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Analytical Method Development and Validation of Esomeprazole and Levosulpiride in their Combined Capsule Dosage Form by RP-HPLC

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ABSTRACT

A new simple, accurate, rapid and precise isocratic Reverse Phase High performance liquid chromatographic (HPLC) method was developed and validated for the determination of Esomeprazole (ESO), and Levosulpiride (LEVO) in capsule formulation. The Method employs Shimadzu HPLC system on Hypercil BDS C18 (25 cm × 4.6 mm i.e., 5 µm) and flow rate of 1 ml/min with a load of 20µl. Acetonitrile and Phosphate buffer was used as mobile phase in the composition of 50:50 at 3.5 PH. The Detection was carried out at 240 nm. Linearity ranges for Esomeprazole and Levosulpiride were 20-60 µg/ml, 37.5-225 µg/ml respectively. Retention Time of Levosulpiride and Esomeprazole were found to be 3.367 min, 4.320 min respectively. Percent Recovery study values of Esomeprazole and Levosulpiride were found to be within 98-102%. This newly developed method was successfully utilized for the Quantitative estimation of Esomeprazole and Levosulpiride in pharmaceutical dosage forms. This method was validated for accuracy, precision, linearity and Robustness as per ICH guidelines.

KEYWORDS

Esomeprazole, Levosulpiride, RP-HPLC, Validation, Simultaneous Estimation

INTRODUCTION

Esomeprazole (ESO)[1-4], (S)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) Methyl sulfinyl]-3H-benzoimidazole. Esomeprazole is a proton pump inhibitor which reduces acid secretion through inhibition of K+/H+ ATPase in gastric parietal cells. By inhibiting the functioning of this enzyme, the drug prevents formation of gastric acid. Gastro esophageal Reflux Disease (GERD) is a Condition in which the digestive acid in the stomach comes in contact with the esophagus (food pipe). The irritation caused by this disorder is known as heartburn. Long term contact between the acid and esophagus can cause permanent damage to the Esophagus.

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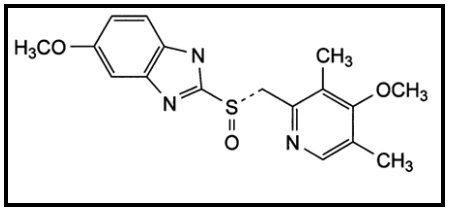
Esomeprazole reduces the production of digestives acids, thus minimizing their effect on the esophagus. Levosulpiride, (LEVO) [5-7]: N-[[((S)-(-)-5-aminosulfonyl-N-[(1-ethyl pyrrolidin-2-yl) methyl]-2-methoxybenzamide. A substituted benzamide anti-psychotic, reported to be a selective antagonist of central dopamine (D-2, D-3 and D-4) receptors. Levosulpiride is used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome and premature ejaculation. (Infant vomiting).Chemical structures are shown in Fig 1.



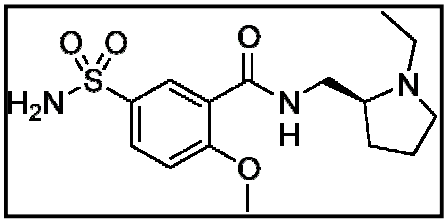


Figure: 1 Chemical Structure of Esomeprazole and Levosulpiride

MATERIAL AND METHOD

Instrumentation

A High performance liquid chromatography Shimadzu LC 20 AT system Equipped with SPD – 20 A Shimadzu UV – VIS Detector with the Spin chrome Software. All weighing's are done on Shimadzu – libror AGE – 220 balance.

Reagents and Standards

Esomeprazole powder was kindly supplied by Sun Pharma Advanced Research Centre Vadodara. Levosulpiride powder was kindly supplied by Amanath Pharmaceuticals, Pondicherry.

