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Research Article

A SIMPLE AND VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RABEPRAZOLE AND LEVOSULPIRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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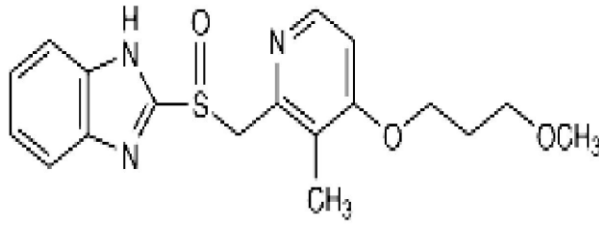
ABSTRACT
A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Rabeprazole and Levosulpiride in Tabletdosage form. A Thermo Hypersil ODS C18 5mm column having 250 x 4.6mm id in Isocratic mode with mobile phase containing 0.1M Ammonium Acetate : methanol (50:50 %v/v pH: 7.0) was used. The flow rate was 1.5ml/min and effluents were monitored at 290nm. The retention time of Levosulpiride and Rabeprazole was 2.2min and 6.8min respectively. The concentration curves were linear in the concentration range of 5-15 µg/mL and18.75-56.25 µg/mL. The developed method was validated for specificity, precision, linearity, accuracy, LOD,LOQ, robustness . Recovery of Rabeprazole and Levosulpiride in formulations was found to be in the range of 98.0% -103.0% and 99%-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision, the proposed HPLC method may be used for the simultaneous determination of these two drugs in pharmaceutical dosage forms

Keywords: RP-HPLC, Rabeprazole and Levosulpiride

INTRODUCTION

Rabeprazole sodium (RBP) is a potent proton pump inhibitor[1] that suppress gastric acid secretion by specific inhibition of the gastric H+,K+-ATPase enzyme system at the secretory surface of the gastric parietal cell and is used in the treatment of GERD[2] and duodenal ulcers[3]. It has a faster onset of action and lower potential for drug interaction compared to omeprazole. RBP is known chemically[4] as 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1*H*-benzimidazole sodium salt.

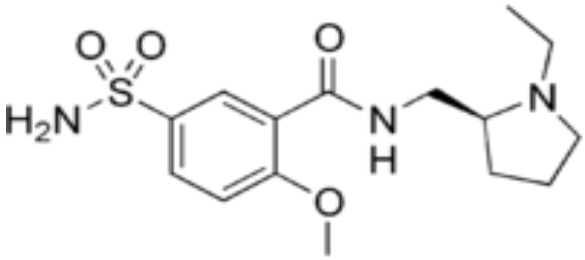
RABEPRAZOLE



Levosulpiride

It is an antipsychotic and prokinetic agent. It is the (*S*)-enantiomer of sulpiride. A substituted benzamide anti-psychotic, reported to be a selective antagonist of dopamine D2 receptors activity on both central and peripheral levels. It is an atypical neuroleptic and a prokinetic agent. Levosulpiride is also claimed to have mood elevating properties. Levosulpiride is used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome and premature ejaculation.

It is chemically known as *n*-{[(2*s*)-1-ethylpyrrolidin-2-yl]methyl}-2-methoxy-5-sulfamoyl benzamide.



LEVOSULPIRIDE

The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC.

Other Apparatus: Electronic balance, Sonicator, 0.45µ membrane filter.

Reagents and Chemicals

Pharmaceutical grade Rabeprazole and Levosulpiride were kindly supplied as a gift sample by Dr.Reddys Laboratory, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Disodium hydrogen orthophosphate were analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was

Several analytical procedures have been proposed for the quantitative estimation of Rabeprazole separately and in combination with other drugs. HPLC and UV methods for estimation of Rabeprazole alone in pharmaceutical preparation have been reported. Rabeprazole in combination with domperidone are also available. Rabeprazole in combination with other drugs is estimated by UV and HPLC have also been reported.

To our knowledge simple and economical analytical method for simultaneous determination of Rabeprazole and Levosulpiride has not been reported so far. So attempt was taken to develop and validate an economic, rapid reversed-phase high performance liquid chromatographic method for the quality control of Rabeprazole and Levosulpiride in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated and found to be accurate, precise and reproducible.

MATERIAL AND METHODS

APPARATUS

Waters e2695Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software.

obtained from a Milli-QRO water purification system.

Commercial Formulation

Rabeprazole and Levosulpiride (SR) Tablets available in the market as ReKool-L in composition of Rabeprazole Sodium (20mg), Levosulpiride (75mg). The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Preparation and Selection of mobile phase

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of Ammonium Acetate buffer pH 7.0 and Methanol were studied for simultaneous separation of both the drugs. The optimal composition of mobile phase determined to be Buffer: Methanol (50:50 v/v) and filtered through 0.45µ membrane filter.

