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Research Article

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

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the esomeprazole and levosulpiride in Capsule dosage form. Chromatogram was run through ODS (150mm 4.6mm, 5 μ). Mobile phase containing Buffer and Acetonitrile in the ratio of 32B:68A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.01N KH₂PO₄ pH 5.4 buffer. Temperature was maintained at 30°C. Optimized wavelength for Esomeprazole and Levosulpiride was 290nm. Retention time of Esomeprazole and Levosulpiride were found to be 2.2min and 4.0min. %RSD of the Esomeprazole and Levosulpiride were found to be 0.97 and 0.50 respectively. %Recover was Obtained as 100.08% and 101.16% for Esomeprazole and Levosulpiride respectively. LOD, LOQ values are obtained from regression equations of Esomeprazole and Levosulpiride were 0.10ppm, 0.34ppm and 1.04ppm, 0.29ppm respectively. Regression equation of Esomeprazole is $y = 10568.x + 307.3$, and of Levosulpiride is $y = 11649.x + 1207$.

Keywords: Esomeprazole, Levosulpiride, RP-HPLC

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1. Introduction

Esomeprazole is a proton pump inhibitor and Levosulpiride is substituted benzamide anti-psychotic drug. The International Journal of Medicine and Pharmaceutical Research

formulation of both these drugs in combined dosage (Capsule) form is recently launched in market by Torrent

Pharmaceuticals [1]. Levosulpiride is used for the treatment of psychosis and Esomeprazole to overcome adverse reaction of acidity caused by Levosulpiride [2]. Esomeprazole is a proton pump inhibitor which is used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GORD/ GERD) and Zollinger-Ellison syndrome. Esomeprazole is the S-enantiomer of omeprazole. It reduces acid secretion through inhibition of ATPase in gastric parietal cells. By inhibiting the functioning of this enzyme, the drug prevents formation of gastric acid [3]. Levosulpiride is a substituted benzamide anti-psychotic, reported to be a selective antagonist of dopamine D₂ receptors activity on both central and peripheral levels[4,5]. It is an atypical neuroleptic and a prokinetic agent[6,7]. It is also claimed to have mood elevating properties. Levosulpiride is used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome and premature ejaculation [8,9].

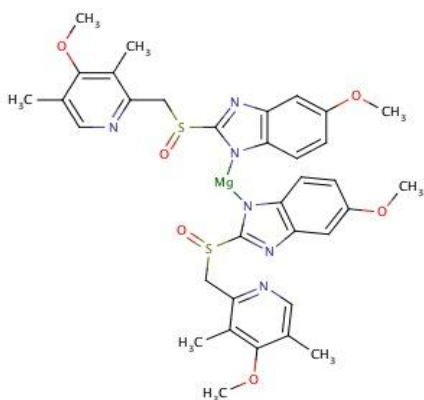


Figure 1: Structure of Esomeprazole

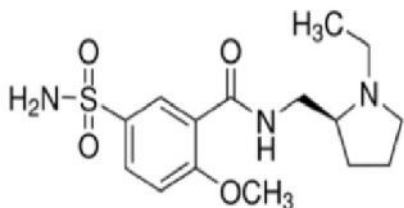


Figure 2: Structure of Levosulpiride

2. Materials and Methods

Materials:

Esomeprazole and Levosulpiride, Combination of Esomeprazole and Levosulpiride capsules, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument:

HPLC instrument was used of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special band width of 2mm and 10mm and matched quartz was be used for measuring International Journal of Medicine and Pharmaceutical Research

absorbance for Esomeprazole and Levosulpiride solutions [10, 11,12].

Methods:

Preparation of buffer:

Buffer: (0.01N KH₂PO₄)

Accurately weighed 1.36gm of sodium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 5.4 with dil. OPA[13,14,15].