HPLC grade Methanol and Acetonitrile were purchased from Rankem, India RFCL ltd, New Delhi .Analytical Water (HPLC grade) were purchased from (Rankem, India, RFCL ltd, New Delhi) Triethylamine and Orthophosphoric acid were purchased from Merck pvt LTD.

Esomeprazole and Levosulpiride combination capsule (Sompraz-L), Esomeprazole 40mg, Levosulpiride 75mg are manufactured by Sun Pharma ltd, Sikkim, India) were purchased from the local Pharmacy.

Optimized Chromatographic Conditions

Chromatographic conditions were optimized after using mobile phase, acetonitrile: buffer (Phosphate buffer: pH 3.5 ± 0.1) (50:50). The flow rate was 1 ml/min and the detector was set at 240 nm. The separation was achieved on Hypercil BDS C18 (25 cm × 4.6 mm i.e., 5 µm).

Preparation of Standard Stock Solution

ESO: Accurately weighed amount of standard of Esomeprazole 40 mg was transferred to 50 ml of volumetric flask. It was dissolved and diluted up to mark with mobile phase (S_1=800 mcg/ml).

LEVO: Accurately weighed 75 mg of Levosulpiride to 50 ml of volumetric flask. It was dissolved and diluted up to mark with mobile phase (S_2=1500 mcg/ml).

Preparation of Sample Solution (Marketed formulation)

ESP: 10 ml of S1 solution was taken in 100 ml volumetric flask and diluted up to mark with mobile phase (S3=80 mcg/ml).

LEVO: 10 ml of S2 solution was taken in 100 ml volumetric flask and diluted up to mark with mobile phase (S4=150 mcg/ml).

Assay Procedure

Inject 20 µL of the standard and sample solutions into the chromatographic system and measure the areas for the Esomeprazole and Levosulpiride peaks (Fig.2). The amount of Esomeprazole and Levosulpiride present in commercial Capsule was calculated by comparing the peak area of standard and sample.

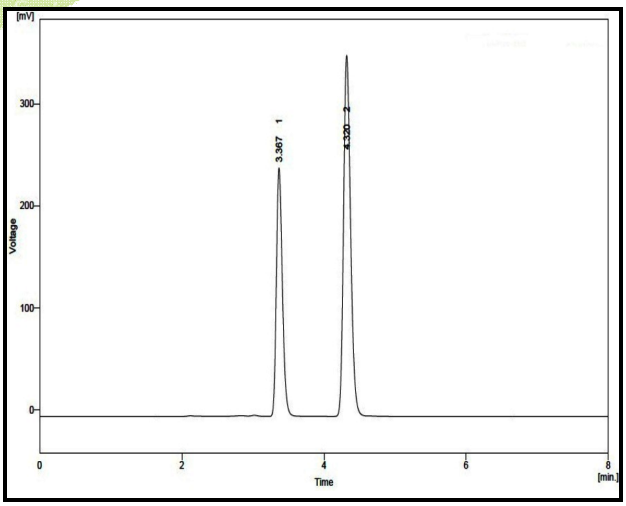


Figure: 2 Chromatogram of Sample Solution (ESO 40µg/ml & LEVO 150µg/ml)

VALIDATION OF ANALYTICAL METHOD [8-13]

Linearity

Aliquots of standard solutions of Esomeprazole and Levosulpiride in 20-120 µg/mL and 37.5-225 µg/mL respectively, was prepared from working standard solution and injected to chromatographic system and analyzed. The graph of peak area obtained versus respective concentration was plotted. The mean area with its standard deviation and % relative standard deviation of peak were calculated. (Fig-3, 4)

