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High performance thin layer chromatographic estimation of ranolazine

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ABSTRACT

A simple, sensitive and validated high performance thin layer chromatographic method has been developed for the estimation of Ranolazine in pure drug and its formulation. Aluminium plates precoated with Silica gel G 60 F_{254} was used as stationary phase, and methanol **:** 10 milli molar ammonium acetate solution in the ratio of 6:4 was used as mobile phase. Quantification was carried out by the use of densitometric absorbance mode at 271 nm. The content of Ranolazine in the marketed formulation was estimated and found to be 99.9%. The proposed HPTLC method was quantitatively evaluated in terms of precision, repeatability, accuracy and calibration correlation proving its utility in routine analysis of its dosage form.

Key words: HPTLC, Densitometric Absorbance mode.

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INTRODUCTION

Ranolazine N-(2, 6 dimethylphenyl)-2-[4-[2-hydroxy-3-(2methoxy phenoxy) propyl] piperazine-1- yl] acetamide [6], belongs to the category of Anti anginal drug. Ranolazine affects the sodium-dependent calcium channels during myocardial ischemia [8].

Literature survey reveals that several methods like HPLC [13-15], LC-ESI [9] and LC-MS-MS [10-12] have been reported for the estimation of Ranolazine in its pure form and in the formulation. There were no reported methods for its estimation in tablet dosage form by HPTLC. The present study described the development and validation of a simple, specific, accurate and precise HPTLC method for the determination of Ranolazine in pharmaceutical dosage form.

EXPERIMENTAL

A CAMAG HPTLC system comprising of CAMAG Linomat IV semiautomatic sample applicator, CAMAG TLC scanner 3, CAMAG twin trough chamber(10 x 10 cm), CAMAG CATS 4 software, Hamilton syringe (100µL) were used during the study. Tablets were purchased from local market. Acetonitrile and Water of HPLC grade (E.Merck India Ltd.) were used for preparing the mobile phase.

Chromatographic conditions [3-5]:

Following chromatographic conditions were uniformly followed in the experiment.

Stationary Phase: HPTLC precoated Silica gel G60 F_{254} (Merck)
Size: 10 x 10 cm
Mode of application: Band
Band size: 4.0 mm
Separation technique: Ascending
Temperature: Ambient
Saturation time: 15 min.
Migration time: 70 mm
Detection: UV
Scanning wavelength: 271 nm
Scanning mode: Absorbance/Reflectance
Slit dimension: 3 x 0.45 nm.

Linearity of detector response

Aliquots of working standard (1000 µg/ml) solution (1,2,3,4,5,6 µl) of Ranolazine were spotted as sharp bands on the precoated TLC plate, using Camag linomat IV semiautomatic applicator under nitrogen stream. The plate was developed under chromatographic conditions mentioned above. The plate was removed from the chamber and dried in hot air dryer.

Densitometric measurements were performed at 271 nm in absorbance mode. Data peak height and peak area of each band were recorded. The calibration curve was prepared by plotting peak area vs. concentration corresponding to each spot. The densitogram of Ranolazine was presented in figure – 1.

Assay

Stock solution A: An accurately weighed quantity of Ranolazine (50mg) was transferred into a 50ml volumetric flask. It was dissolved and diluted up to the mark with methanol to give a standard stock solution of 1 mg/ml. This 1mg/ml solution can be used as a working standard solution.

Preparation of sample solution

20 tablets were accurately weighed and average weight was calculated. Accurately weighed quantity of tablet powder equivalent to 50mg of the drug was transferred to 50ml volumetric flask. To it 8ml of methanol was added and shaken for 10 min and the volume was adjusted upto the mark with methanol and then filtered through Whatmann filter paper no.40. This solution is used as the sample solution.

On the HPTLC plates spots of the standard and sample were applied. The plates were developed and after development the bands of the drugs were scanned at 271 nm. The peak height and area of the standard and sample bands were compared to obtain the concentration.

Method Validation [1-2]

Accuracy: Accuracy of the method was ascertained by performing the recovery studies using standard addition method. To a fixed amount of preanalysed drug were added at different levels. The total amount of the drug was determined by the above proposed method and the amount of pure drug was calculated. The average percent recovery was found to be nearly 100%(Table-3).

Precision: Precision of the analytical method was expressed as SD or %RSD of series of measurements by replicate estimation of the drugs by the proposed method (Table -1).

Ruggedness: It was ascertained by analyst to analyst variation. The result was presented in Table -1.