Standard Preparation:

Accurately Weighed and transferred 15mg of levosulpiride and 8mg of Esomeprazole working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

5 capsules were weighed and powdered and transferred into a 50mL volumetric flask, 35mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2 ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity:

Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Esomeprazole and Levosulpiride are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 20ppm, 40ppm, 60ppm, 80ppm, 100ppm, 120ppm of Esomeprazole and 37.5ppm, 75ppm, 112.5ppm 150ppm, 187.5ppm, 225ppm of Levosulpiride.

Standard Preparation:

Accurately Weighed and transferred 15mg of levosulpiride and 8mg of Esomeprazole working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

5 capsules were weighed and powdered and transferred into a 50mL volumetric flask, 35mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2 ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Accuracy:

Standard Preparation:

Accurately Weighed and transferred 15mg of levosulpiride and 8mg of Esomeprazole working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample preparation:

50%: 5 capsules were weighed and calculate the average weight of each capsule then 750mg capsule powder was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution

0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

100%: 5 capsules were weighed and calculate the average weight of each capsule then 1500mg capsule powder was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

150%: 5 capsules were weighed and calculate the average weight of each capsule then 2250mg capsule powder was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

3. Results and Discussion

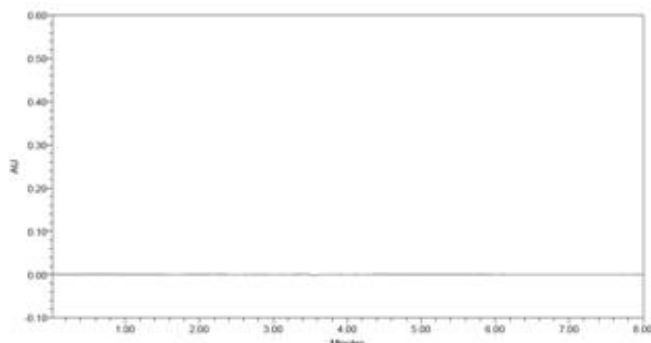


Figure 3: Chromatogram of blank

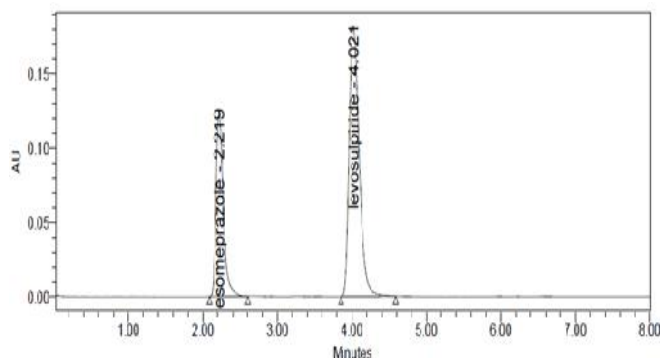


Figure 4: Typical chromatogram of Esomeprazole and Levosulpiride.

