World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers © All Rights Reserved
Available online at: http://www.wjpsonline.org/
Original Article

Method development and validation for simultaneous estimation of rabeprazole and levosulpride in bulk and pharmaceutical dosage forms

Challa Sudheer[1], B. Tirumaleswara Rao[1] and C. Rambabu[2*]

[1]Department of Chemistry, Vikas Degree College (P.G Courses), Vissannapeta, Krishna Dist.-521215
[2]*Department of Chemistry, Acharya Nagarjuna University, Nagarjunanagar, Guntur, AP, India.

Received: 21-09-2015 / Revised: 03-11-2015 / Accepted: 20-11-2015

ABSTRACT

A rapid, sensitive, efficient and reproducible method for the determination of rabeprazole and levosulpride has been developed using reverse phase high performance liquid chromatographic method. This method involves separation of rabeprazole and levosulpride on a reversed phase Aligent, Zorbax C18 column (150 mmx4.6 mm I.D; particle size 5µm) using UV detection at 221nm. The elution was done using a mobile phase consisting of buffer and acetonitrile in the ratio of 600:400 v/v on Shimadzu LC-2010 HPLC equipment. An external standard calibration method was employed for quantitation. The developed RP-HPLC method of rabeprazole and levosulpride was validated with respect to linearity, accuracy, precision, specificity and robustness respectively.

Keywords: Rabeprazole, Levosulpride RP-HPLC Validation

INTRODUCTION

Rabeprazole [1] is chemically 2- [[[4-(3methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H–benzimidazole sodium salt. Rabeprazole is an antiulcer drug in the class of proton pump inhibitors, used in the treatment of duodenal ulcers. It is a prodrug in the acid environment of the parietal cells it turns into active sulphonamide form. Rabeprazole inhibits the H+, K+ATPase of the coating gastric cells and dose-dependent oppresses basal and stimulated gastric acid secretion. [**Fig.1]**

Levosulpiride [2] is chemically 5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxy benzamide is a D2-dopamine receptor antagonist and commonly prescribed to patients with psychosis, depression and functional dyspepsia. **[Fig.2].**

These two drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin. Literature survey revealed that several papers [3-9] have been reported for have been reported for estimation of the above selected drugs in single or in combination forms.As, only few RP-HPLC methods have been reported for the simultaneous determination of rabeprazole and levosulpride in combined dosage form, in the present section an attempt have been made to develop a RP-HPLC method for assay of rabeprazole and levosulpride and in combined dosage form and was validated following ICH guidelines.

MATERIALS AND METHODS

Reagents & Materials: Rabeprazole and levosulpride was obtained as gift samples from Dr.Reddy's Laboratory, Hyderabad. Commercial formulations of RABIM-LS containing a combination of LEVO (75mg) and RABE (20mg) manufactured by Qualite Pharmaceuticals, Dehradun was purchased from local market. Milli-Q Water, Acetonitrile and ortho phoshphoric acid (Merck) were used as solvents. All other reagents used were of analytical grade.

Instrumentation: A HPLC (LC-2010 (SHIMADZU) with UV/VIS Detector/PDA detector equipped with Aligent Zorbax C18 (150mm × 4.6mm × 5/m) column and auto sampler injector with Empower 2 software for data processing was used in the present assay .

Mobile Phase Preparation: The mobile phase consisted of buffer and acetonitrile in the ratio of 600:400 v/v respectively. The buffer solution used in the present assay was prepared by dissolving

***Corresponding Author Address: C. Rambabu,** *Department of Chemistry, Acharya Nagarjuna University, Nagarjunanagar, Guntur, AP, India;* **E-mail:** *sudheervikas@gmail.com*

1.0ml of ortho phosphoric acid in 1000ml of Milli-Q Water. The mobile phase was filtered through a 0.45µm membrane filter (Millipore Pvt. Ltd. Bangalore, India) and degassed using an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai).

Diluent Preparation: Mobile phase was used as a diluent in the present assay.

Standard Preparation: Standard stock solution of rabeprazole and levosulpride containing 1.0mg/ml was prepared in a 100ml volumetric flask by dissolving 100mg of rabeprazole and levosulpride containing 10ml of the diluent. Later this solution is diluted to volume with the same diluent. Further, dilute this stock solution in 25mlvolumetric flask and make up to mark with diluent (standard solution concentration range of 150-450µg/ml for rabeprazole and 40-120µg/ml for levosulpride).

