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Stability Indicating Method Development and Validation for the simultaneous Determination of *Levosulpiride* and *Esomeprazole* in Bulk and Formulation

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ABSTRACT

Present study deals with the development and validation of a rapid, simple and efficient method for the simultaneous determination as well as stability studies of Esomeprazole and Levosulpiride in bulk and formulations. The method involves reverse phase High Performance Liquid Chromatography (HPLC) using stationery Phase ODS C_{18} column (250mm x 4.6 mm, 5m), Mobile phase as Phosphate buffer, Acetonitrile and methanol at the ratio of 65:30:5, subjected to isocratic elution, observed the peaks with PDA detector wavelength 254nm, maintaining the mobile phase flow rate at 1ml/minute, keeping the run time fixed for 8 minutes. Column temperature was maintained at 30^0C, p^H of the mobile phase was 3.0; complete separation of both the compounds took place within four minutes. Retention time was found 2.41 minutes and 3.56 minutes for Esomeprazole and Levosulpiride respectively. The developed new method was validated as per ICH guideline taking the parameters like accuracy, precision, linearity, limit of detection, limit of quantification, intermediate precision and robustness. In the linearity test Correlation Coefficient was found to be 0.999 for both the molecules, percentage relative standard deviation results from precision studies were 0.34 and 0.44; mean percentage recoveries in accuracy studies were found to be 100.35% and 100.14% for Esomeprazole and Levosulpiride respectively. Very low concentrations of LOD and LOQ indicate the method was highly sensitive enough. The designed validated method can be used effectively in the laboratory for regular determination of Levosulpiride and Esomeprazole in formulation and bulk form.

Keywords: Simultaneous, HPLC, Stability studies, Esomeprazole, Levosulpiride.

INTRODUCTION

Esomeprazole[1] is a gold standard proton pump inhibitor, used up to a great extant in gastroenterology, chemically known as (S)-5-methoxy-2-((4-methoxy-3, 5-dimethyl pyridine-2-yl) methyl sulfinyl)-3H-benzoimidazole. Figure I. represents the structure of Esomeprazole.

Levosulpiride[2] a substituted derivative of benzamide, GI motilator and anti-psychotic drug, was reported to be a selective antagonist of central dopamine (D-2, D-3 and D-4) receptors, Levosulpiride also has shown to have mood elevating properties. Chemically the molecule is named as N-(((2s)-1-Ethylpyrrolidin-2-yl) methyl)-2-methoxy-5-suifamoylbenzamide. Figure II. Represents structure of the molecule Levosulpiride.

Literature survey[3-9] shows that there are few methods available for determination of Esomeprazole and Levosulpiride. As such for Levosulpiride and Esomeprazole there is no stability indicating method available. Hence we developed a rapid and simple method for the above mentioned compounds.

MATERIALS AND METHOD

Instruments

HPLC make-waters 2690 detector PDA -2996. ODS C18 column (250mm x 4.6 mm, 5m). Analytical Balance- ER-180A, Microbalance-Sartorius-M500P, p^H Meter- Thermo scientific, Sonicator– Sartorius, Software- Empower V 1.2.2.1

Chemicals

HPLC grade water (Merck), Methanol (Merck), Ortho-phosphoric acid (Merck), Acetonitrile (Rankem), reference Standards (S. L. Drugs Hyderabad). Sample (Nexpro-L capsule).

Preparation of Solutions

Diluent

First the compounds were dissolved in small amount of solution of water and Acetonitrile in the ratio of 1:1 and then made up the volume with Buffer.

Buffer

(0.1% Ortho phosphoric acid): Transferred 1ml of Concentrated Ortho phosphoric acid in a 1000ml volumetric flask, added about 900ml of milli-Q water and sonicated for 15 minutes and finally made up the volume with water.

Standard Preparation

Stock solution Preparation

(800 µg/ml Esomeprazole, 1500 µg/ml Levosulpiride): Transferred 8 mg of Esomeprazole, 15 mg of Levosulpiride Standards into a 10 ml clean dry volumetric flask, added 7 ml of diluent, sonicated for 30 minutes and made up to the final volume with mobile phase.

