Int J Pharm Bio Sci 2012 Oct; 3(4): (P) 718 – 726

Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOSULPIRIDE AND RABEPRAZOLE SODIUM

NANDAKISHORE AGARWAL[1] AND B.JAGADEESH[*2]

1, 2.Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawada-521456.

ABSTRACT

A simple, economic, selective, precise, and stability-indicating Reverse phase High Performance Liquid Chromatography method for analysis of Levosulpiride and Rabeprazole sodium, was developed and validated according to ICH guidelines. The quantification of the drug was carried out using Hypersil BDS C_{18} 250mm × 4.6mm × 5µm or its equivalent in isocratic mode, with mobile phase compressing of Buffer: Acetonitrile (72:28) the flow rate was 1.5ml/min and the detection was carried at 282 nm. The retention time for Levosulpiride and Rabeprazole sodium was found to be 2.23 and 7.27min respectively. The percent assay was found to be 99.7%. The method was also applied for the determination of Levosulpiride and Rabeprazole sodium in the presence of their degradation products formed under variety of stress conditions. Proposed method was validated for precision, accuracy, linearity range, specificity and robustness.

KEY WORDS: Levosulpiride and Rabeprazole sodium, RP-HPLC, stability-indicating, Validation.

B.JAGADEESH
Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawada-521456.

Corresponding author

INTRODUCTION

Levosulpiride is chemically 5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxy benzamide. Levosulpiride is a purified levo-isomer of sulpiride. It is not official in any pharmacopoeia. Compared with racemic and dextro-forms, the levo-form of sulpiride has greater central antidopaminergic activity, antiemetic and antidyspeptic effects and lower acute toxicity. Rabeprazole is chemically[1] 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[2], used in the treatment of GERD[3] and duodenal ulcers[4]. It is a prodrug – in the acid environment of the parietal cells it turns into active sulphenamide form. Rabeprazole inhibits the H+, K+ATPase of the coating gastric cells and dose-dependent oppresses basal and stimulated gastric acid secretion.

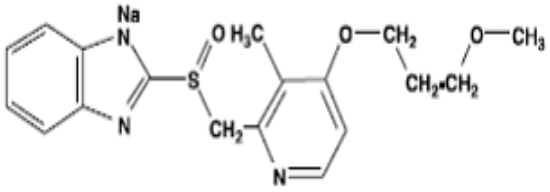

Figure 1
Rabeprazole Sodium

Levosulpiride is a D2-dopamine receptor antagonist and commonly prescribed to patients with psychosis, depression and functional dyspepsia. At low doses, Levosulpiride increases dopaminergic neurotransmission, primarily by blocking of the dopamine autoreceptors, which inhibits the pre-synaptic dopamine synthesis and release of dopamine[5]. Compared with racemic and dextro-forms, the levo-form of sulpiride has greater central anti-dopaminergic activity[6], antiemetic and anti-dyspeptic effects and lower acute toxicity[7].

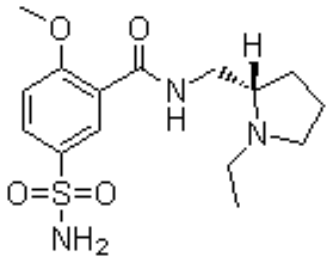

Figure 2
Levosulpiride

Literature review reveals that UV[8, 9], HPLC[10, 11] methods for Levosulpiride alone or in combined dosage forms and various UV[12, 13], HPTLC[14, 15], HPLC[16, 17] methods for Rabeprazole sodium alone or in combined dosage forms. The aim of the present study was to develop accurate, precise and selective reverse phase HPLC methods for the simultaneous analysis of Levosulpiride and Rabeprazole sodium.

MATERIALS AND METHODS

Chemicals and Reagents

Levosulpiride and rabeprazole sodium was obtained as gift samples from Dr.Reddys Laboratory, Hyderabad, and Andhra Pradesh, India. Acetonitrile (HPLC grade), Methanol (HPLC grade), Potassium dihydrogen ortho phosphate (AR grade) and Sodium hydroxide (AR grade) were of reagent grade.

