ISSN: 2279 - 0594

Available Online at www.jbpr.in

Journal of Biomedical and Pharmaceutical Research 3 (3) 2014, 75-80

RESEARCH ARTICLE

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LEVOSULPIRIDE AND PANTOPRAZOLE IN TABLETS BY RP-HPLC METHOD

Kalaiselvi P.[1], K. G. Lalitha[2]*

[1]Asistant Professor, Department of pharmaceutical chemistry, JKKMMRF College of pharmacy, B. Komarapalayam, Tamil Nadu, India

[2]Professor, Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai, Tamil Nadu, India

Received 05 May 2014; Accepted 28 May 2014

ABSTRACT

A simple, precise, accurate, rapid and economical reverse phase high-pressure liquid chromatographic method has been developed as per ICH norms for the simultaneous estimation of Levosulpride and pantoprazole sodium from pharmaceutical formulation. The method was carried out on a Column – C_{18} (250mm x 4.6mm x5 µ) with a mobile phase consisting of buffer (adjusted to pH6.8 with 1% triethylamine): Acetonitrile (65:35v/v) and filtered through 0.45µ cellulose nitrate filters. The flow rate 1.0mL/min. Detection was carried out at 280 nm. The retention time of LVS and PNT was 2.41 and 6.86 min respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Key words: RP-HPLC, Levosulpride, Pantoprazole, Validation

INTRODUCTION:

Levosulpiride[1] (LVS) is an N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxy-5-sulfamoylbenzamide. It is a new orally effective antipsychotic and a prokinetic agent reported to be a selective antagonist of dopamine D_2 receptor activity on both central and peripheral levels. Levosulpride is also claimed to have mood elevating property and used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome, and premature ejaculation. Pantoprazole sodium[10] (PNT) is a (RS) 5-(difluoromethoxy)-2-[[(3, 4-dimethoxy]-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole. It is a proton pump inhibitor drug that inhibits gastric acid secretion used for short-term treatment of erosion and ulceration of the esophagus caused by gastro esophageal reflux disease.

Levosulpiride and pantoprazole sodium of combined tablet dosage form in the ratio of 75mg and 40mg. Literature survey reveals that Levosulpride can be estimated by spectrophotometric methods[2-5] HPLC [6-8] and by HPTLC[9] individually or with other drugs in bulk. In pantoprazole sodium can estimated by Rp- HPLC[11-18] .But combination of Levosulpiride and pantoprazole sodium not estimated by HPLC method .So we planned to develop a RP- HPLC method and to a validate according to ICH norms [19]-[20]

MATERIALS AND METHODS:

Reagents and chemicals:

Acetonitrile HPLC grade was procured from E. Merck Ltd. Mumbai. Methanol, Triethylamine AR grade were procured from Qualigens Fine Chemical, Mumbai. Water HPLC grade was obtained from a milli - QRO water purification system. Reference standards Levosulpiride and pantoprazole were procured from Sunglow Pharmaceutical Ltd., Pondicherry, India respectively.

Apparatus and chromatographic conditions

Chromatographic separation was performed on HPLC-shimodzo module prominence with UV- Visible SDP 20A detector. Column – C_{18} (250 x. 4.6mm, 5 µm) was used for the separation. Mobile phase of 0.05M potassium dihydrogen ortho phosphate of buffer (Adjusted to pH6.8 with1% triethylamine): Acetonitrile in the ratio (65:35v/v) and filtered through the 0.45 µ cellulose nitrate filter and degassed and used for the separation. The flow rate was 1ml/min and inject volume was 20µL with detection at 280 nm and analysis was performed at ambient temperature.

Preparation standard Solution:

Standard stock solution was equivalent to 150 mg of Levosulpiride and 90 of Pantoprazole sodium were prepared separately using 10ml of methanol. The solutions were sonicated for 15 mins and make up with

*Corresponding author: Dr. K.G.Lalitha | Email: kg.lalitha@gmail.com

100ml volumetric flask by using diluent. From above the stock solutions 300µg/ml of LVS and 180µg/ml of PNT concentrations were prepared.