Preparation of standard solution

20mg Rabeprazole and 75mg Levosulpiride was dissolve in 100 ml of Diluent (1:1,Methanol:Na2HPO4) and was further diluted to get stock solution of Rabeprazole and Levosulpiride (47.5µg/ml). This is taken as a 100% concentration. Solution containing mixture of Rabeprazole and Levosulpiride of five different concentrations (50%, 75%, 100% 125%, and 150% of target concentration) were prepared in the same way.

Preparation of Sample Solution

Sample solution containing both the drugs was prepared by dissolving tablet powder into Diluent (1:1, Methanol: Na2HPO4) Ten tablets were weighed separately. Their average weights were determined. Powder of tablets equivalent to one tablet weight were weighed and taken in a 100 ml volumetric flask, dissolved in diluent and shaken and sonicated for about 10 minutes then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration of working sample equivalent to 100% of target concentration.

Chromatographic Conditions

The mobile phase, a mixture of Ammonium Acetate buffer and methanol (50:50v/v) pumped at a flow rate of 1.5 ml/min through the column (C18; 5µ, 4.6 X 250 mm, Thermo Hypersil ODS) at 50ºC.The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane filter. Both drugs showed good absorbance at 290 nm, which was selected as wavelength for further analysis.

Development and validation of HPLC method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Rabeprazole and Levosulpiride in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2002.The method was validated for the parameters like system suitability , selectivity, linearity, accuracy, precision ,LOD, LOQ, and robustness.

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Rabeprazole and Levosulpiride. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates

(Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of Rabeprazole and Levosulpiride were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of Rabeprazole and Levosulpiride of different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

Accuracy (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 Rabeprazole and Levosulpiride were added to pre-analyzed samples and were subjected to the proposed HPLC method.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºc change in temperature[5-8].

RESULTS AND DISCUSSION

Results of system suitability study are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

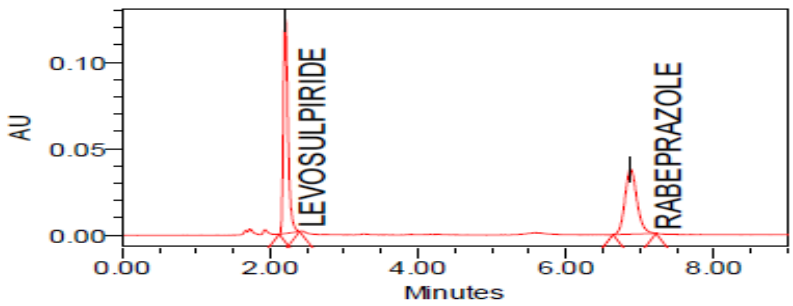


Figure 1: Typical chromatogram of Levosulpiride and rabeprazole sodium in marketed formulation.

Name	Retention Time	Area	% Area	Height	USP Resolution	s/n	USP Tailing	USP Plate Count
LEVOSULPIRIDE	2.207	541504	56.08	123700		64.553036	1.45	6389
RABEPRAZOLE	6.875	424015	43.92	37645	22.62	19.645177	1.14	8745

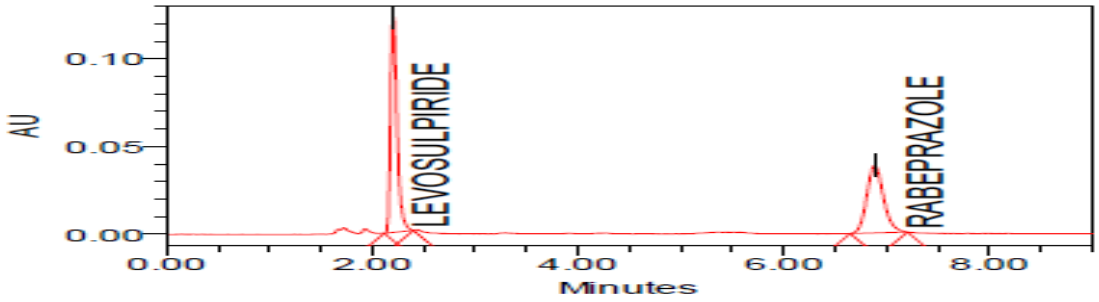


Figure 2 Typical Chromatogram of standard Levosulpiride and Rabeprazole

Name	Retention Time	Area	% Area	Height	USP Resolution	s/n	USP Tailing	USP Plate Count
LEVOSULPIRIDE	2.206	551141	56.08	122803		61.150778	1.47	6001
RABEPRAZOLE	6.886	431551	43.92	38405	22.39	19.124200	1.14	8696