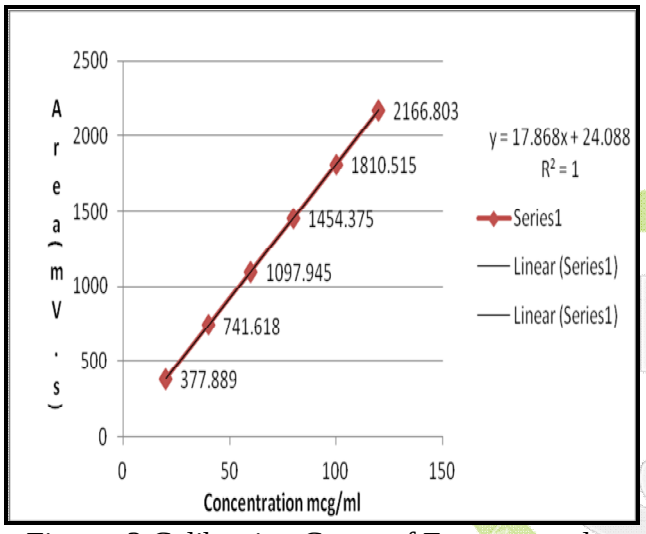


Figure: 3 Calibration Curve of Esomeprazole

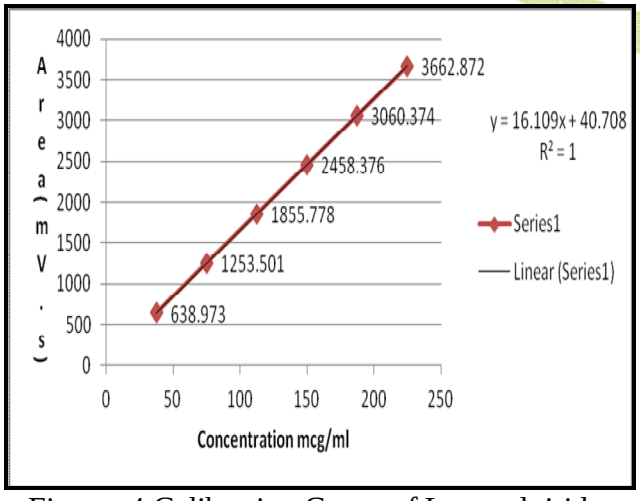


Figure: 4 Calibration Curve of Levosulpiride

Precision

Precision study was assessed by Replicate injection of any one concentration. For injection mixed standard solution of ESO & LEVO was injected in replicate. In this method was confirmed by low %RSD values of peak area for all components.

Intraday Precision

Standard solutions Esomeprazole (20,80 and 120 µg/mL) and Levosulpiride (37.5, 150, and 225µg/mL) were prepared from working standard solution and injected in to system with stated chromatographic conditions5.2.1.3 and analyzed as described under 1.9.4.1 three times in a day. (Table-5.5)

Interday precision

Table: 1 Intraday Precision of esomeprazole and Levosulpiride

Esomeprazole			Levosulpiride		
Conc. (µg/mL)	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/mL)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
20	631.1603 ± 2.224047	0.35238	37.5	373.235 ±1.300059	0.34832
80	2430.54 ±8.572182	0.35269	150	1432.644 ± 5.044776	0.35213
120	3538.161 ±21.40112	0.60498	225	2092.961 ± 12.56823	0.6005

Standard solutions Esomeprazole (20,80 and 120 µg/mL) Levosulpiride (37.5, 150, and 225µg/mL) were prepared from working standard solution and injected in to system with stated chromatographic conditions 5.2.1.3 and analyzed as described under 1.9.4.2 up to three days (Table-1, 2).