Prerparation of sample solution:

Weigh 20 tablets (Pantocid-L) contain 75mg LVS and 40mg PNT were weighed and finely powdered. A quantity of powder equivalent to 958 mg for weighed and transferred to 100ml standard flask. Then 10ml of methanol was added and sonicated for 15 mins. Then the volume was making up with diluent and filtered. Further 300µg/ml of LVS and 180 µg/ml of PNT solutions were prepared. Then 20 µL of standard and sample solutions were injected.

ASSAY METHOD:

With optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The typical chromatogram of sample solution was shown in Fig No.1 the retention time of LVS and PNT was found to be 2.41 and 6.86 min respectively. This procedure was repeated with the sample solution obtained from the formulation. The concentration of the drug was calculated using the following formula.

$$\frac{\text{Sample area X Std dilution X Potency X Average Wt of the tablet}}{\text{Std Area X Sample Dilution X100}}$$

METHOD VALIDATION:

Accuracy:

The Accuracy of the method was performed by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table No. II

System Suitability Studies:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and presented in Table No I. The values obtained demonstrated the suitability of the system for the analysis of the drug combinations. System suitability parameter may fall within ±3% standard deviation range during routine performance of the method.

Specificity:

The specificity defined as the ability of method to measure the analyte accurately and specifically in the presence of components present in the sample matrix, was determined by analysis of chromatograms of drug free and drug a formulation. There was no interference form sample and peak purity of LVS and PNT were 0.9994 and 0.9997 respectively. It showed that developed analytical method was specific for LVS and PNT in tablet dosage form and were presented in Table No III and Fig No 1.

Precision

- *Method precision:*

The method precision of the developed method was established by carrying out the analysis of analyte (n=6) using the proposed method. The low value of the relative standard deviation showed that the method was precise. The percentage % RSD was calculated by LVS and PNT was 1.38 and 0.55 respectively.

- *System Precision:*

The system precision of the developed method was established by six replicate injections of the standard solution containing both the analytes of interest. The percentage RSD was calculated by LVS and PNT was 0.47 and 0.25 respectively.

Linearity and Range:

Linearity and range five point standard curves for both compounds were constructed by drawing peak area versus Levosulpiride and pantoprazole sodium concentration using ranging from 240 - 360µg/ml (for LVS) and 145 - 215µg/ml (for PNT) processed separately and run in duplicate daily for 3-consecutive days and the results were tabulated in Table No 4 and Fig No 2, 3. The slope and intercept value for calibration curve was Y=9.89x +138.72 (R^2=0.9994) for LVS and Y=30.229x+274.26(R^2=0.9997) for PNT .The results shows that an excellent correlation exists between response factor and concentration of drugs within the concentration range.

Standard and Sample Solution Stability:

Standard and sample solution stability was evaluated at room temperature and refrigerator temperature for 24h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were stable up to 24h at room temperature and refrigerator temperature.

Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instruments like HPLC- Shimodzo module prominence. UV- Visible SDP 20A detector. Column – C_{18} (250 x 4.6mm x5 µ) by different operators using different columns of similar type like Kromacil C_{18} column and Inertial-ODS C_{18} column. Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The results of ruggedness studies, the percentage of LVS were calculated as 98.54-100.10%w/w and PNT was calculated as 100.21-100.72% w/w. The percentage %RSD robust for

LVS at flow rate of ±0.2ml/min were 0.23 and 1.04 respectively and wavelength ±2 nm were 0.63 and 0.47 respectively. The percentage %RSD robust for PNT at flow rate of ±0.2ml/min was 0.12 and 0.16 respectively and wavelength ±2 nm was 0.07 and 0.77 respectively.

