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Research Article

HPTLC AND HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RABEPRAZOLE SODIUM AND LEVOSULPIRIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: Two methods are described for the simultaneous estimation of Rabeprazole sodium and Levosulpiride in the binary mixture.

Methods: The two methods are HPTLC and HPLC methods.

Results: The first method was based on the HPTLC of the two drugs. The separation was carried out on Merck HPTLC precoated silica gel 60 F_{254} aluminium sheets, using Toluene: Methanol: Triethylamine (7:3:0.2, v/v/v) as mobile phase. Quantitation was achieved with UV detection at 280 nm based on peak area with calibration curves at concentration ranges 100-500 ng/spot and 375-1875 ng/spot for Rabeprazole sodium and Levosulpiride, respectively. The Rf for Levosulpiride and Rabeprazole Sodium was found to be 0.52 ± 0.02 and 0.71 ± 0.02 respectively. The second method was based on the HPLC separation of the two drugs on the reverse phase, Grace smart [C_{18} (5µm, 25 cm × 4.6 mm)] using a mobile phase consisting of Acetonitrile: Water: Triethylamine (35:65:1.0 v/v/v) and adjusting the pH to 7.0 with 10 % orthophosphoric acid. Quantitation was achieved with UV detection at 290 nm with linear calibration curves at 4-14 µg/mL and 15-52.5 µg/mL for Rabeprazole sodium and Levosulpiride, respectively. The retention times for Levosulpiride and Rabeprazole Sodium were found to be 3.98 ± 0.02 min and 5.84 ± 0.02 min respectively.

Conclusions: The developed HPTLC and HPLC methods are precise, specific and accurate. These methods can be used for routine quality control process in the laboratory.

Keywords: Rabeprazole Sodium, Levosulpiride, HPTLC, Reverse phase HPLC.

INTRODUCTION

Rabeprazole sodium (RABE) [2-({[4-(3-methoxypropoxy)-3-methyl-2-pyridyl]methyl}sulfinyl)-1H- 1, 3 benzimidazole sodium is an antiulcer drug in the class of proton pump inhibitors. RABE belongs to a class of antisecretory compounds that do not exhibit anticholinergic or histamine H_2-receptor antagonist properties, but suppress gastric acid secretion by inhibiting the gastric H^+/K^+ ATPase at the secretory surface of the gastric parietal cell. Because this enzyme is regarded as the acid (proton) pump within the parietal cell, RABE has been characterized as a gastric proton-pump inhibitor. RABE blocks the final step of gastric acid secretion.

Levosulpiride(LEVO)N-[[(2*S*)–1-Ethylpyrrolidin-2-yl]methyl]-2-methoxy-5-sulfamoylbenzami-ide is a substituted benzamide antipsychotic, reported to be a selective D_2 antagonist on both central and peripheral levels. It is an atypical neuroleptic and a prokinetic agent. It blocks the D_2 dopaminergic receptors, preferentially located in the presynaptic membranes in the dopaminergic pathways of the brain. [1, 2]

RABE is a proton pump inhibitor and LEVO is a prokinetic agent, so combination is used in the treatment of Gastroesophageal reflux disease.

Several methods have been reported in literature for individual estimation of RABE and LEVO like HPLC, UV, LC-MS, HPTLC, capillary zone electrophoretic method but very few methods have been reported for simultaneous estimation of RABE sodium and LEVO in combined dosage form [3-11]. The present work presents two new methods for simultaneous determination of RABE and LEVO in tablets using HPTLC and reverse phase HPLC.

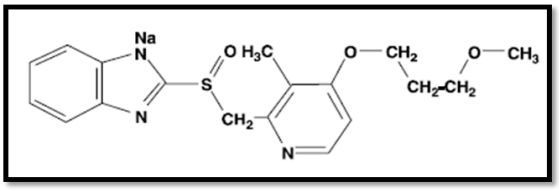

Fig. 1: Structure of RABE

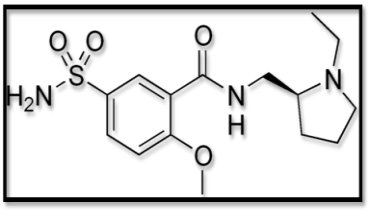

Fig. 2: Structure of LEVO

MATERIALS AND METHODS

Chemicals and reagents

Authenticated standards of RABE and LEVO were procured from Alembic Pharmaceuticals Ltd. (Vadodara, India). For HPTLC, methanol, toluene, triethylamine were of analytical grade. For HPLC, acetonitrile, orthophosphoric acid, triethylamine (RFCL ltd., India) were of HPLC grade. Triple distilled water was used throughout experiment. The marketed formulations of RABE and LEVO (RABE 20mg and LEVO 75mg) procured from local market.

