rizatriptan

Amount ng spot $^{-1}$	Amount found*	S. D.	% R. S. D.
Intra-day			
200	200.13	1.63	0.29
400	399.05	2.43	0.17
800	799.32	1.02	0.42
Inter-day			
200	199.26	3.05	0.13
400	400.11	2.04	0.68
800	800.05	1.19	0.54

* mean of three determinations

Assay of the Marketed Formulation: For the assay of marketed formulation, 1 mL of the marketed sample solution was pipetted out using a volumetric pipette and transferred to a 10 mL of volumetric flask and diluted with methanol to get the concentration of 100 ng/µL. Two µL of this solution was applied on the plate. After chromatographic development peak areas of the bands were measured at 250 nm and the amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample. When the developed chromatographic plate is exposed to atmosphere, the analytes are likely to decompose. Hence it is necessary to

conduct stability studies. Stability of the analyte on the plate was studied at different time intervals and peak areas were compared with the peak area of freshly scanned plate.

RESULTS AND DISCUSSION

The HPTLC procedure was optimized with a view to develop a stability indicating assay method used for the quantification of the Rizatriptan in pharmaceutical tablets. This method utilizes the active analogue principle that lies at the HPTLC [9-16]. Both the pure drug and the degraded products were spotted on the TLC plate and run in toluene-methanol-triethylamine 9:3:1 (v/v/v) were employed as mobile phase gave spot lacked of compactness and considerable less R_f value. 0.48 of Rizatriptan (Table 2). A representative calibration curve obtained by plotting peak area of compound against the Concentration over the range of 200 to 800 ng/spot. The slope, intercept and correlation co-efficient values were found to be 2.732, 43.154 and 0.9999 respectively (Table 2). It showed that good correlation between regression coefficient and concentration of the drug. The intra-day and inter-day relative standard deviations were found in the range 0.76-1.84% and 0.89-1.79% respectively (Table 1). The smaller values of intraday and inter-day variation in the analysis indicate that the method is precise. RSD for repeatability of measurement of peak area and repeatability of sample application were found to be 0.354% and 1.654%, respectively. The RSD values for measurement of peak area and sample application were both below the instrumental specifications (1% and 3%, respectively), ensuring proper functioning of HPTLC system. 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 (% RSD < 2). The results are given in (Table 2). The% assay (Mean ± S.D., n = 6) was found to be 100.88 ± 0.32. The linear regression data for the calibration curves showed good linear relationship over the concentration range 200 - 800 ng (co-relation co-efficient, $r^2 = 0.9999$) with slope 1.87 and intercept 12.61 (Table 2).The RSD for measurement of peak area was calculated and was found to be 0.66%. In repeatability of sample application the% RSD for the peak area values were calculated and found to be 0.42%. The RSD values for measurement of peak area and sample application were both below the instrumental specifications (i.e.1%); ensuring proper functioning of HPTLC system. The% recovery of Rizatriptan was found to be 101.25; 102.11 (at 100 & 50% levels respectively),

Table 2: Summary of Validation Parameters of Proposed HPTLC method

Parameter	Value
R_f(SD)	0.63±0.01
Linearity and range (ng\spot)	200-800ng/spot
Limit of detection(ng\spot)	46 ng/spot
Limit of quantification (ng\spot)	88 ng/spot
% Accuracy ± SD[a] (n=6)	99.89%±0.54
Precision (% RSD[b])	
(a) Repeatability of sample application (n=6)	0.543
(b) Repeatability of sample measurement (n=6)	0.621
Intraday (%RSD)	0.76-1.84%
Inter day (%RSD)	0.89-1.79%
% Assay ± SD[a] (n=6)	100.88 ± 0.32.
LOD[a]	0.132
LOQ[b]	0.326
Robustness	Robust

aSD = Standard deviation, [b]RSD = Relative standard deviation

Table 3: Recovery Studies of Rizatriptan

	Rizatriptan		
Label claimed	Excess drug added to the analyte (%)	Amount recovered (ng)	% recovery
10	0	10.03	100.09
	80	9.98	99.97
	100	10.11	100.06
	120	10.27	100.21

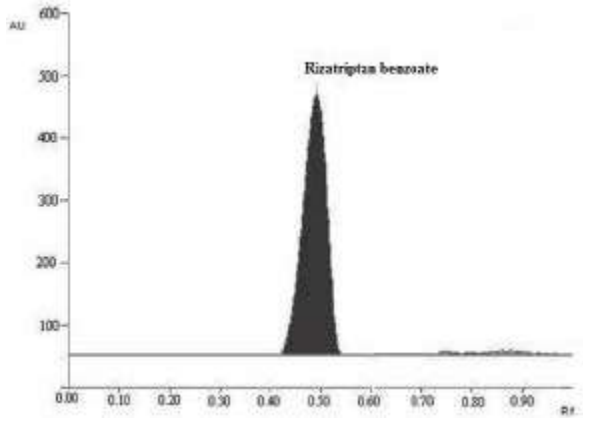

Fig. 1: Representative Densitogram of Rizatriptan (R_f = 0.48±0.04)

which is satisfactory (Table 3).The results of recovery study indicate that the proposed method is accurate for estimation of drug in tablet dosage form. Robustness tests examine the effect of the operational parameters on the analysis results. By introducing small changes in mobile phase composition.

CONCLUSION

The developed HPTLC method for the determination of Rizatriptan is simple, precise, specific, accurate, selective, sensitive and reproducible. The amounts found in formulations well agreed with label claim. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of Rizatriptan from bulk drug and formulations.

ACKNOWLEDGEMENT

We are grateful to Prof.Abhay Kumar Singhei Department of Pharmaceutical Sciences Dr.Hari Singh Gaur.SagarUniversity Sagar (M.P) India, for given valuable suggestion.

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