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Original Article

DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR DETERMINATION OF REBAMIPIDE FROM ITS TABLET DOSAGE FORM

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ABSTRACT

Objective: The present work was carried out with the aim to develop and validate a simple High Performance Thin Layer Chromatographic method for the estimation of Rebamipide (REB) from its tablet dosage form.

Method: In this method the mention drug was spotted on silica gel F_{254} TLC plates under pure nitrogen stream by Linomat TLC spotter. Separation was carried out by using methanol,Ethyl acetate, and glacial acetic acidas mobile phase in ratio of 3:7:0.5 v/v/v. Developed TLC plates were scanned by CAMAG TLC scanner and detection was carried out at 229 nm.

Results: r_f value of separated drug was found to be 0.56 for Rebamipide. The developed method was validated as per ICH guidelines by studying various validation parameters like Accuracy, Precision, Robustness, LOD, LOQ and solvent stability. The developed and validated method was successfully applied for determination of Rebamipide from its tablet dosage form.

Conclusion: The developed method was simple, accurate, precise, specific and applicable for determination of rebamipide from its tablet dosage form.

Keywords: Rebamipide,High performance Thin Layer chromatography, Analytical method validation.

INTRODUCTION

[1-8]Rebamipide is chemically 2-(4-Chlorobenzamido)-3-[2(1H)-quinolinon-4-yl] propionic acid (BCS Class IV drug)[Fig.1] [1].Rebamipide is an antiulcer drug used for the treatment of gastric ulcer. It is also an excellent drug for the treatment of dry eye.The drug also attenuates the activity of neutrophils and the production of inflammatory cytokines stimulated by NSAIDs and/or H. pylori[7].REB is official in Japanese pharmacopoeia[1].

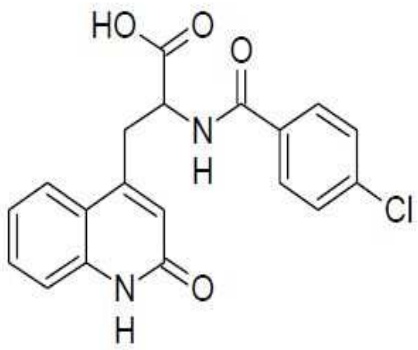

Fig. 1: It shows the chemical structure of Rebamipide

Review of literature revealed that various analytical methods involving HPLC[9-13], LC MS MS[14], stability indicating RP-HPLC[15], UV [16]has been reported for the estimation of REB from its tablet dosage form. The aim of study is to develop and validate high performance thin layer chromatographic methodfor quantitation of Rebamipide in tablet dosage form as per ICH guidelines. Finally the developed method was successfully applied for determination of tablet containing 100 mg of Rebamipide.

MATERIALS AND METHODS

Materials

Analytically pure REB was kindly provided by Torrent Pharma Pvt. Ltd, Dist. Ahmedabad,India as gift sample. Analytical grademethanol was purchased from RFCL limited, New Delhi, India. Tablet of REB, (REBATOR)® was procured from local market.

Instrument and Experimental Conditions

HPTLC analysis was carried out onsilica gel $60F_{254}$ HPTLC plates (10 × 10 cm) by means of a Linomat V automatic spotter equipped with a 100 µl syringe and operated with settings of band length, 6 mm; distance between bands, 5 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin-trough chamber previously saturated for 30 min with the mobile phase for a distance of 7 cm. For densitometry analysis, the spots on the air-dried plate were scanned with the Scanner III at nm using the deuterium source. Photograph of developed plate wastaken by REPROSTAR camera at 254 nm.

Preparation of working solutions

Based upon trial and error at laboratory scale finally it was decided to prepare stock solution of 500 µg/ml. Final mobile phase consisting of methanol, Ethyl acetate, and glacial acetic acid mobile phase in ratio of 3:7:0.5 v/v/v was placed in CAMAG TLC chamber and saturation was performed for 30 min. Final Separation was achieved using above mobile phase.

Analytical Method validation

Preparation of calibration curves/ Linearity and range

For preparation of linearity and range, Synthetic mixture containing 500µg/ml of REB was prepared and 100 µl Hamilton syringe was filed and aliquots of 1µl, 2µl,3µl, 4µl, 5µl, 6µl was applied under pure nitrogen stream to give rise to spots containing REBin range of 500-3000 ng/spot. Spotted plates were developed under stated condition and dried plates were scanned at 229 nm. Procedure was repeated

for further 5 times (total n=6). Finally mean area was plotted against concentration (ng/spot) with help of WINCATS software.

