

Development and Validation of a stability indicating method for the simultaneous determination of Levosulpiride and Rabeprazole by High Performance Liquid Chromatography

Atulita Mullapudi ¹, Sirisha Pingali ² Tata Santosh^{3*}

³Pharmaceutical Research & Development Laboratory, Corpuscule Research Solutions, Visakhapatnam-530003, India.

Date Received:
6TH Sep 2013

Date of Accepted:
1ST Oct 2013

Date Published:
14TH Oct 2013

Abstract:

A simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method has been developed for the simultaneous estimation of Levosulpiride and Rabeprazole in pharmaceutical Tablet dosage form. The mobile phase consisted of 60:40 % (v/v) of Methanol & 0.1% v/v orthophosphoric acid operated on isocratic mode. The flow rate is 1.0 ml/min. Chromatographic separation of Levosulpiride and Rabeprazole was performed on AGILENT POLARIS C₁₈ column (150 X 4.6 mm id, ODS 2, 5µm). The wavelength of detection is 232 nm. The injection volume is 20µL. The retention time of Levosulpiride and Rabeprazole are 2.1 ± 0.10 minutes and 4.1 ± 0.10 respectively. The run time of analysis is 6 minutes. The developed method was validated for parameters such as accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The influence of acid, alkaline, oxidative Stress and photolytic stress conditions on both the drugs was studied. Results indicated complete degradation in alkaline medium for Levosulpiride and Rabeprazole. The proposed method has been successfully used for the estimation in tablet dosage forms.

Keywords: Levosulpiride, Rabeprazole, HPLC, Proton Pump Inhibitors, Dopaminergic antagonist

Introduction

Levosulpiride is chemically 5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidiny) methyl]-2 methoxy benzamide[1] (Fig. 1a). 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 anti-dopaminergic activity, antiemetic and anti-dyspeptic effects and lower acute toxicity. Rabeprazole is chemically 2- [[[4-(3methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole sodium salt[2]. 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[3] Compared with racemic and dextro-forms, the levo-form of sulpiride has greater central anti-dopaminergic activity[4], antiemetic and anti-dyspeptic effects and lower acute toxicity[5]

Rabeprazole is an antiulcer drug in the class of proton pump inhibitors used in the treatment of GERD [6]and duodenal ulcers[7] (Fig. 1b). 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 oppress basal and stimulated gastric acid secretion.

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.

Fig-1a: Structure of Levosulpiride

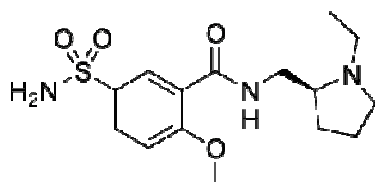
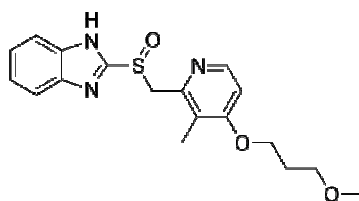


Fig-1b: Structure of Rabeprazole



2. EXPERIMENTAL

2.1. Reagents and chemicals

Orthophosphoric acid (AR Grade, Merck Ltd), Methanol (HPLC grade, Merck Ltd), Milli-Q water, Levosulpiride (99.8 % w/w is a gift sample from Unichem Laboratories Ltd) and Rabeprazole (100% w/w purchased from Sigma, glacial acetic Acid (GR Grade, SD Fine Chem Ltd). All other chemicals are of the highest grade commercially available unless otherwise specified. RABONIK tablets for evaluation of the assay content were purchased from a local pharmacy.

2.2. Apparatus and chromatographic conditions

The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

The mobile phase consisted of 60:40 % (v/v) of Methanol & 0.1% v/v orthophosphoric acid operated on isocratic mode. The flow rate is 1.0 ml/min.