Table: 2 Intermediate precision of esomeprazole and Levosulpiride

Esomeprazole			Levosulpiride		
Conc (µg/ml)	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
20	628.6577 ±5.976821	0.950728	37.5	371.77 ±3.5422812	0.952815
80	2402.335 ±9.598572	0.399552	150	1421.251 ± 5.681505	0.399754
120	3608.45 ±21.727	0.602115	225	2134.479 ± 12.81653	0.600452

Accuracy

Accuracy may often be expressed as percentage recovery. The accuracy was determined by standard addition method. To a fixed amount of pre-analyzed sample (1.0 ml) mixture of Esomeprazole (800µg/mL) and Levosulpiride (1500 µg/mL) increasing amount of its working standard solution (0.8,1.0,1.2ml of 800µg/mL of Esomeprazole and 0.8,1.0,1.2 ml of 1500µg/ml Levosulpiride) were added in three different 10 ml volumetric flask and made up to mark with Mobile phase. Samples were injected to system and analyzed as described in 1.9.3 respectively. The mean % recovery from of peak areas calculated. Accuracy of method was determined by standard addition method at three different concentrations Esomeprazole and Levosulpiride in the range of calibration. Percent Recovery was found to be 99.604%-101.91% and 99.60%-101.365% of Esomeprazole and Levosulpiride, respectively, depicted in (Table-3, 4.)

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak height with a signal-to-noise ratio higher than 10. LOD and LOQ values of ESO & LEVO were reported in (Table-5.)

Table 3: Accuracy data of Esomeprazole

Level of Recovery (%)	Conc. of Sample (µg)	Conc. Added (µg/ml)	Total conc. (µg/mL)	Conc. Recovered (µg/mL)	% Recovery	Mean % Recovery	%RSD
0	80	0	80	79.638	99.604	---	---
80	80	64	144	146.63	101.83	101.183	1.006
80	80	64	144	146.48	100.01		
80	80	64	144	146.47	101.71		
100	80	80	160	162.89	101.81	101.367	0.671
100	80	80	160	162.86	101.79		
100	80	80	160	162.77	100.583		
120	80	96	176	179.36	101.91	101.343	0.9345
120	80	96	176	179.29	101.87		
120	80	96	176	179.28	100.25		

Table: 4 Accuracy data of Levosulpiride

Level of Recovery (%)	Conc. of Sample (µg)	Conc. Added (µg/mL)	Total conc. (µg/ml)	Conc. Recovered (µg/ml)	% Recovery	Mean % Recovery	%RSD
0	150	0	150	149.4	99.6	---	---
80	150	120	270	274.833	101.79	101.176	0.999
80	150	120	270	274.671	101.73		
80	150	120	270	274.206	100.01		
100	150	150	300	305.94	101.96	101.46	0.769
100	150	150	300	305.88	101.86		
100	150	150	300	305.67	100.56		
120	150	180	330	337.275	101.203	101.149	0.339
120	150	180	330	337.261	101.89		
120	150	180	330	337.25	100.365		

Table: 5 LOD and LOQ of Esomeprazole and Levosulpiride

Parameters	ESO	LEVO
Standard deviation of the Y- intercepts of the 5 calibration curves	0.4136	0.0619
Mean slope of the 5 calibration curves	0.0558	0.4675
L.O.D. = 3.3 × (SD/Slope) (µg/mL)	0.0934	0.04557
L.O.Q. = 10 × (SD/Slope) (µg/mL)	0.2831	0.1381

Robustness

To evaluate robustness of the method few parameters were deliberately varied.

The parameters included variation of flow rate, change in pH of buffer and change of mobile phase ratio.

The change was made at 3 levels (-2, 0, +2) and replicate for 3 times. The average value of %

Table: 6 Robustness data of Esomeprazole and Levosulpiride

Parameters varied	Drug name	Parameters (n=3)		
		Mean retention time ± %RSD	Mean Area ± %RSD	Theoretical plate± %RSD
As per method optimized	ESO	4.326±0.150	2424.056±0.55	9088.666±0.281
	LEVO	3.371±0.152	1434.094±0.55	7227.333±0.301
At flow rate 1.2	ESO	4.134±0.248	2325.596±0.507	8868.66±0.493
	LEVO	3.221±0.223	1375.655±0.516	7096.333±0.432
At flow rate 0.8	ESO	4.568±0.306	2562.066±0.711	8568.333±0.878
	LEVO	3.559±0.297	1515.78±0.711	7470.667±0.690
ACN: Buffer (52:48)	ESO	3.903±0.658	2183.32±1.084	8626.33±0.853
	LEVO	3.064±0.715	1294.907±0.911	7469±0.972
ACN: Buffer (48:52)	ESO	4.781±0.293	2675.851±0.807	8828.333±0.745
	LEVO	3.724±0.289	1583.192±0.805	7218.667±0.619

