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Determination of Ranolazine in Tablet Formulation by HPTLC Using Reflectance Scanning Densitometry

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Abstract

A new quantitative densitometric High Performance Thin Layer Chromatographic method was developed and validated for the analysis ranolazine in formulations. Ranolazine the formulations was separated and identified on a silica gel 60 F254 HPTLC plates with Butanol: Acetic acid: Water (6:2:2 v/v), as mobile phase. Densitometric quantification was performed at λ=195 nm by reflectance scanning, which facilitated well-resolved band for the main drug (R_f 0.56+0.02). Response to ranolazine was a linear function of concentration in the range of 100-400ng, with a correlation coefficient, slope and intercept of 0.99912±0.00017, 8.684±0.582 and 492.147±2.67 respectively. The minimum amount of ranolazine that could be authentically detected and quantified was 14.90 and 49.67 ng/spot, respectively. The proposed method was validated with respect to linearity, precision, accuracy, specificity and robustness.

Keywords: Determination of Ranolazine, Tablet Formulation, HPTLC, Reflectance Scanning Densitometry, Linearity, Precision

Introduction

A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention, or treatment of diseases in human beings or animals or for alternating any structure or function of the body of human being or animals. Pharmaceutical chemistry is a science that makes use of general laws of chemistry to study drugs i.e., their preparation, chemical natures, composition, structure, influence on an organism and studies the physical and chemical properties of drugs, the methods of quality control and the conditions of their storage etc [1,2]. Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids. The components monitored include chiral or achiral drug, process impurities, residual solvents, excipients such as preservatives, degradation products, extractables and leachables from container and closure or manufacturing process, pesticide in drug product from plant origin, and metabolites. The aim o fthe present investigation is to develop quantitative densitometric High Performance Thin Layer Chromatographic method and validated for the analysis ranolazine both in bulk and formulations [3-5].

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Material and Methods

Materials

Ranolazine (RZ) was a kind gift from Dr.Reddy's Laboratories, A.P India,, and was used without further purification. Rancad (label claim 50 mg per tablet – Lupin Laboratories Ltd, India) was purchased from local pharmacy. All chemicals and reagents used were of Chromatographic grade and were purchased from Merck Chemicals, India.

Standard solutions and Calibration plots

A stock solution of RZ (1mg/ml) was prepared by dissolving the authentic sample in methanol. This solution was used to construct a calibration plot by applying 1,1.5,2, 2.5,3,3.5 and 4 µl (equivalent to 100-400 ng per band) to a TLC plate. The data of peak area versus drug concentration was treated by linear least square regression to obtain the calibration graphs.

HPTLC instrumentation

TLC was performed on 10×10 cm HPTLC plates pre coated with 60F-254 (With 0.25mm thickness; Merck, Darmstadt, Germany) and the plates were washed with methanol before use. The samples were spotted in the form of bands of 6mm width using Linomat V applicator (Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai) equipped with 100 µl syringe. A constant application rate of 6 µl/sec was employed and the space between two bands was 10mm. The slit dimension was kept at 5× 0.45 mm and a scanning speed of 20 mm/sec was employed. The mobile phase consisted of butanol: acetic acid: water (6:2:2 v/v) and 10 ml of mobile phase was used for chromatography. Densitometric scanning was performed with Camag TLC scanner III in the absorbance-reflectance mode at 254 nm and operated by WINCATS software (v 143 Camag) resident in the system [6].

Analysis of a marketed formulation

To determine the concentration of Ranolazine in tablets (Brand name: RANCAD-50, label claim: 50 mg per tablet), five tablets were weighed, their mean weight determined and finely powdered. The powder equivalent to 1mg of ranolazine was transferred into a 10ml volumetric flask containing 5ml methanol, sonicated for 30 min (Fast Clean Ultrasonic Cleaner, Enertech Electronics Pvt. Ltd, Mumbai) and the volume was made up to 10ml i.e.100ng/µl solution. The resulting sample stock solution was centrifuged at 3000 rpm for 5 min. at 25°C and the supernatant that was filtered with Whatman Filter Paper No. 41 was analyzed for drug content. Then 2µl of this solution (200ng per spot) was applied on a TLC plate which was developed and scanned. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined [7].

Method Validation

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

Precision

Additionally, the precision of the developed HPTLC method was further evaluated in another experiment by separation studies of the reaction mixtures using different chromatographic conditions again on different days.

Limit of detection and limit of quantification

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), concentrations of RZ in the lower part of the linear range of calibration curve were used. Further, the detection limit was calculated by (3.3 x S.D)/b and quantification limit was calculated by (10 x S.D)/b, where "b" corresponds to the slope obtained in the linearity study of the method [8,9].

Robustness

The different composition of mobile phase i.e., butanol: acetic acid: water (6:2:2 v/v and 6.5:2.5:2 v/v) were attempted with two run lengths of 8.5 cm and 9cm.

Recovery studies

The recovery study was carried out by standard addition method. RZ corresponding to 50%, 100% and 150% of label claim has been added to the preanalysed capsule sample solution. Three determinations were performed at each level of recovery [10].

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The peak for RZ test sample was confirmed by comparing the R_f value and the spectrum of the peak with that of the standard [11-13].

Results and Discussion

Selection of the optimum mobile phase

TLC procedure was optimized with a view to develop a stability-indicating assay method and a mixture of butanol: acetic acid: water (6:2:2 v/v)

improved the spot characteristics and facilitated a distinct and selective separation (Fig. 1).

Calibration curves

The linear regression data for the calibration curves as shown in Table 1 offered a good linear relationship over the concentration range of 100-400ng per spot with a correlation coefficient, r=0.99912±0.00017 but there was no significant difference observed in the slopes of standard curves .