Table 1: Result of system suitability tests of Rabeprazole and Levosulpiride

PARAMETERS	LEVOSULPIRIDE	RABEPRAZOLE
Linearity range	5-15 µg/mL	18.75-56.25 µg/mL
Correlation coefficient	0.999	0.999
Slope	5545.7x-3449.1	4365.4x-6285
Retention time	2.2	6.8
Resolution Factor		22.62
USP plate count	6389	8745
Tailing factor*	1.45	1.14
Limit of Detection(LOD)	1 µg/mL	1 µg/mL
Limit of quantification(LOQ)	6 µg/mL	5 µg/mL

*=%Mean

Chromatograms shown in figure1 and figure 2 explain that retention time for standard sample and commercial product of Rabeprazole and Levosulpiride are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas (average peak areas of six replicates) versus concentrations was observed for Rabeprazole and Levosulpiride in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both the drugs which prove that the method is linear. Calibration curve of Levosulpiride and Rabeprazole are shown in Fig 3 and 4.

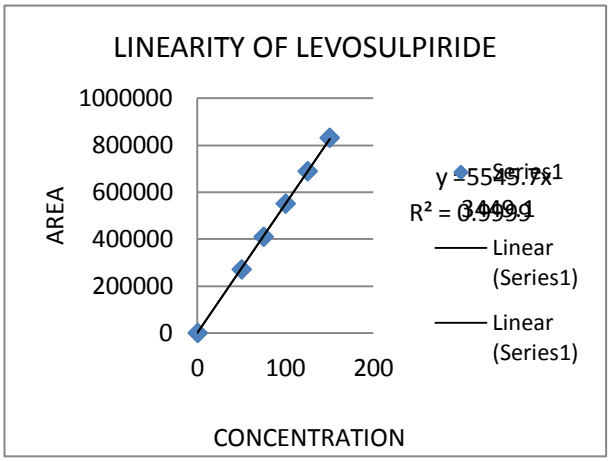


Figure 3 Linearity of Levosulpiride

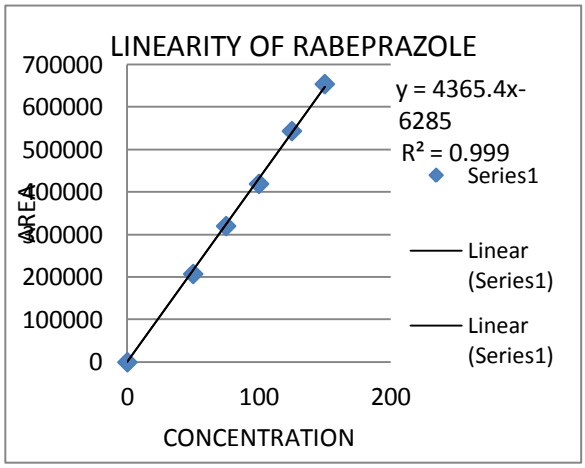


Figure 4 Linearity of Rabeprazole

Table2: Intra day and inter day precision result of Rabeprazole and Levosulpiride

Drug	%RSD (intra-day)	%RSD (inter-day)
Levosulpiride	0.14	0.4
Rabeprazole	1.12	1.5

Table 3:Accuracy (%recovery) results of Levosulpiride and Rabeprazole

Sample No	Levosulpiride			
	Spiked Amount (mg)	Recovered Amount (mg)	% Recovered	%Average recovery
1	37.5mg	36.75mg	98	98.6%
2	75mg	74.25mg	99	
3	112.5mg	111.3mg	99	
	Rabeprazole			
1	10mg	9.85mg	98.5	98.7%
2	20mg	19.6mg	98	
3	30mg	29.91mg	99.7	

Results of Intra day and inter day variability were summarized in table 2. Intra day variability was done from 9.00 am to 6.00 pm on the same day. % RSD of peak areas was calculated for various run .The method is highly precise as % RSD of peak area was less than 1% in all tests.

Table 4: Results for robustness test of Levosulpiride and Rabeprazole

Parameters count	Changes	RT	USP Tailing	USP Plate
LEVOSULPIRIDE				
Flow rate(ml/min)	1	2.5	1.44	6059
	1.4	1.9	1.42	6017
Temperature	45ºc	1.9	1.42	5751
	55ºc	1.95	1.40	6001
RABEPRAZOLE				
Flow rate(ml/min)	1	7.8	1.13	9996
	1.4	6.0	1.11	8033
Temperature	45ºc	6.2	1.09	8221
	55ºc	6.01	1.13	10184

Results of accuracy study are presented in table 3. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 98.6% for Levosulpiride and 98.7% for Rabeprazole. All the results indicate that the method is highly accurate. The results of robustness of the present method showed that small changes were made in the flow rate and temperature did not produce significant changes in analytical results which are presented in Table 4. As the changes are not significant we can say that the method is robust[9-10].

CONCLUSION

The new HPLC method developed and validated for simultaneous determination of Levosulpiride and Rabeprazole pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

ACKNOWLEDGEMENT

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