Standard solution Preparation

(64 µg/ml Esomeprazole, 120µg/ml Levosulpiride)

From the above stock solution, 0.8 ml was pipetted out in to a 10 ml volumetric flask and then made up to the final volume with mobile phase.

Sample Preparation

Stock sample solution Preparation

(800 µg/ml Esomeprazole, 1500 µg/ml Levosulpiride) 10 tablets were weighed and calculated the average weight of each tablet, then the weight equivalent to 1 tablet (380 mg) was transferred into a 50 ml volumetric flask, 30 ml of diluent was added and sonicated for 30 min, further the volume was made up with mobile phase and filtered off.

Standard sample solution Preparation

(64 µg/ml Esomeprazole, 120 µg/ml Levosulpiride) From the filtered stock solution 0.8 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with mobile phase.

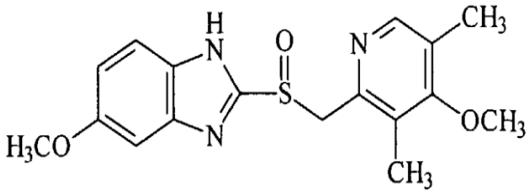

Fig.1: Structure of Esomeprazole

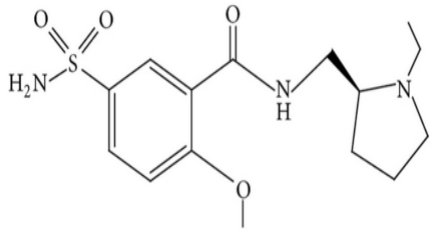

Fig. 2: Structure of Levosulpiride

Label Claim

40 mg of Esomeprazole + 75 mg of Levosulpiride

Method development

To develop a new method[10] for estimation and degradation studies several trials were conducted so that we can achieve most suitable chromatographic condition. The initial attempt was to employ as much low proportion of organic solvents for elution of the compounds. More part of aqueous solvents in mobile phase resulted in prolonging of retention time of both the compounds. Reasonable retention time, number of theoretical plates, value of tailing factors and all were found within the validation limit by using optimized Chromatographic condition.

Method Validation

The developed stability-indicating HPLC analytical method was validated following ICH guidelines[11]

Accuracy

It was conducted by recovery studies, using spiking method. 50%, 100%, and 150% of standard of Levosulpiride and Esomeprazole were spiked to pre-quantified sample solutions and the quantity recovered was estimated.

Test for Precision

It was checked by applying same concentration of compounds repeatedly for six times