HPTLC conditions

Chromatography was performed on 20 cm × 20 cm aluminium plates precoated with layers of silica gel 60 F_{254} (E. Merck, Germany). The plates were prewashed with methanol and activated at 50˚C for 5 mins prior to chromatography. Samples were spotted in the form of band (3 mm wide) by means of a Camag Linomat IV sample applicator equipped with a 100 µL syringe. The mobile phase consisted of Toluene: Methanol: Triethylamine (5:3:0.2 v/v/v). Linear ascending development was carried out in a 20 cm × 20 cm twin trough chamber (Camag, Mutteenz, Switzerland) saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ± 2˚C) and development distance was 80 mm. After chromatography plates were dried in a current of air, scanning was performed using Camag TLC scanner in the absorbance- reflection mode at 280 nm and operated by CATS software (ver. 4.01). [12] Peaks are shown in figure 3.

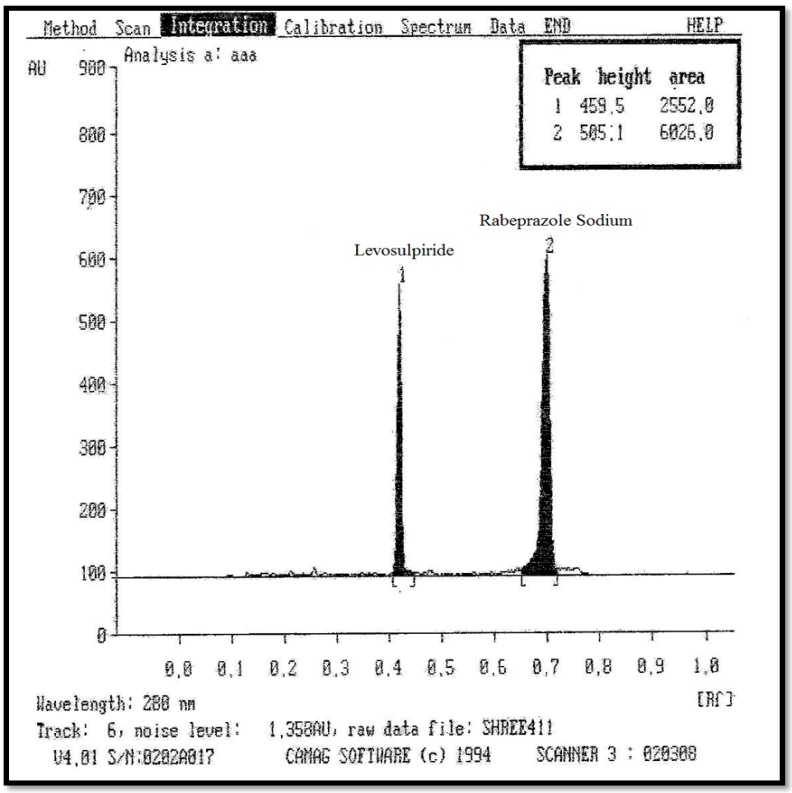

Fig. 3: It shows HPTLC chromatogram of (1) LEVO Rf = 0.42 ± 0.02 and (2)RABE Sodium Rf = 0.71 ± 0.02

HPLC conditions

HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with a model series LC-10 AS pump, Rheodyne 7725i injector with a 50 µL loop and SPD 10A UV-Visible detector. A Grace Smart reversed-phase C18 column (Grace Discovery and Division; 5 µm, 25cm × 4.6mm id) was used as the stationary phase. Class CR10 software was used for data acquisition. The detector was set at 290 nm. Different mobile phases were tested in order to find the best conditions for separating both the drugs simultaneously. The optimal composition of the mobile phase was determined to be acetonitrile:water:triethylamine (35:65:1 v/v/v). The pH was adjusted to 7.0 with orthophosphoric acid. Flow rate kept at 1.0 mL/min and UV detection carried out at 290 nm. [13] Peaks are shown in figure 4.