Validation of the method

Precision

The results of the repeatability and intermediate precision experiments are shown in Table. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies respectively were around <2%, as recommended by ICH guidelines (Table 1 & 2).

LOD and LOQ

Limit of Detection and limit of Quantification were calculated by the method described in the Experimental section and found to be 14.90 ng/spot and 49.67 ng/spot respectively.

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and the %RSD was found to be less than 2%. The low values of the %RSD (Table 3).

Recovery studies

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard RZ was added to pre-analyzed samples and were subjected to the proposed HPTLC method (Table 4).

Specificity

The specificity of the method was ascertained by analyzing standard drug and the sample. The spot for RZ in sample was confirmed by comparing the R_f and the spectra of the spot with those of the standard sample.

Analysis of a marketed formulation

The drug content was found to be 0.99912±0.00017 with a RSD of 0.41% for analysis in triplicate.

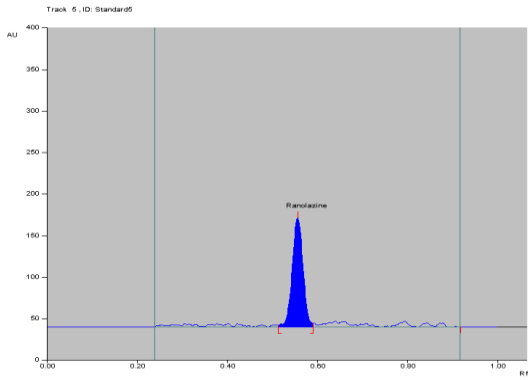

Fig.1: HPTLC chromatogram of standard Ranolazine using Butanol: Acetic acid: Water (6:2:2 v/v) as mobile phase at detection wavelength of 195nm.

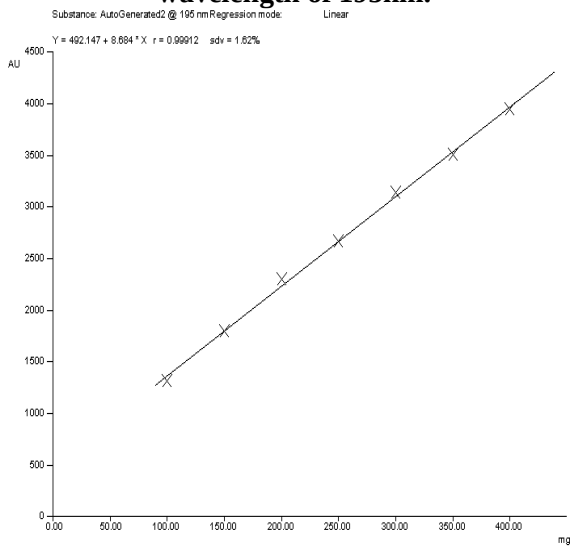

Fig. 2: Calibration plot for standard solutions of Ranolazine by HPTLC

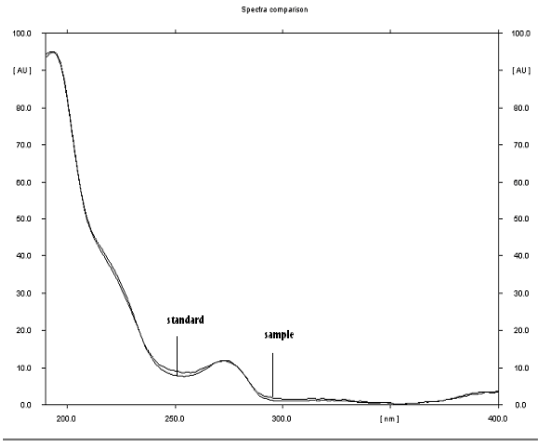

Fig. 3: Insitu UV spectrum of standard Ranolazine and batch sample.

Table 1: Linear regression data for the calibration curves (n=6)

Parameters	**TLC densitometry**
Linear range	100-400 ng/spot
Correlation coefficient (r) ±SD	0.99931±0.00017
Slope±SD	8.68±0.582
Confidence limit of slope [a]	9.26-8.09
Intercept±SD	492.1±2.67
Confidence limit of intercept [a]	494.7-489.4
LOD (ng/spot)	14.90
LOQ (ng/spot)	49.67

[a] 95% confidence limit

Table 2: Intra- and inter- day precision by HPTLC method

Amount	**Repeatability**		**Intermediate precision**	
(ng/spot)	**Mean Area (AU) ±SD**	**%RSD**	**Mean Area (AU) ±SD**	**%RSD**
150	1789.6±14.3	0.79	1751.3±23.7	1.35
250	2664.5±19.5	0.73	2637.1±29.3	1.11
350	3505.2±28.6	0.81	3486.9±37.2	1.06

Table 3: Robustness of the method

Parameter	**% Recovery**	**%R.S.D. [b]**
Mobile phase composition		
I. BU-AA-WA (6:2:2)	101.4	0.67
II.BU-AA-WA (6.5:2.5:2)	99.23	0.51
Plate run length		
I. 8.5 cm	101.1	0.84
II. 9 cm	99.19	1.36
BU=butanol, AA=acetic acid, WA=water.		

[a] n = 6, [b] Average of two concentrations 150, 250 ng/spot.

Table 4: Recovery studies of ranolazine (n=6)

Excess of drug[a] added to the analyte (%)	**Theoretical content (ng)**	**Amount of drug found (ng)**	**Recovery (%)**	**% R.S.D.**
0	100	98.19	98.19	0.29
50	150	151.7	103.4	0.62
100	200	202.4	104.7	0.41
150	250	251.6	102.7	0.29

[a] Matrix containing 100 ng of drug

Conclusion

The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of ranolazine in bulk and pharmaceutical formulations.

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