Sample Preparation: Ten tablets of RABIM-LS procured from local pharmacy were finely powdered and an accurately weighed sample of powdered tablets equivalent to LEVO (75mg) and RABE (20mg) were transferred to a 100ml volumetric flask containing 20ml of diluent. This solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drug. Then the contents in the flask were made up to the mark with same diluent and filtered through a 0.4µ membrane filter. From the filtrate, dilution was made in a 10ml volumetric flask to get concentration ranges as said in standard preparation.

Chromatographic Conditions: Chromatographic analysis of rabeprazole and levosulpride was performed on a HPLC column [Aligent, Zorbax(150 mmx4.6 mm I.D; particle size 5 µm)] at ambient temperature. The flow rate of the mobile phase was adjusted to 1.0ml/min and the injection volume was 10µL. Detection was performed by photodiode array detector at a wavelength of 221nm and the chromatographic runtime was 6 minutes for the analysis.

RESULT AND DISCUSSION:

Development and optimization of the HPLC method: The analytical conditions for the present proposed method were selected, basing on the chemical nature of rabeprazole and levosulpride. Initially spectroscopic analysis of compounds showed that rabeprazole and levosulpride showed a maximum UV absorbance (λmax) at 220nm, 223nm respectively. Therefore, the chromatographic detection was performed at 221nm using a photo diode array detector as both the compounds showed good response at this wave length.

Secondly, the column selection has been done on the basis of back pressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between rabeprazole and levosulpride peak. After evaluating all these factors, Aligent ,Zorbax C18 column (150 mmx4.6 mm I.D; particle size 5µm)was found to be suitable as it gave satisfactory results.

Thirdly the selection of buffer based on chemical nature of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Best results were obtained with orthophospharic acid buffer for rabeprazole and levosulpride. Acetonitrile was chosen as organic constituent of mobile phase, to reduce the longer retention time and to attain good peak shape. Preliminary trials using different composition of mobile phases consisting of buffer and acetonitrile in the ratio of 500:500 v/v and 550:450 v/v, did not give good peak shape for rabeprazole and levosulpride.

Finally, the best separation and resolution of rabeprazole and levosulpride was achieved by fixing mobile phase composition consisting of a mixture of buffer and acetonitrile in the ratio of 600:400 v/v. Under these conditions rabeprazole and levosulpride were eluted at 3.056 and 3.682, minutes respectively with a run time of 5 min. The chromatogram for simultaneous estimation of rabeprazole and levosulpride standard by using the aforementioned chromatographic methods was represented in **Fig:3.** System suitability results of the method are presented in **Table.1.**

Method Validation:

a) System Suitability: In the present study system suitability tests of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected in to the column and the parameters i.e, symmetry, theoretical plate, resolution and % RSD of peak area were determined for same. The results reported in **Table.1** indicated that the asymmetrical factor was not more than 2.0, theoretical plate not less than 2000 for rabeprazole and 5000 for and levosulpride and the % RSD of peak area not more then 2.0, were full fill the good suitability of the proposed RP-HPLC method during all validation parameter.

Blank and **Placebo Interference:** In the present assay a study to establish the interference of blank

and placebo were conducted. Diluent and placebo was injected into the chromatographic system with the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. The chromatogram of blank solution showed no peaks at the retention time of rabeprazole and levosulpride peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of rabeprazole and levosulpride in formulations. Similarly chromatogram of Placebo solution showed no peaks at the retention time of rabeprazole and levosulpride peak revealing that the placebo used in sample preparation do not interfere in estimation of rabeprazole and levosulpride in formulations (tablets).

b) Linearity: In this study linearity studies were made with five point's calibration curve at a concentration range of 150-450µg/ml for rabeprazole and 40-120µmg/ml for levosulpride respectively. The response of the drugs was found to be linear in the investigation concentration range and the linear regression equation for rabeprazole was **y = 52422x -6467** with correlation coefficient 1.0[**Fig:4.A]** and for levosulpride was **y = 65417x + 23715** with correlation coefficient 0.999 [**Fig:4.B].**The regression results of rabeprazole and levosulpride are reported in **Tables.2.A&B** respectively. The LOD value for rabeprazole and levosulpride were found to be 2.980µg/ml and 2.835µg/ml, respectively and the LOQ value 9.934µg/ml and 9.414µg/ml, respectively.