Chromatographic determination of Levosulpiride and Rabeprazole was performed on AGILENT POLARIS C₁₈ column (150 X 4.6 mm id, ODS 2, 5µm). The wavelength of detection is 232 nm. The injection volume is 20µL.

2.3. Preparation of standard solutions, Calibration Standards & Quality Control Samples

Stock solutions of Levosulpiride (1mg/mL), & Rabeprazole (1mg/mL) were prepared separately in a volumetric flask using methanol and labeled accordingly. Suitable dilutions were then prepared using 50:50 %v/v Methanol & Milli-Q water as Diluent Solution. A Linear Calibration curve containing 8 non-zero standards were prepared using diluent solution in the concentration range of 5-50 µg/mL for Rabeprazole & 10-100 µg/mL for Levosulpiride. The calibration standard sample is then transferred into the auto sampler for analysis. Samples for Specificity (Sample with Rabeprazole alone, sample with Levosulpiride alone, Blank Sample and sample containing both the drugs)

For the preparation of quality control samples, a separate stock containing approximately the same concentration of the rabeprazole and levosulpiride were prepared and labeled as quality control stocks. From these stocks, quality control samples containing rabeprazole and levosulpiride were prepared at three concentration levels namely LQC, MQC, and HQC so as to obtain low, median and high concentration quality control samples. The performance of the linear calibration curve is then evaluated using quality control samples were also prepared accordingly.

2.4. Assay

The assay of tablets containing Levosulpiride and Rabeprazole (Brand name: RABONIK) is done using the procedure given in Indian Pharmacopoeia under tablets. The active ingredients in each of 10 dosage units is taken by random sampling and analyzed by the developed method. For the current assay ten tablets were randomly taken and transferred separately into 100ml volumetric flasks and dissolved in 20 ml methanol. The solution was then ultrasonicated for 10min and then made up to volume. Required amount of solution is then taken and filtered through 0.45µ nylon membrane and diluted with diluent solution so that the resultant concentrations are within the calibration range of the developed method. The samples are then analyzed

by using the validated method. The sample is then injected in triplicate⁷.

2.5 Method Validation

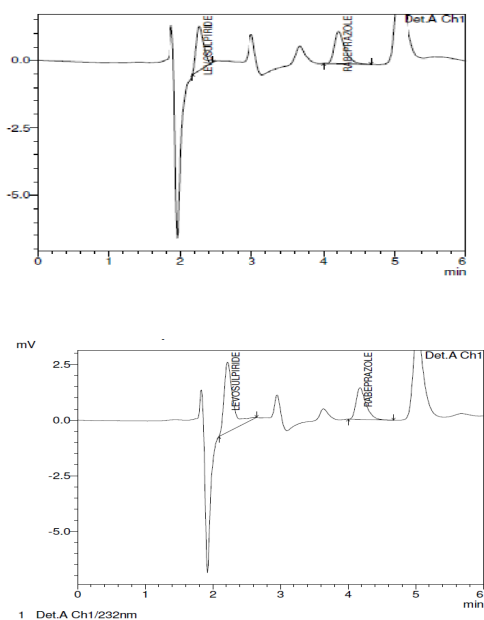
2.5.1 System Suitability

A sample containing mixture of Levosulpiride (at concentration of 50 μ g/ml) and Rabeprazole (at concentration of 50 μ g/ml) was used as system suitability sample. System suitability was assessed by six replicate analysis. A percent coefficient of variation (% CV) less than 1 % for retention times for the drugs is taken as the acceptance criterion.

2.5.2 Detection and Quantitation Limits (Sensitivity)

Limits of detection (LOD) and quantification (LOQ) (Fig-2) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 5, with precision (%CV) and accuracy with (\pm) 20%.

Fig-2: Chromatograms shown below indicate limit of Detection (LOD) above and Limit of Quantitation (LOQ) below.



2.5.3 Linearity (Calibration Curve)

The calibration curve was constructed with eight non-zero standards ranging from 5 to 50.00 μ g/mL for Rabeprazole and 10–100 for Levosulpiride. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (Fig- 3).