Instrumentation

A HPLC (LC-2010 (SHIMADZU) with UV/VIS Detector/PDA detector and Hypersil BDS C_{18} 250mm × 4.6mm × 5µm column was used with auto sampler injector was used. The HPLC system was equipped with Empower2 software for data processing.

Chromatographic Condition

The mobile phase containing Buffer: Acetonitrile (72:28) was found to resolve Levosulpiride and Rabeprazole sodium. Sodium Hydroxide solution was used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 nylon membrane filter and then ultrasonicated for 30 min. The flow rate was set to1.5ml/min. The drug shows good absorbance at 282 nm, which was selected as wavelength for further analysis.

Buffer Preparation

Accurately weighed and transfer 13.6gm of potassium dihydrogen ortho phosphate into 1000ml of water and adjust P^H Sodium Hydroxide to 7.4. Filter the solution through 0.45µm nylon filter paper.

Preparation of Mobile Phase

Preparedly filtered and degassed mixture of buffer and Acetonitrile in the ratio of 72:28 v/v

Diluent solution

Methanol (HPLC grade) and Mobile phase.

Preparation of Standard solution

Weighed and transferred accurately about 25 mg of Rabeprazole sodium and 75mg of Levosulpiride working standard into a 100ml volumetric flask add 50 ml of methanol, sonicated for 15 minutes and make up to the mark with methanol (this is standard stock solution)

Transferred 5.0 ml of the above solution in to 25ml volumetric flask made up to the mark with mobile phase. Filter the solution through 0.45µm nylon filter.

Preparation of Sample solution

Crush 20 tablets and transferred accurately weighed powder equivalent to one tablet into 100ml volumetric flask add 50ml of methanol sonicate to dissolve and make up to the volume with methanol. Filter the solution through whatmann filter paper no.42.

Transfer 5ml of above solution into 25ml volumetric flask and make up to the volume with mobile phase.

METHOD VALIDATION

1) System Suitability/System Precision

System Suitability was performed by injecting six replicate injections of standard solutions of Levosulpiride and rabeprazole sodium at 100% and expressed as %RSD of peak area.

2) Linearity of Levosulpiride and rabeprazole sodium

The linearity of the HPLC method was demonstrated for Levosulpiride and rabeprazole sodium solutions ranging from 60% to 140% of standard concentrations.

3) Specificity

To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system.

4) Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of sample application was carried

out using six replicates of the same sample concentration.

5) Accuracy (%Recovery)

%Recovery studies were carried out at three different levels of 80%, 100% and 120% of standard solution (i.e. Levosulpiride and rabeprazole sodium API spiked to the placebo) in triplicate in each level.

6) Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, column temperature and buffer composition which may differ but the responses were still within the specified limits.

7) Ruggedness

The variability of the results obtained with the analysis of Levosulpiride and Rabeprazole sodium sample solution six times by two different analysts, two different reagents, two different columns, two different instruments on two different days to assess the method ruggedness.

8) Solution Stability

Stability of Levosulpiride and Rabeprazole sodium in analytical solution was verified by analyzing the sample solution initially and also at different time intervals of about 2, 4, 8 and 12 hours by storing at 25^0c and expressed in %RSD of peak areas.

Forced Degradation study

Thermal Degradation

Heat the sample solution at 60°c for 24 hr and analyzed the sample as per the test method.

Acid degradation

Sample solution was treated with 1.0ml of 0.1N hydrochloric acid and kept on bench top for 20minutes and analyze the treated sample.

Base degradation

Sample solution was treated with 0.5ml of 0.1N sodium hydroxide and kept on bench top for 5minutes and analyze the treated sample.

Peroxide degradation

Sample solution was treated with 1.0ml of 3.0%v/v solution of hydrogen peroxide and kept on bench top for 20minutes and analyze the treated sample.

UV Degradation

Sample was exposed to UV light under 254nm for 24 hrs and analyze sample as per the test method.