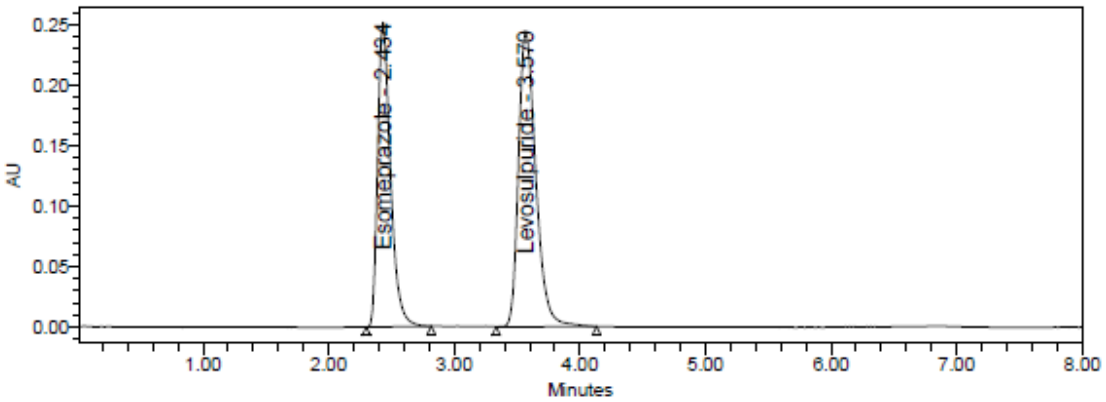

Fig. 3: A typical Chromatogram of Esomeprazole and Levosulpiride

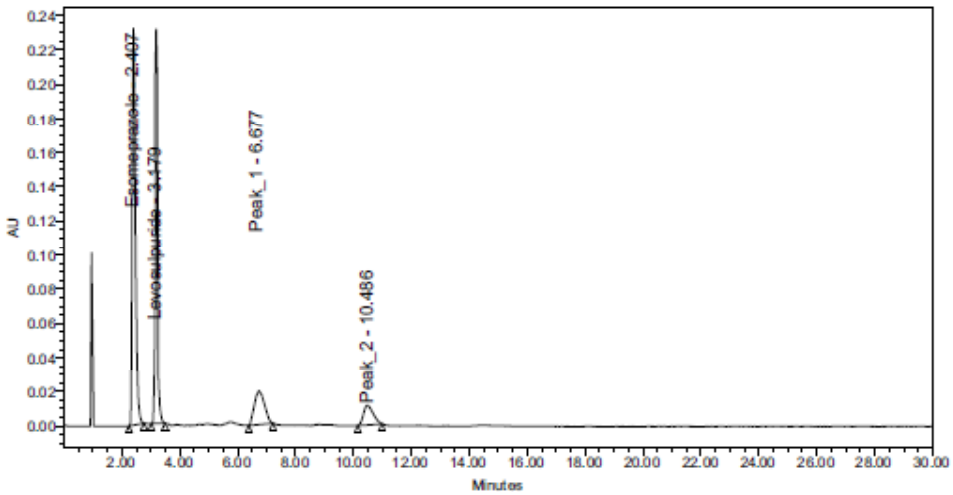

Fig. 4: Chromatogram by degraded compounds after Oxidative stress

calculated standard deviation and relative standard deviation.

Test for Linearity

For Esomeprazole the range was 16ppm to 96ppm and Levosulpiride 30ppm to 180ppm. Each Concentration was injected thrice and calculated correlation coefficient.

Intermediate precisions

The test for Intermediate precisions of this method was determined by experimenting the results in different days keeping a gap of 24 hours.

Test for LOD and LOQ

The limit of detection, the limit of quantification was determined by considering standard deviation of y intercept and slope of regression line and using them into the formula-