2.5.4 Accuracy and Precision

Accuracy of assay method was determined for both intra-day and inter-day variations using triplicate analysis of the QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days).

2.5.5 Specificity

For demonstration of specificity, 4 samples namely blank sample, sample containing rabeprazole alone, sample containing levosulpiride alone and sample containing the mixture of levosulpiride and rabeprazole were prepared separately. Specificity of the method was determined by comparing results of all the samples (Fig-4). The developed method is said to be specific if the % interference calculated as peak area (if any) at the retention time of each of the analytes in the blank sample is less than 20% of peak area at the corresponding retention times of each of the drugs in the lowest calibration standard. Sample Specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

2.5.6 Stability

The stability of the drug is determined by placing the MQC samples for the short term stability at room temperature up to 12 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hrs was studied and established.

2.5.7 Stress Degradation Studies

For Stress Degradation Analysis, 1 mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100 μ L of 0.1N HCl (Acid stress), 0.1N NaOH (Alkaline stress), 5% v/v Hydrogen Peroxide (Oxidative Stress), for 24 Hrs. Samples for Photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples. The analysis is performed in triplicate.

3.0 RESULTS AND DISCUSSION

3.1 Method Development and Validation

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Functional group analysis revealed the presence of acidic character to the molecules. Therefore we evaluated the chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like Hypersil-BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-pack pro, Spherisorb C18, Phenomenex C18 have been tried with different buffer salts such ammonium formate, ortho phosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran. However less tailing and high theoretical plates are obtained with Agilent Polaris column C18 150 X 4.6 cm 5 μ m column. The peak response of Levosulpiride decreased with increased composition of Methanol in the mobile phase. Mobile phase composition consisted of 60:40 % (v/v) of Methanol & 0.1% v/v orthophosphoric acid operated on isocratic mode. The flow rate of the method is 1.0 ml/min. Calibration standards were prepared in diluent solution containing 50:50 % v/v of methanol and milli-Q water. The wavelength of detection is 232nm. The column temperature is maintained at 25 $^{\circ}$ C. At the reported flow rate, peak shape was excellent; however increasing or decreasing the flow rate resulted in unacceptable tailing factor and poor peak shape. Hence 1.0 ml/min was optimized flow rate decreasing the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis. To evaluate the feasibility of the experiment under regular lab conditions, we assessed the stability of Rabeprazole and Levosulpiride under room temperature and under normal light conditions.

3.2 Method Validation

3.2.1 System Suitability

The % RSD of the peak area for both drugs is within the acceptable criteria (**Table-1**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 10883 \pm 1 20 for Rabeprazole and 5153 \pm 65 for Levosulpiride. The USP tailing factor was 1.25 \pm 0.1 for Rabeprazole while that of Levosulpiride is 1.21 \pm 0.08.

3.2.2 Determination and Quantification Limits (Sensitivity)

Fig-2 represents the chromatogram of limit of detection and limit of quantification. The method is found to be sensitive which can be determined from the data obtained from the (**Table-2**).

3.2.3 Linearity

The linearity was demonstrated in triplicate. The results of the best fit line ($y = mx + c$) for the triplicate analysis is given in **Table 3**. The accuracy of the calibration standards was evaluated from the back calculated concentrations (**Table 4**). All the standards were found to be within the range of 90.33-109.23% for levosulpiride and 92.49-105.33% for rabeprazole.

3.2.4 Accuracy and Precision

Accuracy and precision calculated for the QC samples during the intra- and inter -day run are given the (**Table-5**). The intra-day (day-1) and inter-day accuracy for Rabeprazole ranged from 51.8-52.7 % while that of Levosulpiride ranged from 102.78-105.46 %. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

3.2.5 Specificity

Specificity was determined by comparison of the Blank chromatogram with that of the Standard chromatogram (**Fig-4**)

3.2.6 Room Temperature Stability

Stability studies were done for short term stability up to 12 hrs on the bench top for the MQC levels conditions. Stability is calculated as the ratio of the

mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n=6). The room temperature stability was found to be 120.85 % for Rabeprazole and 110.95 % for Levosulpiride. The results are tabulated in **Table-6**.