Humidity Degradation

Sample was exposed in humidity desicator 97%RH for 24 hrs and analyze sample as per the test method.

RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for Levosulpiride and Rabeprazole sodium. The Mobile phase Acetonitrile: buffer (phosphate buffer, 32:68) was found to be satisfactory and gave symmetric and well resolved peak for Levosulpiride and Rabeprazole sodium.

Table 1
System suitability data

Parameter	Levosulpiride	Rabeprazole
Tailing Factor	1.342	1.067
Theoretical Plates	4282	10362
%RSD of Peak area	0.102	0.068

The correlation coefficient (r^2) was found to be 0.999 and shows good linearity. The data of the calibration curve was given in Table.2 & 3.

Table 2
Linearity data for Rabeprazole sodium

Level	Concentration (µg/ml)	Peak Area
60%	24.0	913179
80%	32.0	1209462
100%	40.0	1513750
120%	48.0	1803566
140%	56.0	2102412
	Slope	**37157.1**
	Intercept	**22189.20**
	Correlation Coefficient	**0.99998**

Table 3
Linearity data for Levosulpiride

Level	Concentration (µg/ml)	Peak Area
60%	90.0	658162
80%	120.0	865765
100%	150.0	1078475
120%	180.0	1278526
140%	210.0	1485767
	Slope	**7096.5**
	Intercept	**25888.43**
	Correlation Coefficient	**0.9943**

The resolution between Levosulpiride and Rabeprazole sodium was found to be 23.89 which indicate good separation of compounds & chromatograms were shown in fig.3 & 4.

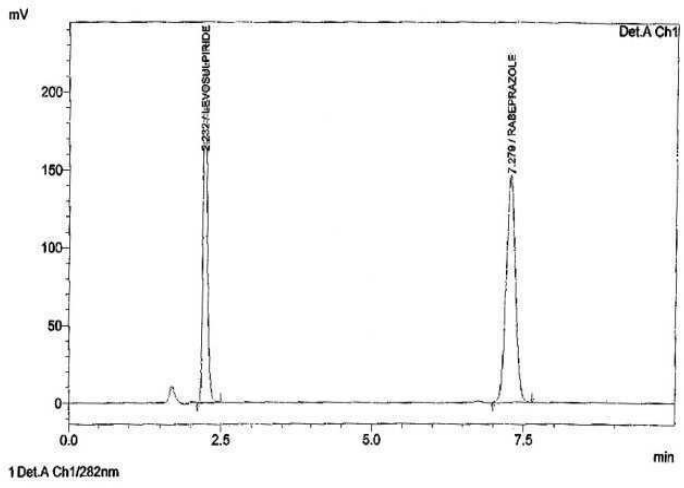

Figure 3 Standard chromatogram

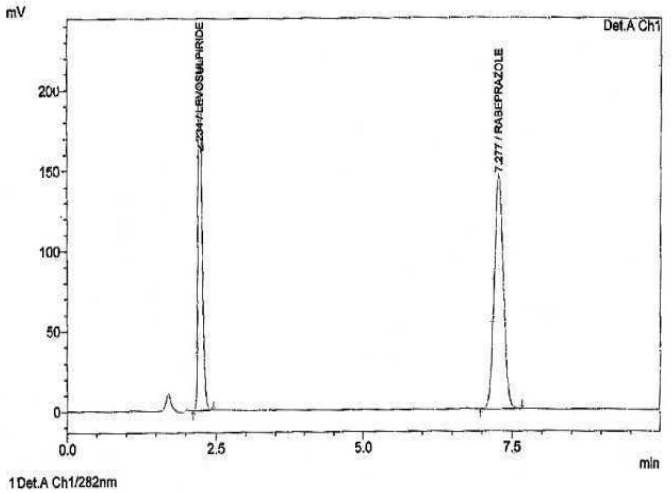

Figure 4 Sample chromatogram

Precision was determined & the results are represented in the form of %RSD which was found to be below 2% & shows that the test method was highly precise and results given in Table-4.