Accuracy studies (Recovery)

Accuracy studies were performed by spiking test solution with standard solution. Accuracy studies were performed at spiking level of 50, 100 and 150% of target concentration. Here stock solution containing 10000 µg/ml of REB was prepared from tablet formulation. Resulting solution was filtered and 1 ml of solution was transferred to each four 10ml volumetric flask. Now from standard stock solution of 5000 µg/ml of REB various aliquots were transferred to each 10 ml volumetric flask. Volume was made up to mark with methanol and 1µl of solution was applied from each volumetric flask on to plate. Procedure was repeated for further 2 times and mean recovery for each level was calculated (n=3).

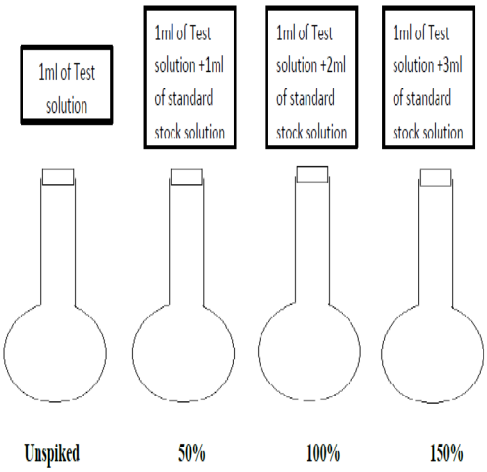

Fig. 2: Preparation of solution for accuracy studies for Rebamipide

Method Precision (Repeatability)

For repeatability studies the linearity studies was repeated for 6 times without changing the syringe and position of plates. Data are collected from each set and Mean area, standard deviation and Coefficient of variance was calculated.

Intermediate Precision

Precisions of the proposed HPTLC methods were determined by analyzing standard solution of REB at 3 different concentrations (500, 1500, 2500 µg/spot for REB) 3 times on the same day (intraday precision) and on 3 different days (Interday Precision). The results are reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantitation (LOD and LOQ)

Limit of detection and Quantitation was performed based upon signal to noise ration of instrument (Instrumental LOD and LOQ) and also performed as per ICH guidelines by using mean of slope and standard deviation intercept from calibration curve.

$LOD = 3.3 \times \sigma / S$, $LOQ = 10 \times \sigma / S$

Where, σ =the standard deviation (SD) of the response and

S = The SD of the *y*-intercept of the regression line.

Specificity Studies

The excipients such as hydroxypropyl cellulose, polyethylene glycol 6000, and lactose monohydrate were spiked into a reweighed quantity of drug to assess the specificity of the methods. The peak area was measured to determine the quantity of the drug.

Robustness

Robustness was performed by changing various method parameters like Composition of mobile phase, Size of TLC Chamber, Saturation time and plate pretreatment. Finally effect of this change was observed for change in $R_F$value and change in peak area.Spot stability was observed by performing 2-dimensional HPTLC development using the same mobile phase.

Analysis of Marketed formulation

Powder 20 tablets (Average weight of tablet 100mg), and take powder equivalent to 100mg Rebamipide. Dissolve power in 100 ml volumetric flaskwith 50 ml of Methanol. Sonicate for 15 minutes and make up volume up to mark withmethanol. Filter above solution for whatmann filter paper (0.45 micron). Stock solution: 500 µg/ml of Rebamipide. From above solution apply 2µl of volume on to pretreated Silica Gel F_{254} plates.

RESULT AND DISCUSSION

Method optimization

Several mobile phases were tried to accomplish good separation ofREBand final separation was achieved usingmethanol, Ethyl acetate, and glacial acetic acidas mobile phase in ratio of 3:7:0.5v/v/v[Fig.3]. The developed plate was analyzed by densitometry and Densitogram was recorded to check resolution was represented infig.4. R_F value of REB was found to be 0.56. For quantitation spots were scanned at 229 nm [Fig. 5]. Finally all chromatographic conditions were optimized [Table.1].