LOD = 3.3 × SD/Slope
LOQ= 10 × SD/Slope

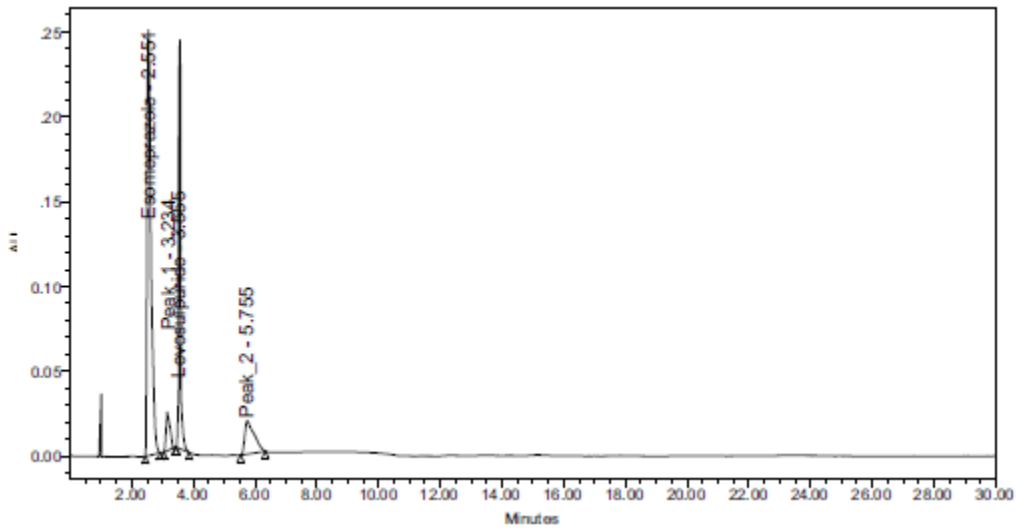

Fig. 5: Chromatogram after Acid Degradation

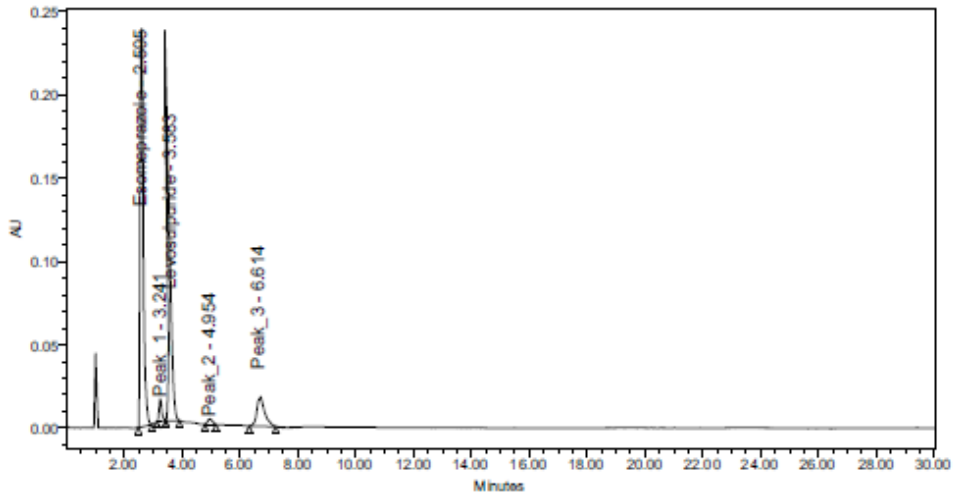

Fig. 6: Chromatogram after Alkali Degradation

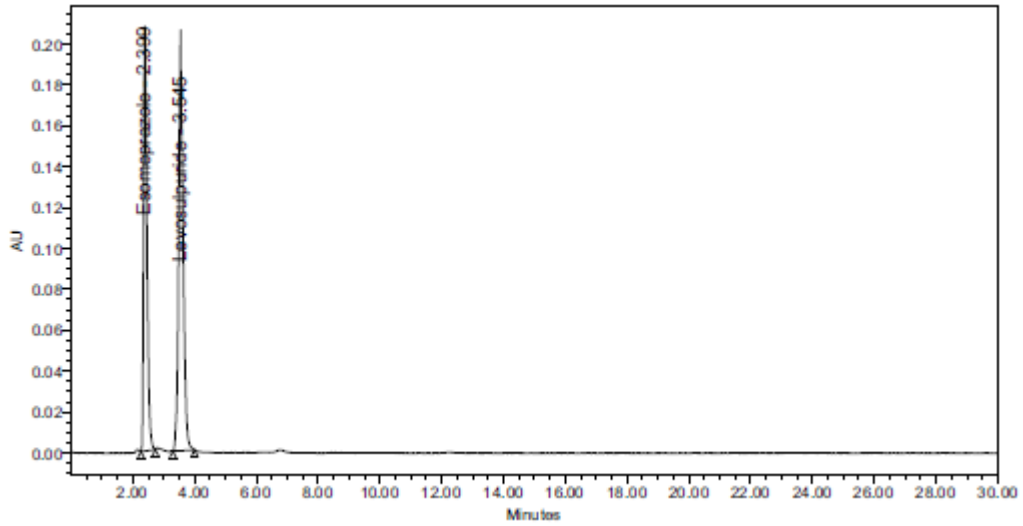

Fig. 7: Chromatogram after Heat Degradation

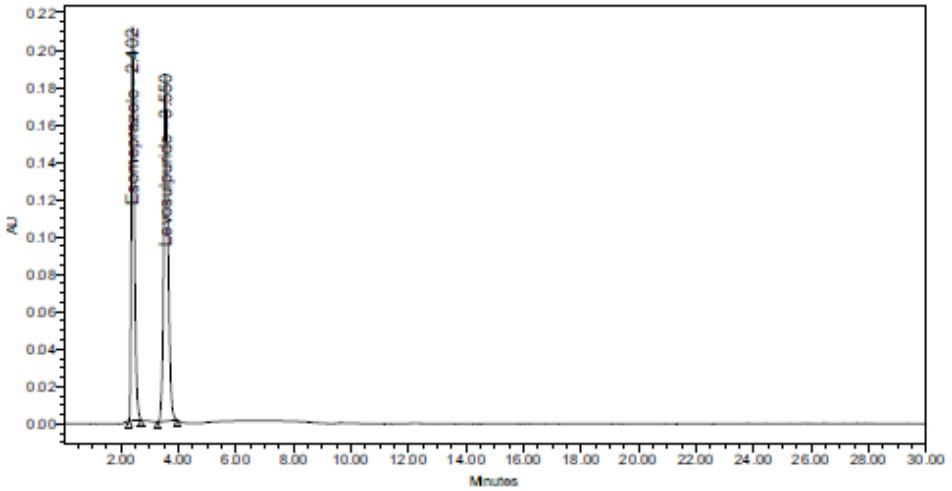

Fig. 8: Chromatogram after UV Degradation

Table 1: Accuracy Results

% Spiked	Amount added		Amount recovered		% of recovery		Mean %	
	Esom	Levos	Esom	Levos	Esom	Levos	Esom	Levos
50%	32 ppm	60 ppm	32.10 ppm	60.04	100.31	100.00	100.48	100.00
100%	64 ppm	120 ppm	64.15 ppm	120.30	100.23	100.25		
150%	96 ppm	180 ppm	96.88 ppm	179.55	100.91	99.75		

Note: number of replicates for each spiking = 3.

Test for Robustness

It was conducted by maling small change in the mobile phase composition (aqueous phase 10% ±), temperature (±5^0 C) and flow rate (± 0.2 ml per minute).

Test for Stability: Oxidation

To 1 ml of stock solution 1 ml of 20% hydrogen peroxide (H_2O_2) was added. The solutions were kept for 30 min at 60^{0}c. For HPLC study, the resultant solution was diluted to obtain 64µg/ml and 120µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of s tock solution 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^{0}c, diluted, and 10 µl was injected into the system.

Alkali Degradation Studies

To 1 ml of stock solution 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}c. diluted, 10 µl was injected into the system.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105^{0}c for 6 hours to study dry heat degradation, diluted, 10µl was injected into the system.

Photo Stability studies

It was conducted by exposing the solution to UV Light by keeping the beaker in UV Chamber for 7days, solution was diluted, 10 µl was injected into the system.

Neutral Degradation Studies

It was studied by refluxing the drug in water for 6hrs at a temperature of 60°C, diluted, 10 µl was injected into the system.

RESULTS AND DISCUSSIONS

Method development

Optimization of chromatographic condition was achieved after several trials. Reasonable retention time for Esomeprazole was 2.4 minutes and for Levosulpiride was 3.5 minutes. Resolution, tailing factor and number of theoretical plates were acceptable enough for quantitative analysis and stability studies.

Optimized Chromatographic conditions

Mobile phase was as Buffer, Acetonitrile and Methanol at the ratio of 65:30:5, Flow rate 1 ml/minute, Column ODS 250 mm x 4.6 mm, 5 m, Detector wave length 254 nm, Column temperature 30°C, Injection volume 10 mL, run time 8 minutes, Diluent: First drug dissolved in water and Acetonitrile at the ratio of 1:1 and then made up the volume with Buffer.