Table 4
Repeatability data

Parameter	Levosulpiride	Rabeprazole
Mean	**99.7**	**99.7**
SD	**0.20**	**0.37**
%RSD	**0.20**	**0.37**

The % mean recovery for Levosulpiride and Rabeprazole sodium was found as 99.54% & 98.94%.The results were summarized in Table.5.

Table 5
Accuracy data

Drug	%Level	Mean Peak Area	% Recovery	Mean recovery
Levosulpiride	80%	866956	99.87	99.54%
	100%	1077567	99.57	%RSD-0.342
	120%	1286388	99.19	**Mean recovery**
Rabeprazole	80%	1204073	98.97	98.94%
	100%	1503390	99.14	%RSD
	120%	1798628	98.70	0.224

As part of the robustness, deliberate changes in the flow, column temp & buffer composition was made to impact on the method. RT was significantly changed but within the acceptance limit and results given in Table.6.

Table 6
Robustness data

Parameter	Peak Area of Levosulpiride	% Assay	Peak Area of Rabeprazole	% Assay
Actual	1079339	99.73	1538578	100.25
High Flow Rate	973181	100.13	1342808	100.60
Low Flow Rate	1190818	100.25	1634062	100.60
High Temperature	1083257	99.97	1459616	100.55
Low Temperature	1065961	99.33	1454245	99.90
High PH	1082646	99.36	1443794	100.10
Low PH	1086658	98.60	1459966	99.60

The % bias for ruggedness was within the limit i.e. less than 2.0 and results shown in Table.7.

Table 7
Ruggedness data

Parameter	Levosulpiride		Bias (%)	Rabeprazole		Bias (%)
	Day-1	Day-2		Day-1	Day-2	
Mean assay	99.7%	99.1%	+0.6%	99.7%	98.98%	+0.72%
SD	0.20	0.49	-	0.37	0.66	-
%RSD	0.20	0.49	-	0.37	0.66	-

Solution Stability was determined and the results are represented in the form of %RSD which is below 2 and shows that the drug was stable in corresponding environment and results given in Table.8.

Table 8
Solution Stability data

Time in hrs	Peak Area Levosulpiride	Peak Area Rabeprazole
Initial	1080547	1416774
2	1069644	1401612
4	1070046	1401826
8	1078970	1406721
12	1072172	1399469
%RSD	0.28	0.15

Result of Force Degradation Studies
The study showed that slight degradation observed when treated with acid, base, peroxides, UV (254nm), Humidity, and Heat and photo stability conditions after heating. The %assay and %degradation of drug is within specified limit and results were given in Tables.9 & 10.

Table 9
Forced degradation data Rabeprazole Sodium

SR.NO.	Condition	%Assay	% Degradation
1	Control sample	100.50	------
2	Acid degradation	100.85	-0.35
3	Base degradation	100.95	-0.45
4	Peroxide degradation	100.95	-0.45
5	Thermal degradation	100.80	-0.35
6	Sun light degradation	100.60	-0.10
7	UV degradation	100.90	-0.40
8	Humidity degradation	100.35	0.15

Table 10
Forced degradation data Levosulpiride

SR.NO.	Condition	%Assay	% Degradation
1	Control sample	99.52	------
2	Acid degradation	99.77	-0.25
3	Base degradation	99.43	0.09
4	Peroxide degradation	99.68	-0.16
5	Thermal degradation	99.92	-0.40
6	Sun light degradation	99.93	-0.41
7	UV degradation	99.93	-0.41
8	Humidity degradation	99.75	-0.23

CONCLUSION

It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust, rugged and reproducible for the simultaneous analysis of Levosulpiride and Rabeprazole Sodium with less tailing and is also economical.

ACKNOWLEDGEMENT

This work was supported by spectrum pharma research solutions,#301,NandiniResidency, Addagutta, NearJNTU, Kukatpally, AndhraPradesh,Hyderabad-500072 India.