c) Precision: The results of Intra-day and inter-day precision studies for rabeprazole and levosulpride are represented in respective chromatograms and the results were reported in **Table.3** respectively. The RSD values for intraday precision and interday precision studies were < 2.0 % for rabeprazole and levosulpride confirming that the developed RP-HPLC method was precise.

d) Accuracy: Recovery studies of rabeprazole and levosulpride were determined at three different concentration levels. The mean recovery for rabeprazole and levosulpride was within the ICH limits and these results indicated that the proposed method is accurate[**Table.4]**.

e) Robustness: The robustness study of the present RP-HPLC assay method for rabeprazole and levosulpride were established in all slight variance in chromatographic conditions. The assay value of the test preparation solution was not affected and it was in accordance with that of actual. In addition the system suitability parameters were also found satisfactory; hence, the analytical method was concluded as robust (**Table.5**).

f) Assay in formulations: This validated method was applied to the determination of rabeprazole and levosulpride in commercially available Rabim-LS tablets. The observed concentrations of rabeprazole and levosulpride were found to be 19.95mg/ml (Mean) and 74.96mg/ml by the proposed method respectively and the results of this assay (n = 6) yielded 99.93% and 99.94% of label claim for rabeprazole and levosulpride, respectively. The results of this assay (**Table.6**) indicated that the developed method is selective for the analysis of both rabeprazole and levosulpride in formulations without interference from the excipients.

CONCLUSIONS

The RP-HPLC method developed for quantitative determination of rabeprazole and levosulpride was found to be linear, accurate, precise, rapid and specific. The method was fully validated showing satisfactory data for all method validation parameters tested. The developed method can be conveniently used by quality control departments to determine the rabeprazole and levosulpride and assay in regular production samples and also in stability samples.

ACKNOWLEDGMENTS

We would like thank to Dept. of Chemistry, Acharya Nagarjuna University, Guntur, A.P., India, for giving good support.