Fig. 3: Photograph of developed Plate in final Mobile phase at 254 nm

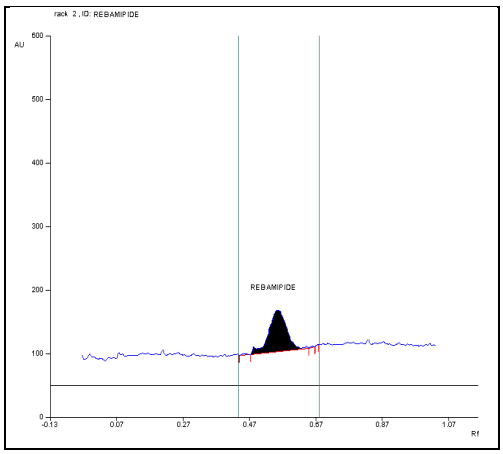

Fig. 4: Densitogram of standard solution of REB containing 1000 ng/spot using mobile phase Methanol: Ethyl Acetate: Glacial acetic acid (3.0: 7.0:0.5, v/v/v)

Table 1: Optimized chromatographic conditions for Rebamipide

Parameter	Condition
Mobile phase	Methanol: Ethyl acetate: Glacial acetic acid (3:7:0.5 v/v/v)
Diluent	Methanol
Stationary phase	Silica gel G F254
Distance run	70 mm
Chamber dimensions	20 x 20 cm
Saturation time	30 minutes
Temperature	Ambient
Detection wavelength	229 nm
R_F value	0.56

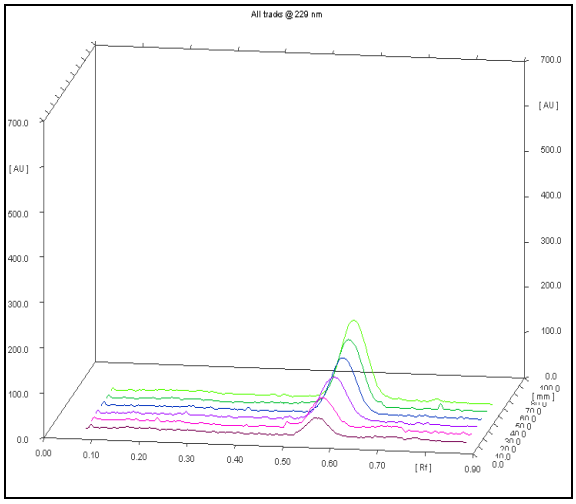

Fig. 5: Overlay views of all tracks of REB at 229nm

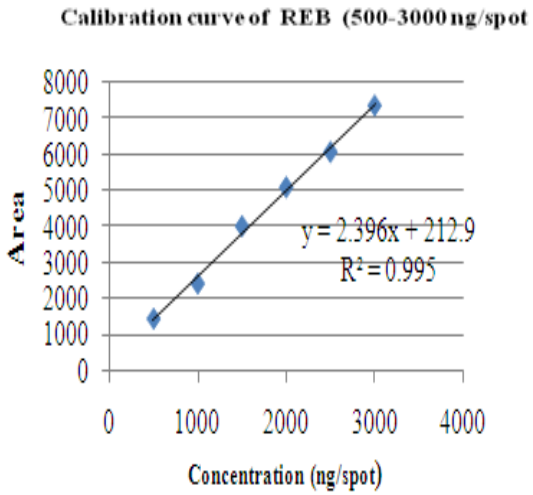

Fig. 6: Linearity curve of REB by HPTLC method

Table 2: Linearity data for REB

Concentration (ng/spot)	Area Mean ± S.D. (n=6)	C.V.
500	1427.85 ± 18.54	1.29
1000	2412.18 ± 21.85	0.90
1500	4016.4 ± 28.82	0.71
2000	5104.95 ± 49.59	0.97
2500	6092.01 ± 59.70	0.98
3000	7391.15 ± 35.04	0.47

Analytical Method Validation

Linearity and range

The method was found to be linear with concentration of 500 -3000 ng/spot of REB. The data were represented in Table 2. The correlation coefficient obtained was 0.9958 for Rebamipide[Fig. 6]. Overlain spectra in #D view also showed a good linearity [Fig. 7].

Method Precision (Repeatabilitystudies)

Method was found to be repeatable as value of coefficient of variance was found to be less than 2.For Rebamipide at all concentration.

Intermediate Precision

Method was found to be reproducible as value of coefficient of variance was found to be less than 2.For Rebamipide at all given concentration for both Interday and intraday.The data were represented in Table 3.

Accuracy Study

Accuracy was performed by spiking method at 50, 100,150 % of target concentration. Recovery was found in the range of 100.04-100.52% for REB. The data were represented in Table 4.