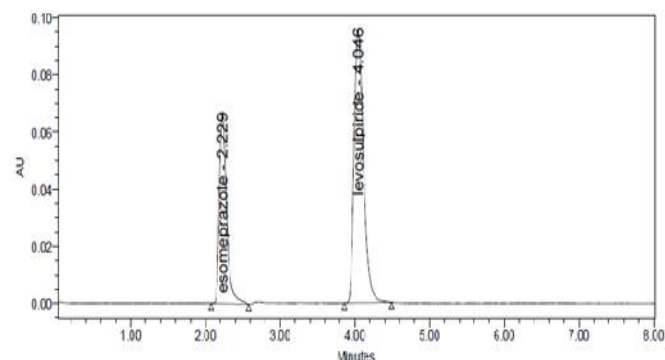


Figure 5: Linearity 50% Chromatogram of Esomeprazole and Levosulpiride method

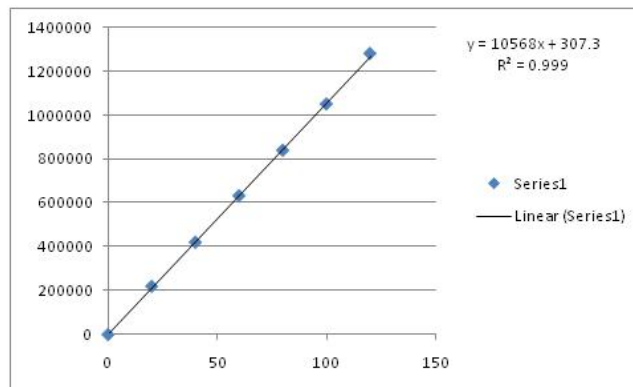


Figure 6: Calibration curve of Esomeprazole

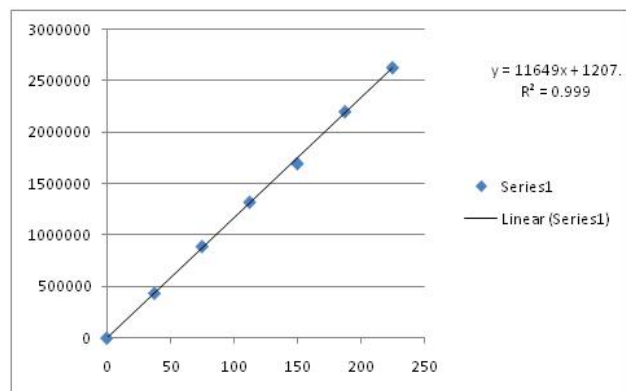


Figure 7: Calibration curve of Levosulpiride

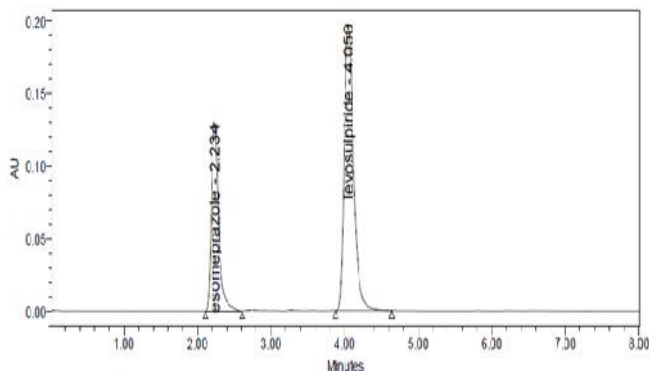


Figure 8: Linearity 100% Chromatogram of Esomeprazole and Levosulpiride method

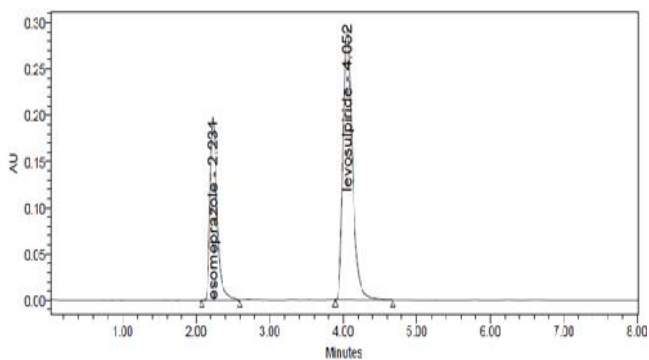