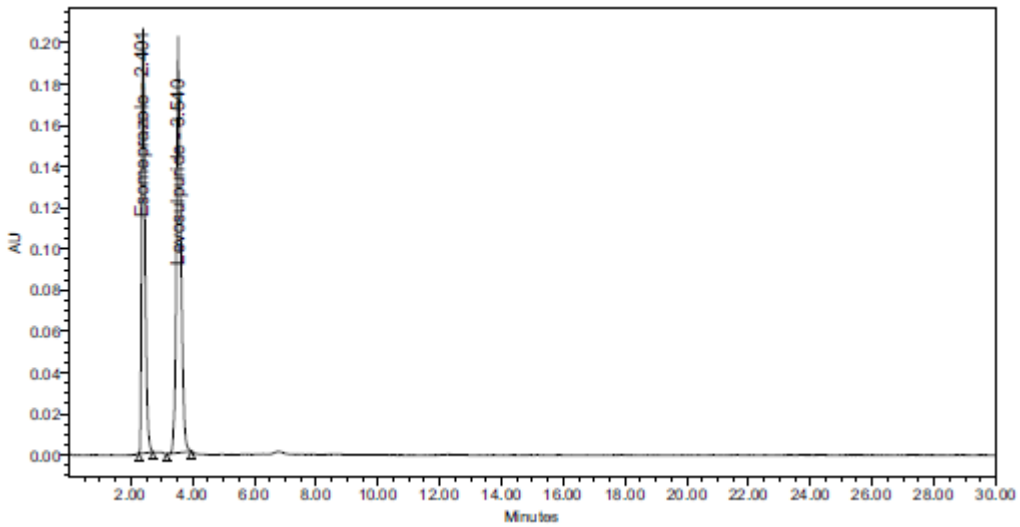

Fig. 9: Chromatogram after Neutral Degradation

Validation Results: Accuracy Results

Number of replicates were three for each trial. Mean percentage recovery was found to be 100.48% and 100.0 % for Esomeprazole and Levosulpiride respectively. Table 1 contains details of accuracy observations

Precision Results

SD was found to be 6568.0 and 1325.8; percentage relative standard deviation 0.38 and 0.60 for Esomeprazole Levosulpiride respectively. Table 2 contains details of precision/System suitability results.

Linearity Result

Linearity test was conducted by applying Levosulpiride (30ppm-180ppm) and Esomeprazole (16ppm-96ppm), calculated the value of Correlation Coefficient, and was found to be 0.999 for both the compounds. Details of Linearity results are given in Table 3.

Table 2: System suitability

Compound	Retention time (Average)	Response area (Average)	Plate count (Average)	Tailing factor (Average)
Esomeprazole	2.41	1714592	2888	1.42
Levosulpiride	3.56	2268541	3228	1.33

Note: Number of replicates =6

Table 3: Regression Analysis

Parameters	Esomeprazole	Levosulpiride
Linearity (µgm/ml)	16- 96 µgm/ml	30- 180 µgm/ml
Correlation Coefficient.(r)	0.999	0.999
Slope of Regression (mean)	511.7	446.6
%RSD of Slope	4.03	23.02
Regression Intercept (mean)	27831	19270
%RSD of Intercept	0.01	0.34

Note: number of replicates = 6.

Table 4: Intermediate Precision Results (Intraday and Inter day)

Compound	Results	Standard Area	Sample Area (Intraday)	Sample Area (Inter day)
Esomeprazole	Average	1717440	1714592	1712288
	SD	6568.0	5874.4	5055.0
	%RSD	0.38	0.34	0.41
	%Assay	—-	99.83	99.70
Levosulpiride	Average	2266038	2268541	2265528
	SD	13258.6	9949.0	8852.2
	%RSD	0.60	0.40	0.44
	%Assay	—-	100.11	99.97

Note: number of replicates = 6.

Intermediate precision Results

The test for Intermediate precisions of this method was determined by conducting the trials in different days, significant change was not observed. Table 4 contains details of intermediate precision results.

