International Research Journal of Pharmaceutical and Applied Sciences
Available online at www.irjpas.com
Int. Res J Pharm. App Sci., 2012; 2(4):49-55

Research Article

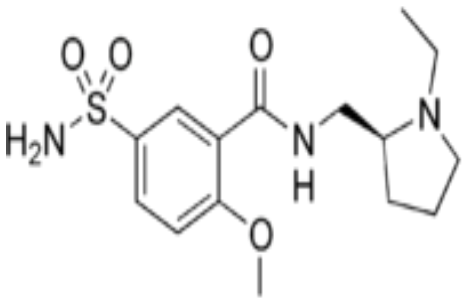
Method development and validation of simultaneous estimation of levosulpiride and rabeprazole in bulk and pharmaceutical dosage form by RP-HPLC

A. Sirisha*, A. Ravi Kumar
Department of Pharmaceutical Analysis and Quality assurance, Bapatla College of pharmacy, Bapatla-522301, Andhra Pradesh, India
(Received: 01 August, 2012; Accepted: 13 August, 2012; Published: 29 August, 2012)
Corresponding Author: Email: **sirisharagala@gmail.com**

ABSTRACT
A new simple**,** precise, accurate and selective RP- HPLC method has been developed and validated for estimation of Levosulpiride and Rabeprazole in pharmaceutical formulation. The detection was carried out at 216nm for both drugs. The retention time for LEVO and RAB were found to be 4.918 min. and 5.873 min. respectively. The method was validated for precision, recovery, robustness, specificity, and detection and quantification limits, in accordance with International Conference on Harmonization guidelines. Linearity was observed in the concentration range from 50% to 150% of nominal concentration of Rabeprazole and Levosulpiride Correlation coefficient was 0.999 for both the drugs .The limit of detection and quantification of LEVO were 0.021 mg/ml and 0.0731 mg/ml respectively. While for RAB it was 0.06 mg/ml and 0.20 mg/ml, respectively. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 101.3% for LEVO and 99.3% for RAB. The % RSD below 2.0 shows the high precision of proposed method.
Key words: RP-HPLC, Rabeprazole and Levosulpiride

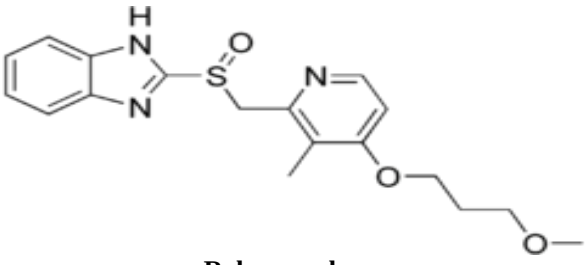
INTRODUCTION

Levosulpiride is chemically *N*-[[(2*S*)-1-Ethylpyrrolidin-2-yl] methyl]-2-methoxy-5-sulfamoylbenzamide having molecular formula $C_{15}H_{23}N_3O_4S$ with molecular weight 341.43. It is white crystalline powder with melting point 177-181^{0}C and soluble in water and ethanol. It is acts as an antipsychotic and prokinetic agent. [1, 2]



Levosulpiride

Rabeprazole is indicated chemically as (*RS*)-2-([4-(3-methoxypropoxy)-3-methylpyridin-2-yl] methylsulfinyl)-1*H*-benzo[*d*]imidazole (Fig. 1). The molecular formula is C18H21N3O3S with molecular weight of 359.44. It is a white or off white crystalline powder with melting point range of 140–145^{0}C and freely soluble in water, soluble in methanol, ethyl acetate, chloroform.