Figure 9: Linearity 150% Chromatogram of Esomeprazole and Levosulpiride method

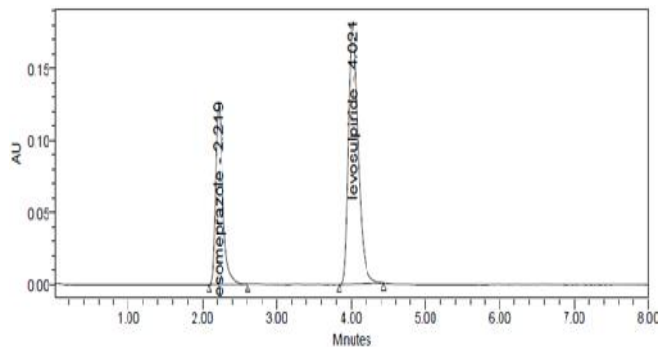


Figure 10: Repeatability Chromatogram of Esomeprazole and Levosulpiride method

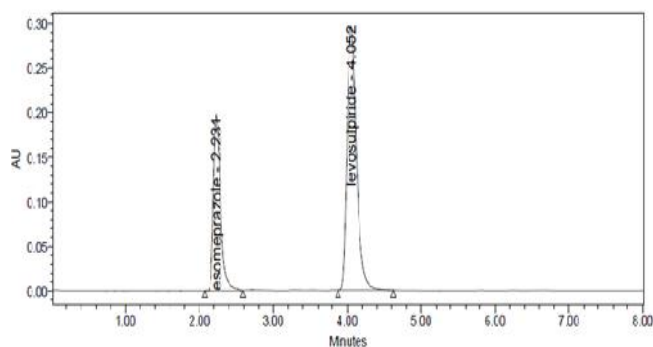


Figure 14: Accuracy 150% Chromatogram of Esomeprazole and Levosulpiride method

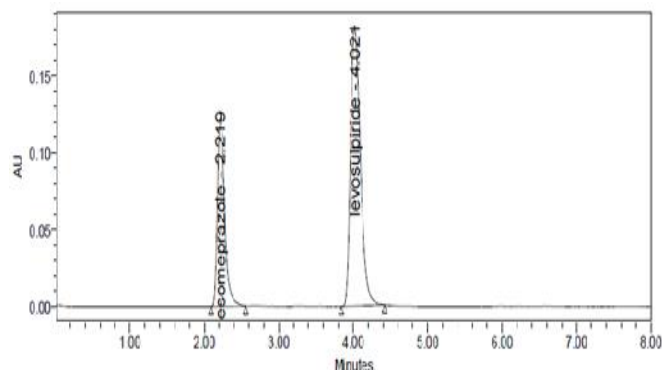


Figure 11: Inter Day precision Chromatogram of Esomeprazole and Levosulpiride method