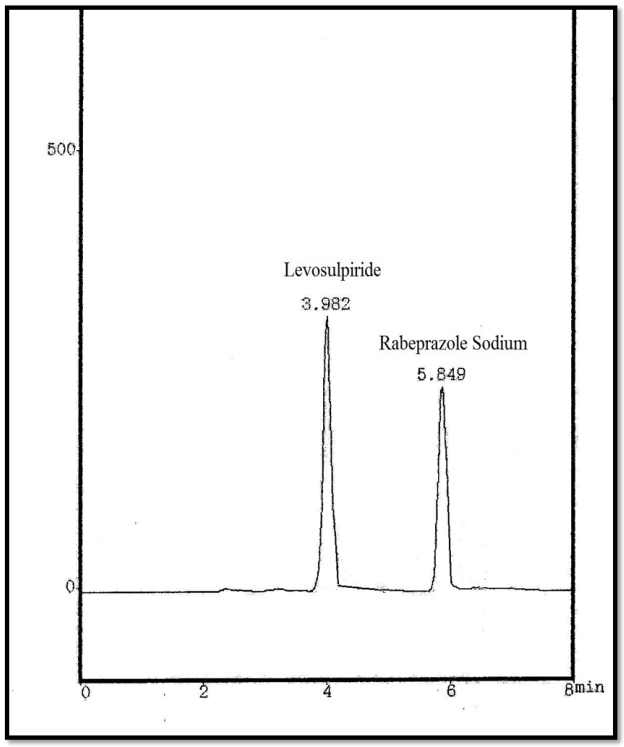

Fig. 4: It shows HPLC chromatogram of standard LEVO (Retention time = 3.982 min) and standard RABE (Retention time = 5.849 min)

Preparation of standard stock solutions

Stock standard solution was prepared by dissolving 37.5 mg of LEVO and 20 mg of RABE in 10 mL methanol.

Preparation of calibration curve for HPTLC

The standard solutions were prepared by dilution of the stock solution to reach a concentration of 75 µg/mL and 20 µg/mL of LEVO and RABE respectively. From the standard solution 5, 10, 15, 20 and 25 µL were used for spotting on the TLC plate. The plate was developed in the previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. Regression equation and correlation coefficient are shown in table 1.

Procedure for assay of marketed formulations by HPTLC

About 20 tablets were weighed and powdered. Powder equivalent to 20 mg RABE (142 mg) was transferred to 100 mL volumetric flask. Methanol (10 mL) was added and sonicated for 45 mins and filtered through Whatman filter paper no 42. The filtrate was made upto the mark with methanol to 25 mL. Aliquot quantity 1 mL was transferred to 10 mL volumetric flask and diluted with methanol upto the mark. The solution (17 µL) was spotted along with the calibration curve and analysed.

Preparation of calibration curve for HPLC

The standard solutions were prepared by dilution of the stock solution with mobile phase to obtain a concentration range of 15-52.5 µg/mL and 4-14 µg/mL for LEVO and RABE respectively. 20 µL injections were made for each concentration of LEVO and RABE and chromatographed under the conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. Regression equation and correlation coefficient are shown in table 1. System suitability parameters are shown in table 2.

Procedure for assay of marketed formulations by HPLC

Twenty tablets were weighed and powdered. The quantity of RABE equivalent to 10 mg RABE (565 mg) was accurately weighed and transferred into 50mL volumetric flask. It was dissolved in mobile phase by sonication for 30 minutes. The solution was filtered through Whatman filter paper no.42 and the volume was adjusted upto the mark with mobile phase. This solution contains 200µg/mL and 750µg/mL of RABE and LEVO respectively. Aliquot 5.0 mL was taken into 10 mL volumetric flask and volume was adjusted upto the mark with mobile phase (100µg/mL). Aliquot 1.0mL was taken in 10 mL volumetric flask and volume adjusted upto the mark with mobile phase. The resulting solution (10µg/mL) was filtered and injected for analysis.

Validation of optimized HPTLC method was carried out with respect to following parameters as shown in table 3. [14]

HPTLC Method Validation

Linearity

The linearity response for RABE and LEVO were assessed by analysis of five independent levels of calibration curve in range of **100-500 ng/spot** and **375-1875 ng/spot** respectively, in terms of slope, intercept and correlation coefficient values.

Precision

The precision of the instrument was checked by repeated scanning of the same spot (six times) of RABE (400 ng $spot^{-1}$) LEVO (1500 ng $spot^{-1}$) without changing the position of the plate for HPTLC method.

Intermediate precision

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same days over a period of 1 week for 3 different concentration of RABE (100, 300 and 500 ng) and LEVO (375, 1125 and 1875 ng) for the HPTLC method. The results are reported in terms of standard deviation.

Limit of detection and limit of quantification

Different concentrations of RABE (5, 10, 15, 20 ng $spot^{-1}$) and LEVO (50, 60, 70, 80, 90, 100 ng $spot^{-1}$) were spotted on TLC plate for determination of LOD practically. The LOD and LOQ were calculated using following equations.

$$LOD = \frac{3.3\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

Where, σ = Standard deviation of response and S = Slope of calibration curve

Accuracy Along with standard calibration curve, assay 5, 10 and 15 µL of standard solution were added on succeeding spots to obtain final concentration range of 100, 200, 300 ng/spot for RABE and 375, 750 and 1125 ng/spot for LEVO. The amount of drug was calculated by employing corresponding calibration curve equations. Average recovery obtained at all 3 levels was reported as % Recovery.