Rabeprazole

Various methods are reported for the analysis of individual drug and in combination but no method were reported for selective estimation of these two drugs in combined dosage form. Therefore, it was thought worthwhile to develop RP-HPLC methods for analysis of Rabeprazole and Levosulpiride in Pharmaceutical dosage form. Therefore, the objective of this study was,

therefore, to develop a simple, economical, selective, precise, and reproducible high performance liquid chromatography (HPLC) method on both the drugs in bulk and tablet formulation.[3, 4]

MATERIALS AND METHODS

Reagents and chemicals

Levosulpiride (LEVO) and Rabeprazole (RAB) were supplied as a gift sample by Dr.Reddys Laboratory, Hyderabad, and Andhra Pradesh, India. These drugs were used as working standard. All the chemicals used of HPLC Grade collected from E. Merck, Darmstadt, Germany and milliQ water was used for mobile phase preparation.

Apparatus:

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

Commercial Formulation

Rabeprazole and Levosulpiride (SR) Tablets are available in the market as Rabikind plus 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 Dibasic sodium phosphate buffer (PH-9.3) and Acetonitrile were studied for Simultaneous separation of both the drugs. The optimal composition of mobile phase determined.Buffer: Acetonitrile (75:25 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 (water) and was further diluted to get stock solution of Rabeprazole and Levosulpiride . concentration. Solution containing mixture of Rabeprazole and Levosulpiride of five different concentrations (50%, 75%, 100% 125%, and 150% of target concentration) was prepared in the same way.

Preparation of Sample Solution

Sample solution containing both the drugs was prepared by dissolving tablet powder into diluent (water) ten tablets were weighed separately. Their average weights were determined. 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 Dibasic sodium phosphate buffer and Acetonitrile (75:25v/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 216 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 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%. This proves that method is precise.

Robustness of Method [5-8]

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 changes in flow rate and ± 5 º c changes in temperature.

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.

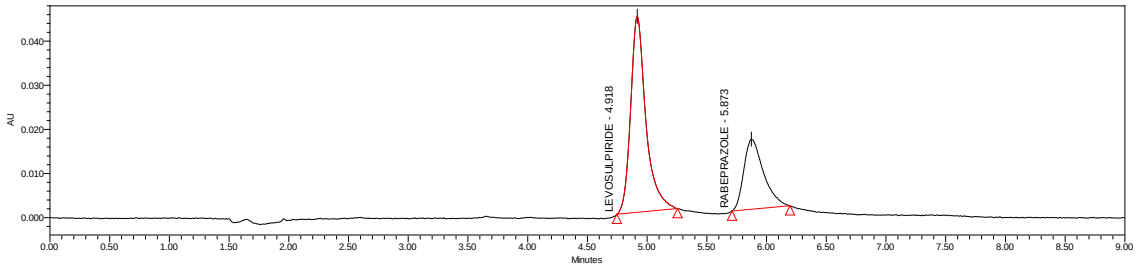


Figure 1: Typical chromatogram of Levosulpiride and Rabeprazole sodium in marketed formulation.

	Name	Retention Time	Area	USP Tailing	USP Plate Count
1	LEVOSULPIRIDE	4.918	395076	1.35	8092
2	RABEPRAZOLE	5.873	182014	1.46	6172

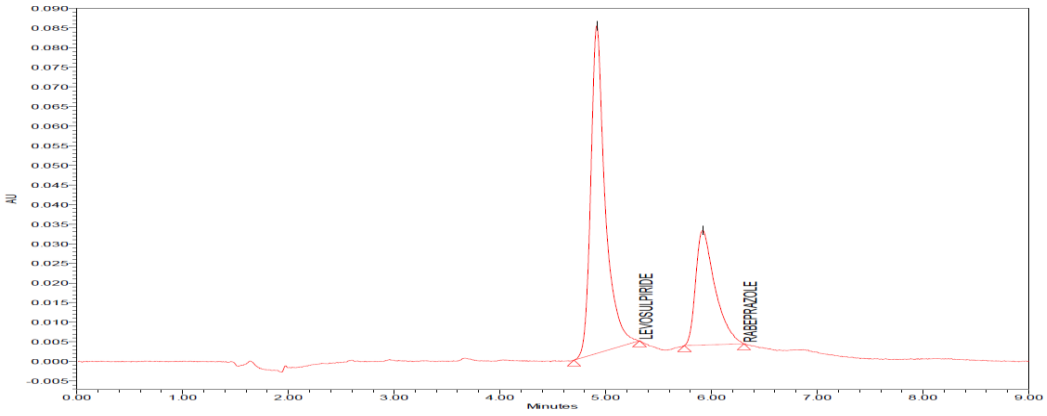


Figure 2 Typical Chromatogram of standard Levosulpiride and Rabeprazole

	Name	Retention Time	Area	USP Tailing	USP Plate Count
1	LEVOSULPIRIDE	4.909	789918	1.4	8030
2	RABEPRAZOLE	5.934	363111	1.6	6962