RESULTS AND DISCUSSION:

Estimation of Levosulpiride and Pantoprazole in dosage forms:

The HPLC procedure was optimized with a view to develop precise and stable assay method. Both the pure drugs Levosulpiride and Pantoprazole were run in different mobile phase compositions to provide an appropriate chromatographic separation, but the proposed method with the column –C_{18} (250 x 4.6 x5µ), with mobile phase of a mixture of 0.05m potassium dihydrogen ortho phosphate buffer (adjusted to pH 6.8 with 1% triethylamine) : Acetonitrile in the ratio (65:35v/v) and delivered at a flow rate 1ml/min with detection at 280nm gave sharp and symmetrical peaks with retention time 2.42 and 6.86 min for LVS and PNT respectively. The resolution factors at the above said condition was the typical chromatogram of sample solution was show in Fig No1 which illustrates the separation of both active ingredients in the system. The assay procedures were repeated for six times and mean peak area and weight of standard drugs were calculated. The percentage of individual drugs found in formulations, mean, standard deviation were calculated and presented in Table No I. The result of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations. The proposed method is simple and does not involve laborious time consuming sample preparation.

Table 1: Analytical Parameters

Parameters	Levosulpiride	Pantoprazole
Linear dynamic range	240-360(µg/ml)	145-215(µg/ml)
Retention time	2.41	6.86
Resolution	12.478	
Theoretical plates	8066	53970
Tailing Factor	1.120	1.321
R^2 value	0.9994	0.9997
LOD (µg/mL)	1.29	1.42
LOQ (µg/mL)	3.94	4.30

Table 2: Accuracy (Recovery Studies)

% Target	Levosulpiride (Area)	% Recovery	(% Recovery and % RSD	Pantoprazole (Area)	% Recovery	(% Recovery and % RSD
80%	915.143	98.14	98.35/0.39	2633.10	97.15	97.24/0.15
	914.861	98.12		2640.72	97.42	
	921.304	98.81		2633.21	97.15	
100%	1156.02	99.19	99.20/0.10	3299.40	97.38	98.35/0.1.64
	1155.04	99.11		3301.77	97.45	
	1157.53	99.32		3395.79	100.22	
120%	1429.02	102.17	102.55/0.32	3952.31	97.21	97.21/0.04
	1441.14	1102.74		3954.77	97.25	
	1434.55	1102.74		3950.93	97.17	

Table 3: The Determination of Levosulpiride and Pantoprazole in tablet dosage form

Components	Label Claim (mg/tablet)	N	Amount Present	Percentage Label Claim (%w/v)
Levosulpiride	75	3	75.26±1.38	100.35± 1.38
Pantoprazole	40	3	40.40±0.55	101.02±0.55

Table 4: Linearity for Levosulpiride and Pantoprazole

Level	Area of Levosulpiride	Area of Pantoprazole
80%	925.016	2671.420
90%	1030.160	3000.450
100%	1138.160	3332.670
110%	1227.050	3596.200
120%	1321.390	3884.990

Table 5: Method Ruggedness of Levosulpiride and Pantoprazole in dosages forms

Day	Analyst	Instruments	%Recovery	
			Levosulpiride	Pantoprazole
1	1	1	98.22	100.12
1	2	1	100.10	100.72
2	1	2	98.54	100.46
2	2	2	99.54	99.32