Specificity

The specificity of an analytical method is ability to measure accurately an analyte in presence of interferences like synthetic precursor, excipients, degradants or matrix component. Purity of spectra was determined at three different levels, at starting, middle and end, correlation between them was considered for determination of peak purity.

HPLC method validation

Linearity

Linearity was studied by preparing standard solutions at 6 different concentrations. Each concentration was repeated 5 times. The linearity range for RABE and LEVO were found to be 4 – 14 µg/mL and 15 - 52.5 µg/mL respectively.

Precision

Interday precision was determined by an assay of sample solution on three different days for three different concentrations (Combined standard samples of concentrations 4, 6 and 8 µg/mL for RABE and 15, 22.5 and 30 µg/mL for LEVO).

Limit of detection and Limit of quantitation

According to the ICH recommendation, the approach based on the standard deviation (SD) of the response and slope was use for the determining the LOD and LOQ values.

Accuracy

Standard drug was added at three different concentrations (15, 22.5 and 30 µg/ml for LEVO and 4, 6 and 8 µg/ml for RABE) to preanalyzed sample and mixture was analyzed by proposed method. The experiment was repeated three times.

Table 1: Regression equations for LEVO and RABE

Method of analysis	Compound	Regression equation	Correlation coefficient (r)
HPTLC	LEVO	$y = -0.000x^2 + 1.171x + 1225$	0.997
	RABE	$y = -0.014x^2 + 18.42x + 1376$	0.996
HPLC	LEVO	$y = 66157x + 14281$	0.999
	RABE	$y = 21062x + 21633$	0.997

Table 2: System suitability parameters for HPLC

Parameters	Results		Recommended as per USP
	LEVO	RABE	
Resolution (Rs)	3.6		0.8 ≤ T ≤ 1.5
Theoretical plates (N)	1074.79	1642.95	
HETP	0.0232 cm	0.0152 cm	
Tailing Factor (T)	1.17	1.42	0.8 ≤ T ≤ 1.5

RESULTS AND DISCUSSION

Analysis of pharmaceutical formulations

The propose HPTLC and HPLC methods were applied to the simultaneous estimation of RABE and LEVO in pharmaceutical formulations. Three repeated determination were made and results are shown in table 4.

Statistical Analysis

In comparative study, both the methods (i.e HPTLC and RP-HPLC), for simultaneous estimation of LEVO and RABE in marketed preparations (i.e. Rabonik Plus and Rekool L tablets), were compared for the significant difference between them. The comparison was done using paired two sample t-test and results are shown in table 5.

Table 3: Validation results obtained by applying proposed method for the determination of LEVO and RABE

Parameter	LEVO		RABE	
	HPTLC	HPLC	HPTLC	HPLC
Linearity range	375-1875 ng/spot	15-52.5 µg/mL	100-500 ng/spot	4-14 µg/mL
Intercept	1225	14281	1376	21633
Correlation coefficient	0.997	0.999	0.996	0.997
Intraday precision (% RSD)	0.85-2.39	0.84-1.47	0.92-2.09	0.42-1.62
Interday precision (% RSD)	0.98-2.72	1.15-1.94	0.82-2.23	0.48-2.31
Accuracy (% Recovery)	100.127±1.69	99.79 ± 0.93	100.254±1.30	100.06 ± 1.25
LOD	70 ng/spot	2.169 µg/mL	10 ng/spot	1.787 µg/mL
LOQ	210 ng/spot	6.574 µg/mL	30 ng/spot	5.361 µg/mL

Table 4: Application of developed methods to pharmaceutical formulations

Product	HPTLC		HPLC	
	LEVO	RABE	LEVO	RABE
	% Recovery Mean ± SD (n=3)	% Recovery Mean ± SD (n=3)	% Recovery Mean ± SD (n=3)	% Recovery Mean ± SD (n=3)
Rabonik Plus	99.29 ± 0.31	101.03 ± 0.66	99.80 ± 0.38	100.18 ± 0.90
Rekool L	101.57 ± 0.87	100.49 ± 0.45	100.25 ± 0.65	100.98 ± 0.99

Table 5: Paired sample t-test result for method comparison

Parameters	LEVO		RABE	
	HPTLC	HPLC	HPTLC	HPLC
Mean	100.57	100.50	100.11	100.02
Variance	1.03	0.39	0.68	0.80
T calculate	1.15		0.88	
t critical	2.57		2.57	
P (T<t) one tail	0.30		0.42	
T calc < t critical	Yes		Yes	

CONCLUSIONS

The developed HPTLC and HPLC methods are precise, specific and accurate. Statistical analysis proved that both the methods are suitable for the analysis of RABE sodium and LEVO as a bulk drug and in pharmaceutical formulation without any interference from the excipients. These methods can be used for routine quality control process in the laboratory.

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