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

PARAMETERS	LEVOSULPIRIDE	RABEPRAZOLE
Linearity range	0.02-0.12mg/ml	0.04-0.24mg/ml
Correlation coefficient	0.999	0.999
Regression equation	Y=7916x-2841	Y=3646x-2388
Retention time	4.918	5.9873
USP Plate count	8092	6172
Tailing factor	1.35	1.46
Limit of detection(LOD)	0.0219mg/ml	0.06mg/ml
Limit of quantification(LOQ)	0.07313mg/ml	0.2mg/ml
Capacity factor	1.001	0.9882

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.

Method precision

Six sample solutions were prepared individually using single batch of Levosulpiride and Rabeprazole tablets as per the test method and injected each solution into HPLC as per methodology.

Acceptance criteria

The results were found to be within the acceptance criteria; hence the method is accurate throughout the selected range.

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 101.03% for Levosulpiride and 99.3% 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

3&4.As the changes are not significant we can say that the method is robust.[9-10]

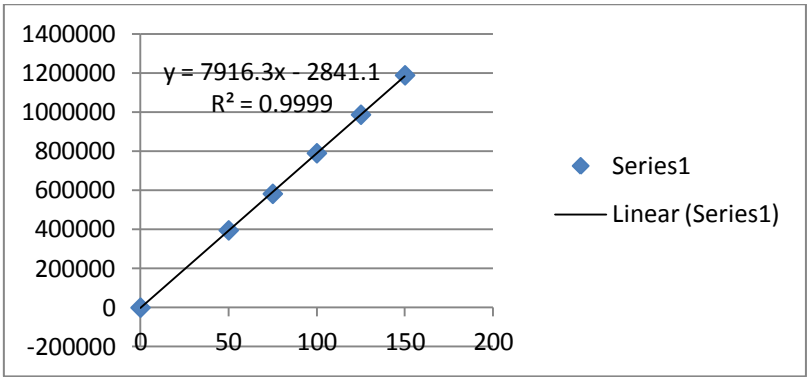


Figure: 3: INEARITY OF LEVOSULPIRIDE

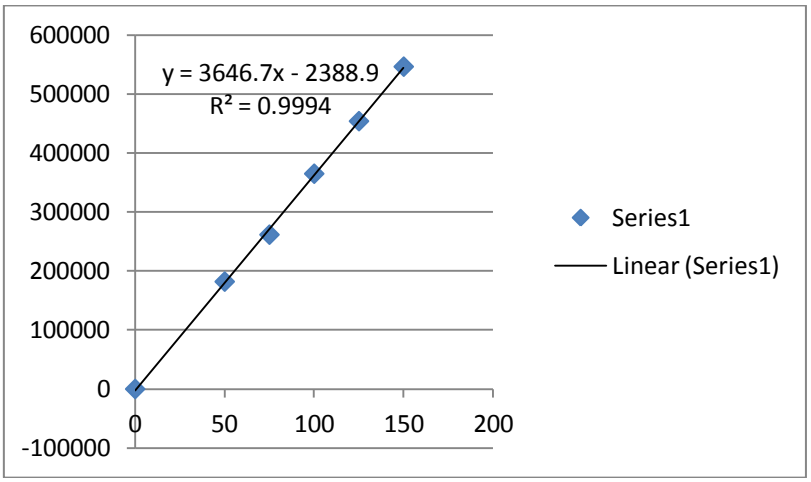


Figure: 4:LINEARITY OF RABEPRAZOLE

Table-2: Results of Method precision

S.No	Number of injections	Sample area-1(Levosulpiride)	Sample area-2(Rabeprazole)	% Assay	% Assay
1	1	788637	371421	100	101
2	2	789921	364573	100	99
3	3	787737	361593	100	99
4	4	789342	364727	100	99
5	5	790676	367965	100	100
6	6	783753	366355	100	100
Average Assay				100	100
STD				2467.85	3358.15
%RSD				0.33	0.92