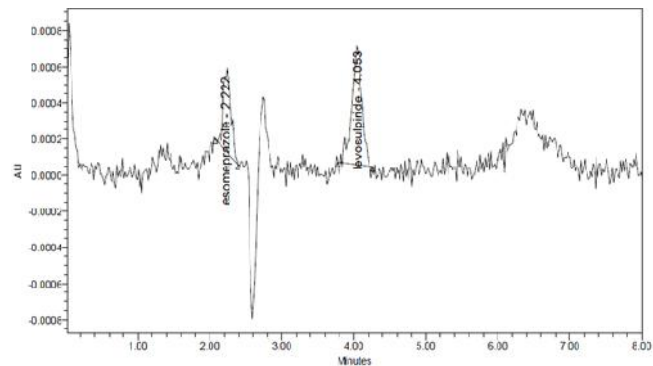


Figure 15: LOD Chromatogram of Esomeprazole and Levosulpiride method

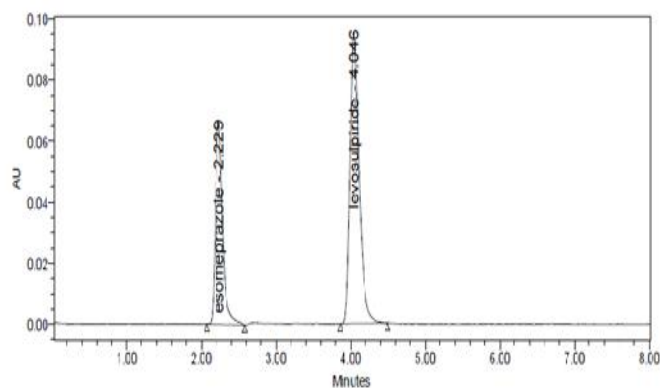


Figure 12: Accuracy 50% Chromatogram of Esomeprazole and Levosulpiride method

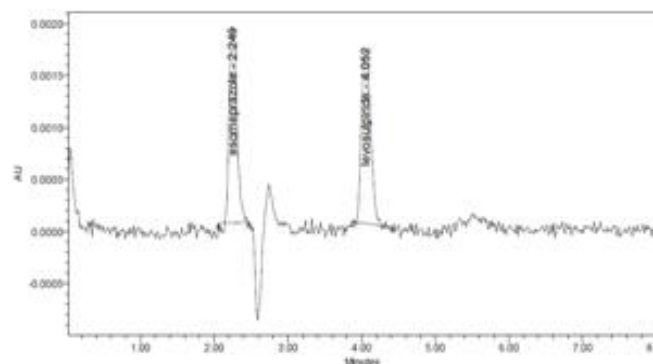


Figure 16: LOQ Chromatogram of Esomeprazole and Levosulpiride method

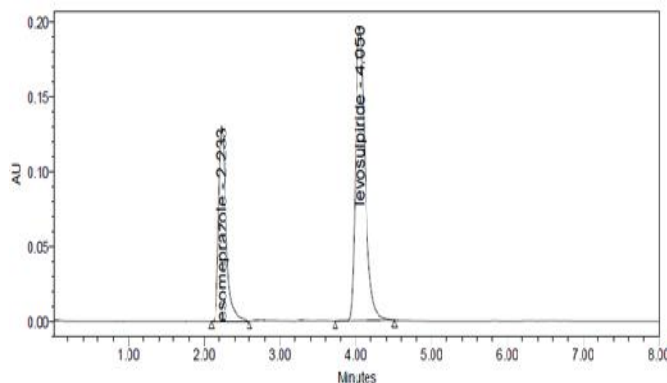


Figure 13: Accuracy 100% Chromatogram of Esomeprazole and Levosulpiride method

4. Conclusion

The method was found to be precise, accurate and linear over the linear concentration range. The method developed is unique in determining the impurities even at low levels than that of specifications. The analytical method validation of Esomeprazole and Levosulpiride in capsule dosage form by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Esomeprazole and Levosulpiride in capsule dosage form. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness and solution stability, Therefore, this HPLC method can be used as a routine analysis of these drugs in pharmaceutical formulations.

Table 1: Calibration data of Esomeprazole and Levosulpiride Method

S.No	Concentration Esomeprazole ($\mu\text{g/ml}$)	Response	Concentration Levosulpiride ($\mu\text{g/ml}$)	Response
1	0	0	0	0
2	20	219743	37.5	436194
3	40	420301	75	891483
4	60	632028	112.5	1323196
5	80	839036	150	1697713
6	100	1049902	187.5	2202932
7	120	1279655	225	2630631

Table 2: Repeatability results for Esomeprazole and Levosulpiride

S. No.	Esomeprazole	Levosulpiride
1	814986	1754081
2	825795	1754437
3	840851	1758333
4	819155	1752184
5	822084	1752359
6	839797	1754366
AVG	827111	1754293
STDEV	10835.5	2217.2
%RSD	1.3	0.1

*Average of six determinations

Table 3: Inter day precision results for Esomeprazole and Levosulpiride

S. No.	Esomeprazole	Levosulpiride
1	834986	1743927
2	843663	1732187
3	830708	1728243
4	848290	1726706
5	832084	1720428
6	848665	1739622
AVG	839732.7	1731852
STDEV	8135.6	8675.3
%RSD	1.0	0.5

Table 4: Accuracy results of Esomeprazole and Levosulpiride

Sample	Amount added ($\mu\text{g/ml}$)	Amount Recovered ($\mu\text{g/ml}$)	Recovery (%)	% RSD
Esomeprazole	40	39.04	99.97	0.2
	80	79.82	99.99	0.48
	120	120.99	100.32	0.71
Levosulpiride	75	75.72	100.250	0.79
	150	150.15	101.58	0.33
	225	225.55	101.48	0.18

5. Acknowledgement

I give my immense pleasure to express my sincere thanks to my guide Ramesh Dhani, M. Pharm, Ratnam institute of pharmacy, for giving guidance at all stages of my work.

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