RESULTS AND DISCUSSION

Ranolazine was completely extracted from the tablet matrix with methanol. Combination of Methanol: 10mmAmmonium acetate (6:4) offered optimum migration and resolution of Ranolazine from other components of the formulation matrix.

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Figure 1 - Densitogram of ranolazine

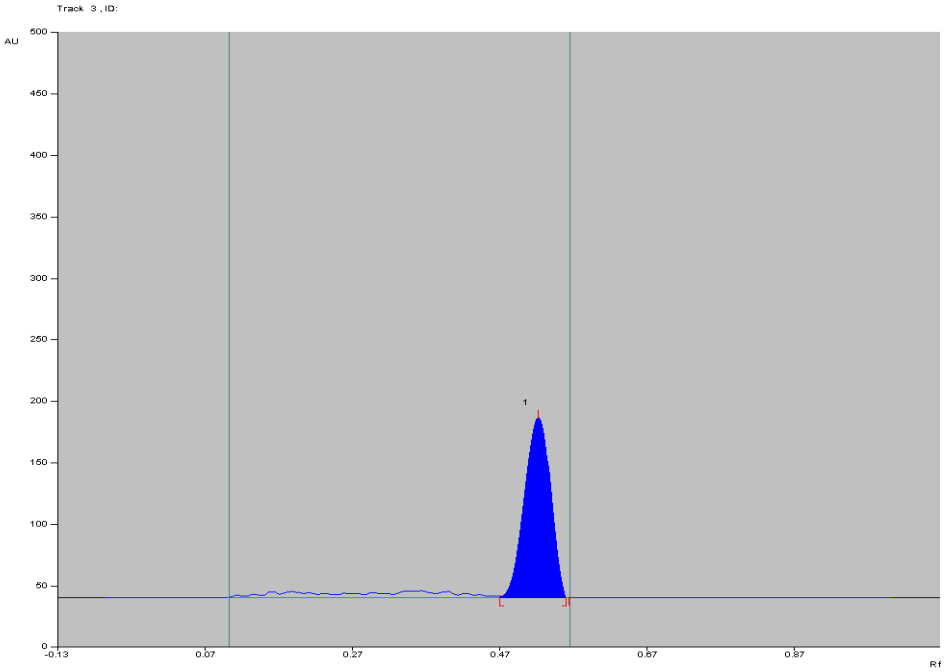

Table-1: Validation parameters of Ranolazine

Parameters	Tolterodine Tartarate
λ_{max} (nm)	271
Beer's law limit(µg/mL)	1000 – 6000
Limit of Detection (LOD)	25.31 ng
Limit of Quantification (LOQ)	76.711 ng
Regression equation(Y*)	
Slope(b)	1.393
Intercept(a)	190.47
Correlation coefficient(r)	0.998
Intra day %RSD **	0.05471
Inter day % RSD	0.07624
Analyst to analyst % RSD	0.04912

*Y = a + bx^7, where 'Y' is the absorbance and x is the concentration of the drug in µg/mL **For six replicates

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Table 2 - Estimation of Ranolazine in formulation

S.No	Label Claim (mg)	Amount Estimated (mg)	%Label Claim
1 2 3	500 500 500	495 497.5 495	99 99.5 99
Mean*			99.1
Standard deviation*			0.2888
% RSD*			0.2912
Standard error*			0.1666

* Average of three determinations

Table 3 - Recovery studies

S.No	Amount Present (µg/µl)	Amount Added (µg/µl)	Amount Recovered (µg/µl)	%Recovery
1 2 3	10 10 10	5 10 15	15.1 19.9 25.1	100.1 99.1 99.4
Mean				100.03
Standard deviation				0.11547
% RSD				0.11505

The amount of Ranolazine in the formulation was calculated on applying suitable dilution factor and comparing peak height and peak area of the standard and sample solutions. The content of the drug in the formulation was found to be within the limits. (Table - 2).

The linearity of response was found to be in the range of 1000 – 6000 µg/ml. Also the percent recovery values were found to be within the limits. Lower values of intra-day and inter-day variation on the analysis indicate that the method is precise. Different validation parameters for the proposed HPTLC method have been summarized in Table-1.

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CONCLUSION

The proposed HPTLC method was found to be simple, specific, precise and accurate. The sample recoveries in the formulations were in good agreement with their respective label claims. Hence this method can be conveniently adopted for the routine analysis of Ranolazine in pure form and in its dosage form.

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