Table 3: Results of Recovery Studies for LEVO and RAB

LEVOSULPIRIDE

Spiked level	Amount added	Amount found	% Recovery	%RSD
50%	148.75	150.6	101.29	0.4
100%	297	300.93	101.26	0.1
150%	445.75	445.75	101.26	0.1
Mean			101.27	

RABEPRAZOLE

Spiked level	Amount added	Amount found	% Recovery	%RSD
50%	40.01	39.76	99.36	0.2
100%	80.00	79.36	99.18	1.4
150%	120.07	119.44	99.47	0.2
Mean			99.33	

Table 4: Results for robustness test of Levosulpiride and Rabeprazole

Parameters	Changes	RT	USP Tailing	USP Plate count
LEVOSULPIRIDE				
Flow rate(ml/min)	1.4	5.66	1.34	8496
	1.6	4.37	1.26	6022
Temperature	45°c	4.98	1.31	6447
	55°c	4.97	1.27	6947
RABEPRAZOLE				
Flow rate(ml/min)	1.4	6.73	1.47	5629
	1.6	5.19	1.46	4326
Temperature	45°c	5.87	1.53	4561
	55°c	5.80	1.48	4776

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:

The authors are thankful to Rainbow Pharma Training Lab, Kukatpally, Hyderabad and Bapatla College of Pharmacy, Bapatla.

REFERENCES

1. The Martindale 35th ed: The complete drug reference, published pharmaceutical press, lambeta high street, londan SEI 75M, UK. 2006.
2. Rena S, Park M, Sah H, and Lee B, Effect of pharmaceutical excipients on aqueous stability of Rabeprazole sodium. *International J. Pharmaceutics*, **2008**; 350: 197-204.
3. Ramakrishna NVS, Vishwottam KN, Wishu S, Koteshwara M, Suresh Kumar S, High Performance liquid chromatography method for the quantification of rabeprazole in human plasma using solid-phase extraction. *J. of Chromatography B*, **2005**; 816: 209-214.
4. Sabnis SS, Dhavale ND, Jadhav VY, Gandhi SV, Spectrophotometric simultaneous determination of Rabeprazole Sodium and Itopride Hydrochloride in capsule dosage form. *Spectrochimica Acta*, **2008**; 69: 849-852.

5. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October **1994.**
6. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March **1996.**
7. Lozano R, Peralta Concha M, Montealegre A, de Leon A, Ortiz Villalba J, Esteban H, et al. Effectiveness and safety of levosulpiride in the treatment of dysmotility-like fuctional dyspepsia. Therapeutics and Clinical Risk Management **2007**; 3: 149-155.
8. Silambarasan S P, Anandakumar K, Venkatalakshmi R, Sasikal C.Development of UV Spectrophotometry and RP-HPLC methods for the estimation of Levosulpiride in bulk and in tablet formulation. *Asian J Res Chem* **2010**; 3(3)
9. Manjunath S, Chouhan V and Sandeep S: 9.Spectrophotometric estimation of levosulpiride in bulk drug and formulations. *International Journal of Pharmacy and Pharmaceutical Sciences* **2011**; 3(2): 135-137.
10. Patel.B.H., B.N. Suhagia, M.M. Patel and J.R. Patel. Determination of pantoprazole, rabeprazole,Esomeprazole, Domperidone and Itopride in pharmaceutical product by reversed phase liquid chromatography using single mobile phase, *journal of chromatographia*, **2007**; 2: 743-748.