REFERENCES

1. 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.
2. The Martindale 35th ed: The complete drug reference, published pharmaceutical press, lambeta high street, londan SEI 75M, UK. 2006.
3. 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.
4. 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.
5. Mucci A, Nolfe G and Maj M: Levosulpiride A review of its clinical use in psychiatry. Pharmacology and Respiratory 1995; 31: 95-101.
6. Tonini M, Cipollina L, Poluzzi E, Crema F, Corazza G and De Ponti F: Clinical implications of enteric and central D2 receptor blockade by antidopaminergic gastrointestinal prokinetics. Aliment Pharmacology and Therapeutics 2004; 19: 379-90.
7. Lozano R, Peralta Concha M, Montealegre A, de Leon A, Ortiz Villalba J and Esteban H: Effectiveness and safety of levosulpiride in the treatment of dysmotility-like functional dyspepsia. Therapeutics and

Clinical Risk Management 2007; 3: 149-155.
8. Venkatesh chouhan*, Shobha manjunath, S.Sandeep, Spectrophotometric estimation of levosulpiride in bulk drug and formulations, Int J Pharm Pharm Sci, Vol 3, Issue 2, 2011, 135-137.
9. Yogesh P. Agrawal, Surya Prakash Gautam, Ajay Verma, Mona Y. Agrawal and Arun K. Gupta, Simultaneous estimation of esomeprazole and levosulpiride in solid dosage form, Pelagia Research Library Der Pharmacia Sinica, 2012, 3 (3):337-342.
10. S.P. Silambarasan, K. Anandakumar, R. Venkatalakshmi and C. Sasikala, Development of UV Spectrophotometry and RP-HPLC Methods for the Estimation of Levosulpiride in Bulk and in Tablet Formulation Asian J. Research Chem. 3(3): July- Sept. 2010, 542-544.
11. Snehalatha.t*, Padmalatha.m, Ramya.s, Kanakadurga.m, A simple and validated rp-hplc method for the simultaneous estimation of rabeprazole and levosulpiride in bulk and pharmaceutical dosage forms, int. res j pharm. app sci., 2012; 2(2):99-106.
12. *N.B. Dobaria, N. H. Vadia and S. J. Rajput, Simultaneous Spectrophotometric Estimation of Rabeprazole Sodium and Domperidone Maleate in their Combined Pharmaceutical Dosage Form, International Journal of Chem Tech Research, Vol.1, No.4, pg.no-1162-1166, Oct-Dec 2009.
13. Patel Vandana. B.*, Baldha R. G, and Mayank Bapna, Simultaneous Spectrophotometric Estimation of Rabeprazole Sodium and Domperidone in combined dosage forms, International Journal of PharmTech Research, Vol.2, No.2, pp 1563-1568, April-June 2010.
14. Janhavi R Rao*, Vishal V Bharekar, Toufik S Mulla, Savita S Yadav, and milind P Raj, validated hptlc method for simultaneous estimation of rabeprazole sodium and aceclofenac in bulk drug and formulation, international journal of comprehensive pharmacy 2011, 5 (06).
15. Mallikarjuna rao.N*, Development and Validation of Stability Indicating HPTLC method for Simultaneous Estimation of Paracetamol, Aceclofenac and Rabeprazole in Combined Tablet Dosage Formulation, International Journal of PharmTech Research, Vol. 3, No.2, pp 909-918, April-June 2011.
16. Prasanna Reddy Battu* and MS Reddy, Development and Validation of RP-HPLC for the Rabeprazole sodium in Pharmaceutical formulations and Human Plasma, Asian J. Research Chem. 2(1):49-51, Jan.-March, 2009.
17. Vaithiyanathan Sree Janardhanan*, Rajappan Manavalan and Kannappan Valliappan, Stability-indicating HPLC method for the simultaneous determination of pantoprazole, rabeprazole, lansoprazole and domperidone from their combination dosage forms, Int. J. Drug Dev. & Res., Oct-Dec 2011, 3 (4): 323-335.