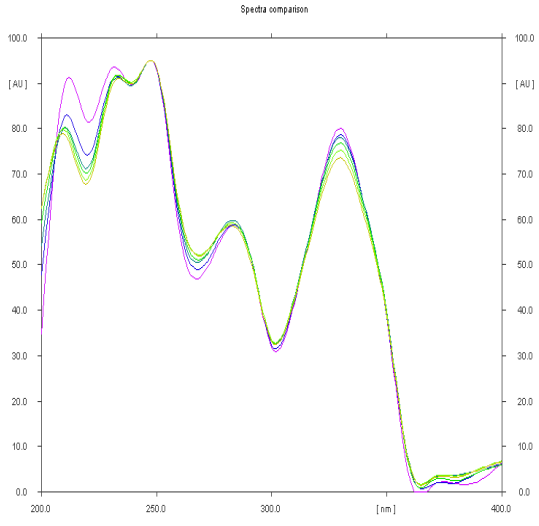

Fig. 7: Overlay Absorbance spectrum of REB (200-400 nm)

Table 3: Precision data for Rebamipide (n=3)

Conc. (ng/spot)	Intraday (Area ± SD)	C.V	Interday (Area ± SD)	C.V
500	1410.16 ± 5.35	0.37	1427.16 ± 5.27	0.36
1500	4006.76 ± 12.88	0.32	4045.93 ± 18.46	0.45
2500	6114.33 ± 29.07	0.47	6048.76 ± 38.46	0.63

Table 4: Accuracy data for REB at 50, 100 and 150 % of target concentration (n=3)

% Level of Recovery	Amount of drug in Sample (ng/spot)	Amount of Standard added (ng/spot)	Amount of drug recovered ± SD (ng/spot)	% Recovery ± SD
	REB (ng/spot)	REB (ng/spot)	REB (ng/spot)	% REB
	1000	-	-	-
50%	1000	500	508.58 ± 7.52	100.28 ± 0.50
100%	1000	1000	1014.69 ± 14.68	100.52 ± 0.73
150%	1000	1500	1505.3 ± 15.48	100.04 ± 0.61

Determination of LOD and LOQ Mathematical LOD was found to be 26.02 ng/spot and LOQ was found to be 78.87ng/spot. The data were represented in Table 5.

Table 5: Determination of LOD and LOQ

	Rebamipide
LOD Based On mathematical Equation	26.02 ng/spot
LOQ Based On mathematical Equation	78.87 ng/spot

Table 6: Robustness studies for Rebamipide

Parameter	Level of Change	Effect on Peak area	
		Rebamipide	
		Area ± S.D.	%Assay ± S.D.
Concentration of Mobile Phase	7 : 3 : 0.5	2596.22 ± 9.40	99.71 ± 0.39
	8.5 : 1.5 : 0.5	2593.87 ± 15.45	99.61 ± 0.65
Size of TLC chamber	20*20 cm	2592.04 ± 9.0	99.53 ± 0.37
	10*10 cm	2594.88 ± 14.21	99.65 ± 0.59
Saturation time	20 min	2592.23 ± 10.97	99.54 ± 0.45
	10 min	2591.59 ± 8.09	99.52 ± 0.32
Plate Pretreatment	100 ˚C	2593.37 ± 14.34	99.59 ± 0.60
	Without Pretreatment	2581.88 ± 8.06	99.11 ± 0.33

Assay of Marketed Formulation The developed method applied for quantitation of REB from its Tablet dosage form value was found to be 99.61±0.31.The data were represented in Table 7.

Table 7: Assay results of Marketed Formulations

Formulation	Drug	Amount taken (ng/spot)	Amount found (ng/spot) (n=3)	Labelled claim (mg)	Amount found per Tablet (mg)	% Label Claim ± S.D
Tablet (Rebator)	Rebamipide	1000	997.95	100	99.79	99.61 ± 0.31
Tablet (Mucopide)	Rebamipide	1000	1000.40	100	100.04	100.0± 0.90

Specificity Studies

The method was found to be specific as there was no interference from the commonly used excipients. Analysis was performed with peak purity analysis and peak purity was found to be greater than 0.997.

Robustness

Minor modification were made in method parameters and changes were observed in peak are and R_F value. And it was found that the method was found to be robust as there was no significant change in peak area and R_F value except in chamber saturation time where less saturation time leads to significant change in peak area and R_F value.The data were represented in Table 6.

CONCLUSION

The HPTLC method was successfully developed and validated as per ICH guidelines and was successfully applied for rapid determination of Rebamipide from its Tablet dosage form. The developed and validated HPTLC method for REB was found to be simple specific and cost effective for analysis of REB in their Tablet dosage form. The additives usually present in the pharmaceuticalformulations of the assayed analytes did not

interfere withdetermination of REB. The method can be used for the routine analysis of REB in pharmaceuticalpreparations.

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