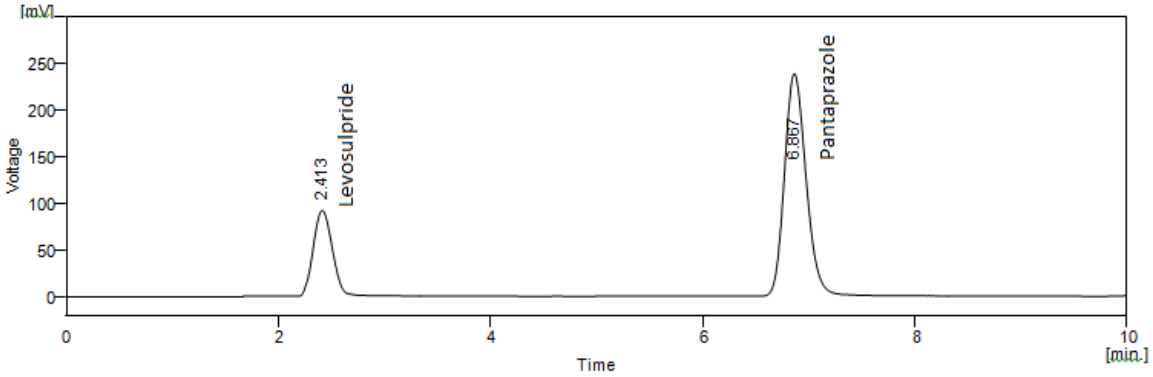

Figure 1: Chromatogram

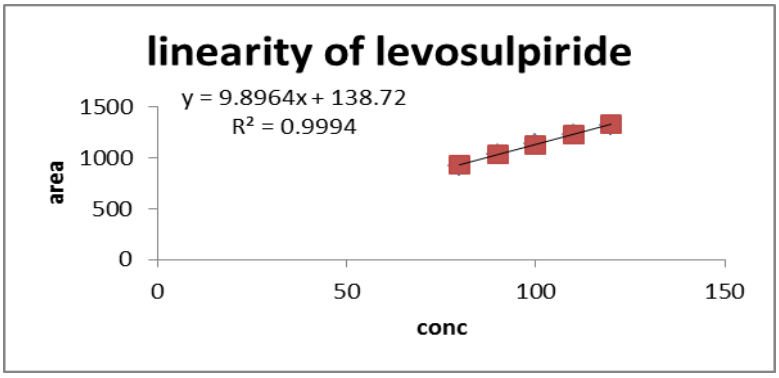

Figure 2: Calibration curve for Levosulpiride

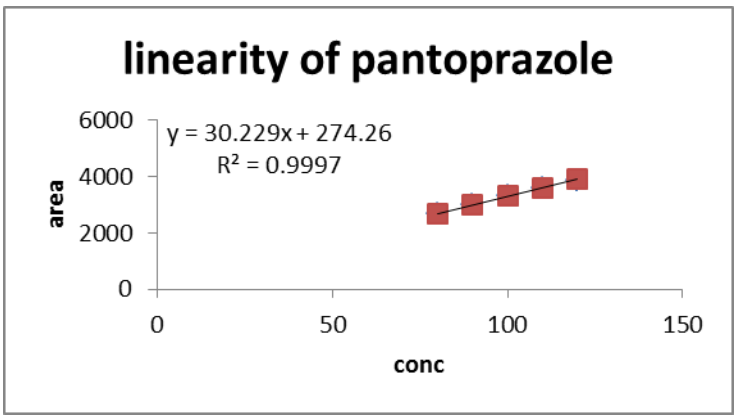

Figure 3: Calibration curve for Pantoprazole

CONCLUSION:

The proposed RP-HPLC method simultaneous estimation of Levosulpiride and Pantoprazole in combined dosages forms has not been reported. The presented method for determination of two ingredients at single wavelength is sufficiently, the way of the two active ingredients was not interfered by excipients in the products. The RP-HPLC method is suitable for the quality control of the raw materials and formulations.

REFERENCES:

1. The Merck Index (2006), "An Encyclopedia of chemicals, Drugs and Biological, 14, Whitehouse station, NJ, USA, 1542.
2. Jain Manu S. Agrawal Yogesh S., Chavhan Randhir B., Bari Manoj M., Barhate S.D.: UV Spectrophotometeric Methods for Simultaneous Estimation of Levosulpiride and Esomeprazole in capsule Dosage From, Asian J. Pharm. Ana, 2012,2(4), 106-109.
3. Shobha Manjunath, Venkatesh Chouhan, Sandeep.S.: Spectrophotometric estimation of levosulpiride in bulk drug and formulations. International Journal of Pharmacy and Pharmaceutical Sciences, 2011, 3(2), 135-137.
4. Dhwani D. Brahmbhatt, Mandev B. Patel, Jignesh C. Patel.: Development and validation for Estimation of Levosulpiride by Area Under Curve and Difference Spectrophotometric Method, International Journal of Chem Tech Research, 2012, 4(3),945-950.
5. Yogesh P. Agrawal, Surya Prakash Gautam, Ajay Verma, Mona Y. Agrawal, Arun K. Gupta. :Simultaneous estimation of esomeprazole and levosulpiride in solid dosage form. Der Pharmacia Sinica, 2012, 3(3), 337-342.
6. Sravan Kumar.K., Prasada Rao. M, Sambasiva Rao. G,: Method Development and validation of rabeprazole and levosulpiride in pharmaceutical dosage form by using Rp-Hplc method. International Journal of Pharmacy and Integrated Life Sciences, 2013, 1(11), 174-182.
7. Rakesh R. Jain, Pravin O. Patil, Sanjay B. Bari. : Simultaneous estimation of esomeprazole and levosulpiride in bulk and in capsule formulation by Rp-Hplc. Chil. Chem. Soc, 2013 58,(3)1846-1849.
8. Atulita Mullapudi, Sirisha Pingali, Tata Santosh.: Development and Validation of a stability indicating method for the simultaneous determination of Levosulpiride and Rabeprazole by High Performance Liquid Chromatography. International journal of pharmaceutical drugs and analysis. 2013 1, (1), 25-36.
9. Shreenidhi surve, Arpit patwari, Jiten patel, Ishwarsinh Rathod, Mahesh Chhabria. : Hptlc and Hplc method development and validation for simultaneous estimation of rabeprazole sodium and levosulpiride in bulk and its pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 2013, 3(3), 65-67.
10. The Merck Index (2006), "An Encyclopedia of chemicals, Drugs and Biological, 14, Whitehouse station, NJ, USA, 1209.
11. Sivakumar.T, Manavalan.R, Valliappan.K. :Development and validation of a reversed-phase hplc method for simultaneous determination of domperidone and pantoprazole in pharmaceutical dosage forms. Acta chromatographia, 2007, 18, 130-142.
12. Prasanna Reddy Battu and N. Kiran Kumar Reddy.:Development and validation of RP-HPLC for the pantoprazole sodium sesquihydrate in pharmaceutical dosage forms and human plasma. International Journal of Chem Tech Research, 2009. 1, (2), 195–198.
13. A. G. Tated, F. A. Khan, C. K. Gadewar, R. L. Bakal, A. P. Dewani, and A. V. Chandewar. :Method development and validation for simultaneous determination of Naproxen and Pantoprazole sodium

in capsule dosage form by RP-HPLC. International Journal of Pharmaceutical Research and Development, 2011, 3, (5), 8–14.

14. Prasanna Reddy.B, Mathsa.Jayaprakash Kotta.Sivaji, G.T.Jyothesh Kuamr E.C.Surendranath Reddy, B.Ravindra Reddy. :Determination Of Pantoprazole Sodium And Lansoprazole In Individual Dosage Form Tablets By Rp- Hplc Using Single Mobile Phase. International Journal of Applied Biology and Pharmaceutical Technology, 2010, I(2), 683-688.

15. Kampati Anil Kumar, Sree.V. Janadharanan, Yarasi Surendranath Reddy, Vanam Naveen Kumar. : Method Development and validation for simultaneous estimation of pantoprazole sodium and itopride hydrochloride in its bulk dosage forms by rp-hplc. International Journal of Pharmacy and Pharmaceutical Sciences, 2013, 5, (1), 190-194.

16. Mohideen. S, Shivakanth. M, SureshKumar. P, Navaneetha Krishnan.S, Surendranath. Y, Satyanarayana. T. : Development and validation of analytical method for naproxen and pantoprazole in capsule dosage form. Der Pharmacia Sinica, 2011, 2(6), 114-121.

17. Suryadevara Vidyadhara, Yarraguntla Srinivasa Rao, Anne Ramu, Reddyvalam Lankapalli Sasidhar, Anne Jaya Ramya. :Method Development and Validation for the Simultaneous Estimation of Cinitapride and Pantoprazole in Solid Dosage Forms By RP-HPLC. Oriental Journal Of Chemistry, 2013,29 (3), 1213-1220.

18. Saurabh Pandey, Preeti Pandey, Durgesh Mishra, Umesh Kumar Singh.: A validated stability indicating HPLC method for the determination of process-related impurities in pantoprazole bulk drug and formulations. Brazilian Journal of Pharmaceutical Sciences, 2013, 49,(1), 175-184**.**

19. ICH, Q2B, (R1): Validation of analytical procedures: Text and methodology, Fedral Register, 1996.

20. ICH, Q2, (R1): Validation of analytical procedures: Text and methodology, Geneva, 2005.