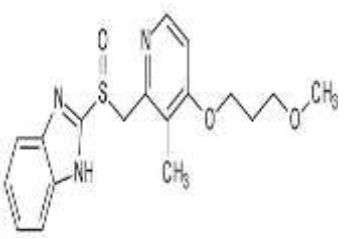

FIG.1: STRUCTURE OF RABEPRAZOLE

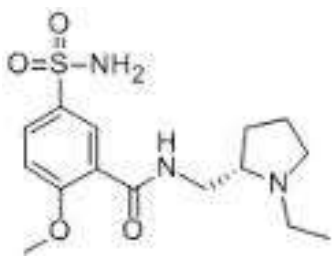

FIG.2: STRUCTURE OF LEVOSULPRIDE

FIG: 3. TYPICAL HPLC CHROMATOGRAM OF RABEPRAZOLE AND LEVOSULPRIDE

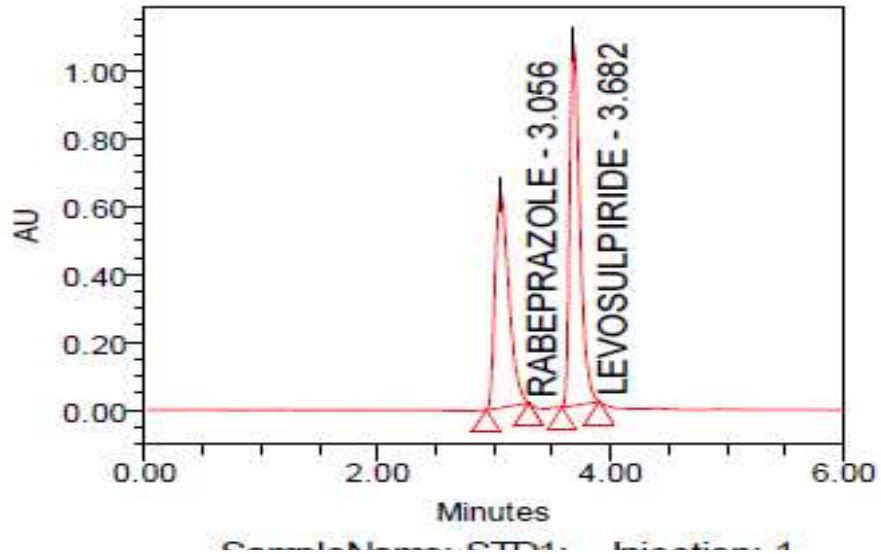

FIG: 4.A. LINEARITY CURVE OF RABEPRAZOLE

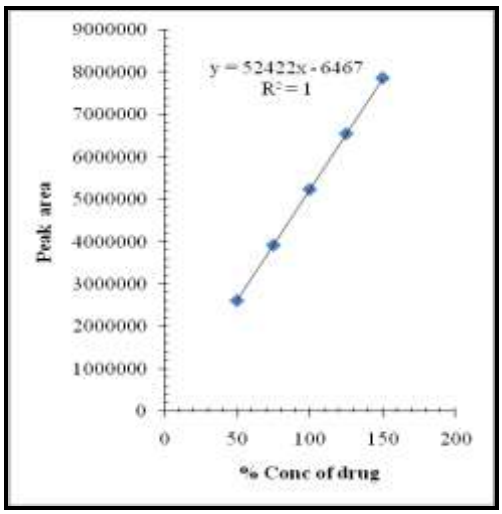

FIG: 4.B. LINEARITY CURVE OF LEVOSULPIRIDE

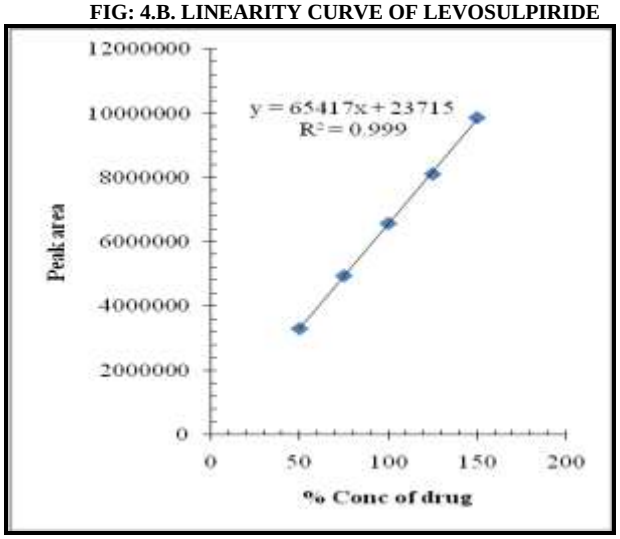

TABLE: 1

SYSTEM SUITABILITY PARAMETERS FOR RABEPRAZOLE AND LEVOSULPRIDE

NAME OF THE COMPOUND	RETENTION TIME	THEORETICAL PLATES	TAILING FACTOR	USP RESOLUTION
RABEPRAZOLE	3.058	3089	1.470	-
LEVOSULPRIDE	3.682	8710	1.541	3.239

TABLE:2.A&B:LINEARITY STUDIES FOR RABEPRAZOLE AND LEVOSULPRIDE BY THE PROPOSED METHOD

LINEARITY STUDY FOR RABEPRAZOLE		
% LEVEL (APPROX.)	CONC. µg/mL	AREA
50	150.00	2616511
75	225.00	3921989
100	300.00	5236655
125	375.00	6546329
150	450.00	7857061
Slope		52422
Intercept		-6467
RSQ(r2)		1.00
LOD (µg/mL)		2.980
LOQ (µg/mL)		9.934

LINEARITY STUDY FOR LEVOSULPRIDE		
% LEVEL (APPROX.)	CONC. µg/mL	AREA
50	40	3295145
75	60	4946086
100	80	6598990
125	100	8236402
150	120	9883633
Slope		65417
Intercept		23715
RSQ(r2)		0.9999
LOD (µg/mL)		2.835
LOQ (µg/mL)		9.414

TABLE: 3:METHOD PRECISION (INTER AND INTRADAY) STUDIES FOR RABEPRAZOLE AND LEVOSULPRIDE

METHOD PRECISION BY PROPOSED METHOD		
FOR RABEPRAZOLE		FOR LEVOSULPRIDE
METHOD PRECISION (INTER & INTRA DAY)		METHOD PRECISION (INTER & INTRA DAY)
Set-1	5237956	6595041
Set-2	5237373	6595506
Set-3	5230127	6593543
Set-4	5231824	6597298
Set-5	5235488	6592308
Set-6	5230792	6596048
Over All Avg.	5233927	6594957
Over All Std Dev.	3441.956	1788.609
Over All %RSD	0.657	0.0271