3.2.7 Stress Degradation

Stress studies revealed that Rabeprazole is not susceptible to degradation under acid, light (UV) and oxidative stress conditions (**Fig 5a**). However, in alkaline conditions (0.1N NaOH), the drug was unstable and the degradation peak eluted earlier accompanied with a drastic peak distortion and increased tailing. Except for alkaline conditions, the drug content was within 95 –105 % for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Stress studies on Levosulpiride indicated instability under alkaline conditions. This has been clearly demonstrated by the help of overlap spectra of all the stress samples as compared with that of freshly prepared sample of similar concentration (**Fig 5b**).

3.2.8 Robustness study

Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to evaluate the robustness of the method. The impact of flow-rate (1.0 ± 0.1 ml/min), and effect of mobile-phase composition (± 5%) on chromatographic parameters such as retention time, theoretical plates, and tailing factor, were studied. At lower flow rate, the retention time of Rabeprazole was 4.63 ± 0.10 minutes while that of Levosulpiride was 2.48 ± 0.10 minutes. At lower flow rate, the tailing factor for Rabeprazole increased to 1.27 ± 0.03 while that of Levosulpiride 1.33± 0.03. At higher flow rate, tailing factor for Levosulpiride is 1.28 and Rabeprazole 1.34. The elution was earlier at higher flow rate; Rabeprazole and Levosulpiride eluted at 3.79 ± 0.01 and 2.06 ± 0.02 minutes respectively. The retention time of Rabeprazole and Levosulpiride were 4.63 ± 0.02 and 2.48 ± 0.03 minutes respectively (n=6) when the mobile phase composed of 75 methanol and 25 parts of 20m orthophosphoric acid (pH 3.0).

3.3 Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of Rabeprazole and Levosulpiride. Also the method is validated for different

parameters; hence it has been applied for the simultaneous estimation in pharmaceutical dosage forms. RABEKIND was evaluated. The % assay of Levosulpiride in the tablet is 99.85% and that of Rabeprazole is . 101.25 %. None of the tablets ingredients interfered with the analyte peak. The spectrum of Rabeprazole and Levosulpiride in the extracted tablet was matching with that of standard compounds indicating the purity of the compounds in the tablets

Conclusions

The method gave accurate and precise results in the concentration range of 5 - 50 µg/mL for Rabeprazole and 10 to 100µg/mL for Levosulpiride. The mobile phase composition consists of (60:40 v/v) of Methanol and orthophosphoric acid (pH adjusted to 3.0 with glacial acetic acid), at the flow rate of 1.0 ml/min. The retention time of Rabeprazole is 4.1 ± 0.10 minutes and that of Levosulpiride is 2.1 ± 0.10 minutes. The column is Agilent POLARIS 2, 150 X 4.6mm, C18 column with the particle size of 5µm. A rapid sensitive and specific method for the simultaneous estimation of Rabeprazole and Levosulpiride in the pharmaceutical tablet formulations has been developed and validated.

Table 1. System Suitability test for Rabeprazole and Levosulpiride

RABEPRAZOLE				
Sample ID	Peak Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	4.22	1062533	11196	1.26
2	4.19	1038058	11318	1.26
3	4.21	1039651	11349	1.26
4	4.16	1045551	11139	1.29
5	4.13	901577	11740	1.30
6	4.13	975363	11648	1.32
MEAN	4.17	1010455.5	11398.3	1.28
STDEV	0.039	61063.98	243.37	0.026
% CV	0.942	6.04	2.135	1.99

LEVOSULPIRIDE				
Sample ID	Peak Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	2.24	1101145	5023	1.24
2	2.22	932987	5029	1.26
3	2.23	978884	5054	1.25
4	2.22	1020780	5062	1.26
5	2.22	910838	5196	1.28
6	2.23	1085389	5149	1.26
MEAN	2.24	1005003.8	5085.5	1.25
STDEV	0.0055	78344.95	70.54	0.013
% CV	0.25	7.80	1.387	1.05

Fig-3a: Linear calibration curve of Rabeprazole.