DETERMINATION OF ESOMEPRAZOLE AND LEVOSULPIRIDE FROM COMBINED DOSAGE FORM

Table: 7 Assay of marketed Formulation Sompraz - L

Drug	Label claim mg/tablet	Conc. estimated* (mg)	% Conc. estimated *±SD	% RSD
Esomeprazole	40mg	39.602	99.007% ± 0.141	1.0
Levosulpiride	75mg	74.252	99.003% ±0.158	1.5

SYSTEM SUITABILITY PARAMETER OF DEVELOPED METHOD

Table: 8 System Suitability Parameters

Sr. No	System Suitability Test	Observed Values		Standards
		ESO	LEVO	
1	Resolution (Rs)	-	5.610	>1.5
2	Number of theoretical plates (N)	9087	7208	Not less than 2000
3	Tailing Factor	1.3	1.5	Not greater than 2.0

RESULTS & DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of Esomeprazole and Levosulpiride in pharmaceutical dosage forms. The Retention Time of Levosulpiride and Esomeprazole were found to be 3.367 min, 4.320 min respectively.Each sample was injected five times and the peak area for drug solution was reproducible as indicated by low coefficient of variation. A good linear relationship (r = 0.999) was observed between the concentrations and respective peak areas. Precision was determined and the results are presented in the form of %RSD which is below 1.00 and shows that the proposed HPLC method was highly precise. The method was robust as observed from insignificant variation in the results of analysis by changes in flow rate, Mobile phase composition. The drug content in the Capsule was quantified using the proposed analytical method. The absence of additional peaks indicates no interference of the excipients used in the Capsule. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

CONCLUSION

A Simple, Rapid, Accurate and Precise RP-HPLC method was developed and validated for estimation of Esomeprazole and Levosulpiride in combined Capsule dosage form.

The assay values of Esomeprazole and Levosulpiride was obtained 99.007% ± 0.141 and 99.003% ±0.158 Assay of this Brand complies with the Esomeprazole assay limit (90 to 110% Esomeprazole) and Levosulpiride assay limit (90 to 110% Levosulpiride) given in the individual monograph for drug formulations in the IP 2010.

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REFERENCES

1. Indian Pharmacopoeia. Volume II, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of India Ministry of Health and Family Welfare, 2010; 1296.
2. The Merck Index. Fourteenth Edition, pp 6945; 8989.
3. http://www.drugs.com/search.php?searchterm=esomeprazole
4. http://www.drugbank.ca/drugs/DB00736

5. http://www.prescriptiondruginfo.com/drug_details.asp?title=Levosulpiride&page=1005821&ad=true
6. http://www.drugs.com/search.php?searchterm=levosulpiride
7. http://www.drugbank.ca/drugs/DB00391
8. Food and Drug Administration, Guideline on general principles of process validation. FDA, Rockville, MD 1984.
9. Validation of Analytical Procedure: Methodology, ICH Harmonized Tripartite Guidelines, 1996, 1-8.
10. Heyrman AN., Henry RA., Importance of Controlling Mobile Phase pH in RP- HPLC. Technical Bull, 1999, 1-7.
11. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedure: Text and Methodology ICH Q2 (R1). 2005.
12. Chandran S, Singh RSP., Comparison of various international guidelines for analytical method validation. Pharmacies, 2007, 62:4-14.
13. Quality Assurance of Pharmaceuticals. A compendium of guidelines and related materials, WHO Geneva, Vol. I, 1997, 19-24.