Table 5: Results of robustness study

Drug	Chromatographic conditions	RT (Minutes)	Mean area	USP Plate	USP Tailing
Esomeprazole	Flow- 0.8ml/minute	2.408	1755236	2337	1.46
	Flow-1.2ml/minute	2.151	1535572	2216	1.44
	Buffer (70%)	2.412	1769940	2380	1.45
	Buffer (60%)	2.433	1779377	2322	1.45
	Temperature-25^{0}C	2.410	1709257	2347	1.44
	Temperature-35^{0}C	2.409	1634134	2347	1.44
Levosulpiride	Flow- 0.8ml/minute	3.555	2291211	2411	1.27
	Flow-1.2ml/minute	3.169	2009069	2335	1.29
	Buffer (70%)	3.553	2322981	2483	1.25
	Buffer (60%)	3.559	2293413	2431	1.47
	Temperature-25^{0}C	3.543	2218891	2430	1.25
	Temperature-35^{0}C	3.534	2123457	2431	1.24

Note: number of replicates = 3.

Table 6: Results of degradation studies

Stress Condition	SL	Peak	RT (Min)	Area	% Area	Purity Angle	Purity Threshold	Plate Count	Tailing
Oxidative	1	Esom	2.407	1609556	40.85	2.159	2.272	2379	1.5
	2	Levo	3.179	2121701	41.13	0.209	0.317	2994	1.5
	3	Peak1	6.677	1791786	11.97	0.223	0.300	3914	1.4
	4	Peak2	10.468	263669	6.05	0.459	0.664	4325	1.3
Acidic	1	Esom	2.551	1659778	65.99	1.162	2.397	2399	1.8
	2	Levo	3.234	393664	11.65	0.515	0.786	5234	1.4
	3	Peak1	3.555	2064263	9.89	0.371	0.408	4280	1.7
	4	Peak2	5.755	421009	12.46	1.170	0.507	1078	2.0
Alkali	1	Esom	2.595	1626253	57.52	1.200	2.087	3043	1.6
	2	Peak1	3.241	88125	2.65	0.276	1.459	6015	1.4
	3	Levo	3.583	2141047	21.12	0.616	0.713	3601	1.4
	4	Peak2	4.954	38620	1.16	2.012	2.412	3975	1.1
	5	Peak3	6.614	615481	18.54	0.545	0.727	2273	1.7
Elevated Temp	1	Esom	2.399	1591177	44.00	1.339	1.702	2387	1.5
	2	Levo	3.545	2187109	56.00	0.231	0.310	2548	1.2
UV Light	1	Esom	2.402	1667213	44.56	1.472	1.685	2383	1.5
	2	Levo	3.550	2204921	55.44	0.258	0.410	2651	1.2
Neutral	1	Esom	2.410	1709709	44.07	1.387	1.682	2375	1.5
	2	Levo	3.540	2226986	55.93	0.181	0.303	2503	1.2

LOD

The limit of detection was found to be 0.04µg/ml and 0.02µg/ml for Esomeprazole and Levosulpiride respectively.

LOQ

The limits of quantification were found to be 0.12µg/ml and 0.05µg/ml for Esomeprazole and Levosulpiride respectively.

Robustness Study

The test for robustness was performed by taking the parameters like flowrate-1.0 ± 0.2 ml per minute, Mobile phase composition - aqueous phase 10% ± and temperature ±5^0 C. Result was found like change in retention time from 2.138 minutes to 2.439 minutes for Esomeprazole and from 3.144 minutes to 3.666 minutes for Levosulpiride, other parameters like theoretical plates, tailing factors etc. were not affected significantly. Table 5 contains details of robustness results.

Results of Stability test

Stability studies indicate that both the compounds were prone to peroxide, acid and alkali degradation. Thermal, UV or Water degradation was not significant. Figure IV to IX represent chromatogram due to force degradation and Table 6 contains details of degradation results.

CONCLUSION

In this research work the method developed for assay and stability studies was found to be rapid, simple, accurate, precise and robust for regular analysis of the drugs simultaneously. The present method has certainly beneficial edges analytically as compared to available methods which makes the method quiet advantageous and unique.

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