TABLE.4: RECOVERY STUDIES (ACCURACY) OF FOR RABEPRAZOLE AND LEVOSULPRIDE

Level (%)	Theoretical Concentration[a] (µg/ml)	Observed Concentration[a] (µg/ml)	% Recovery	% RSD
50	50.34	50.42	100.17	0.49
100	99.40	97.58	98.17	1.68
150	148.73	145.83	98.05	1.38
50	20.85	20.73	100.58	0.32
100	40.03	40.00	100.08	1.23
150	59.64	60.20	99.07	1.37

[a]Average of three determinations

TABLE.5

ROBUSTNESS STUDIES OF THE PROPOSED RP-HPLC METHOD

ROBUST CONDITIONS		RABEPRAZOLE			LEVOSULPRIDE		
		THEORETICAL PLATES	RT	PEAK AREA	THEORETICAL PLATES	RT	PEAK AREA
FLOW RATE	0.8 ml/min	4218	3.063	5230641	6555	3.618	6568898
	1.2 ml/min	3815	3.057	5224994	5982	3.611	6575829
TEMP	40^{O}C	4137	3.057	5264291	6279	3.612	6635470
	45^{O}C	3863	3.052	5235848	5923	3.615	6604124

TABLE.6

ANALYSIS OF MARKETED TABLETS BY THE PROPOSED METHOD[RABIM-LS]

DRUG	LABAL CLAIM	QUANTITY FOUND*	%ASSAY
RABEPRAZOLE	20	19.95	99.93
LEVOSULPRIDE	75	74.96	99.94

*Average of six determinations

REFERENCES

1. The Merck Index-An Encyclopedia of Chemicals, Drugs and Biologicals (14th edition), Merck Research Laboratories, Whitehouse Station,New Jersey,USA,2006.
2. Brahmbhatt D.D, Patel M. B., Patel J.C. Development and validation for Estimation of Levosulpiride by Area Under Curve and Difference Spectrophotometric Method, International Journal of ChemTech Research Vol.4, 945-950,2012.
3. Agrawal Y.P, Gautam S.P, Verma A., Agrawal M.Y., Gupta A.K. Simultaneous estimation of esomeprazole and levosulpiride in solid dosage form, Pelagia Research Library ,(3):337-342, 2012.
4. Manjunath S., Chouhan V., S.Sandeep Spectrophotometric Estimation of Levosulpiride In Bulk Drug And Formulations, International Journal of Pharmacy and Pharmaceutical Sciences Vol 3, Issue 2, 135-137,2011.
5. Jain M.S., Agrawal Y.S., Chavhan R.B., Bari M.M., Barhate S. D. UV Spectrophotometric Methods for Simultaneous Estimation of Levosulpiride and Esomeprazole in Capsule Dosage Form, Asian Journal of Pharmaceutical Analysis; Vol. 2: Issue 4, 106-109,2012.
6. Birajdar A.S, Subramania N.M., Bojraj S. Application of UV-Spectrophotometry and RP-HPLC for Simultaneous Determination of Rabrprazole and Domperidone in Pharmaceutical Dosage Form, Pelagia Research Library, 1 (3): 69-78, 2010.
7. Sahu D.,Agrawal Y.P, Sabarwal N., Jain A.,Gupta A.K. Simultaneous Estimation of Aceclofenac & Rabeprazole Sodium In Solid Dosage Form Asian Journal of Biochemical and Pharmaceutical Research Issue 3 (Vol. 1) ,66-75,2011.
8. Baldha R G., Patel V.B.,Mayank Bapna Simultaneous Spectrophotometric Estimation of Rabeprazole Sodium and Domperidone in combined dosage forms, International Journal of PharmTech Research, Vol.2, No.2, 1563-1568,2010.
9. S. Shilpa, T. Sai Annapurneswari, V. Jayathirtha Rao, B. Rajesh, P.Venkateshwarao, B. China Raju Simultaneous Estimation and Validation of Levosulpiride and Rabeprazole Sodium in Bulk and Pharmaceutical Dosage Form by RP-HPLC Method, Journal of Pharmacy Research,5(10),5010- 5013,2012.