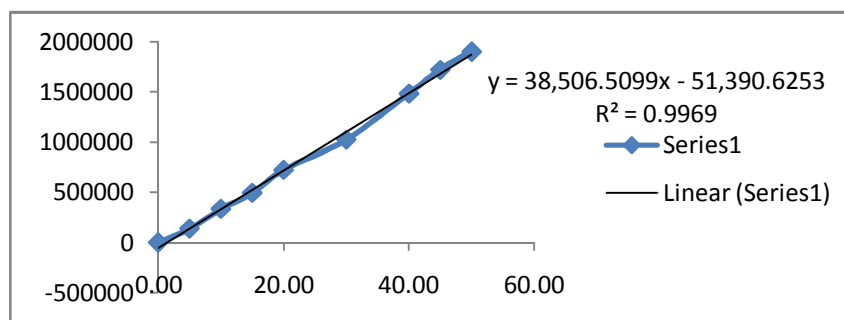


Fig-3b: Linear calibration curve of Levosulpiride.

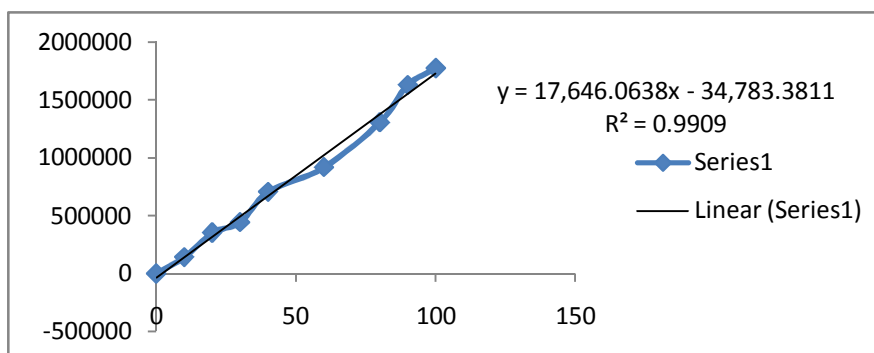


Table 2. Sensitivity

RABEPRAZOLE LOD		
SR NO	DRUG	
	Retention Time	Peak Area
1	4.22	10201
2	4.22	11124
3	4.18	10525
MEAN	4.20	24833.3
ST DEV	0.023	3942.05
% CV	0.54	15.8

LEVOSULPIRIDE LOD		
SR NO	DRUG	
	Retention Time	Peak Area
1	2.26	19985
2	2.26	19788
3	2.22	19739
MEAN	2.24	17170.7
ST DEV	0.02	2275.06
% CV	1.02	13.24

RABEPRAZOLE LOQ		
SR NO	DRUG	
	Retention Time	Peak Area
1	4.17	24641
2	4.21	27367
3	4.21	30266
MEAN	4.19	24091.3
ST DEV	0.023	3562.9
% CV	0.550	14.8

LEVOSULPIRIDE LOQ		
SR NO	DRUG	
	Retention Time	Peak Area
1	2.22	28845
2	2.25	25966
3	2.26	29813
MEAN	2.24	28208
ST DEV	0.02	2001.04
% CV	0.927	7.09

Fig-4: Comparison of (a) Blank Chromatogram, (b) Rabeprazole alone (c) Levosulpiride alone and (d) sample containing both Levosulpiride and Rabeprazole

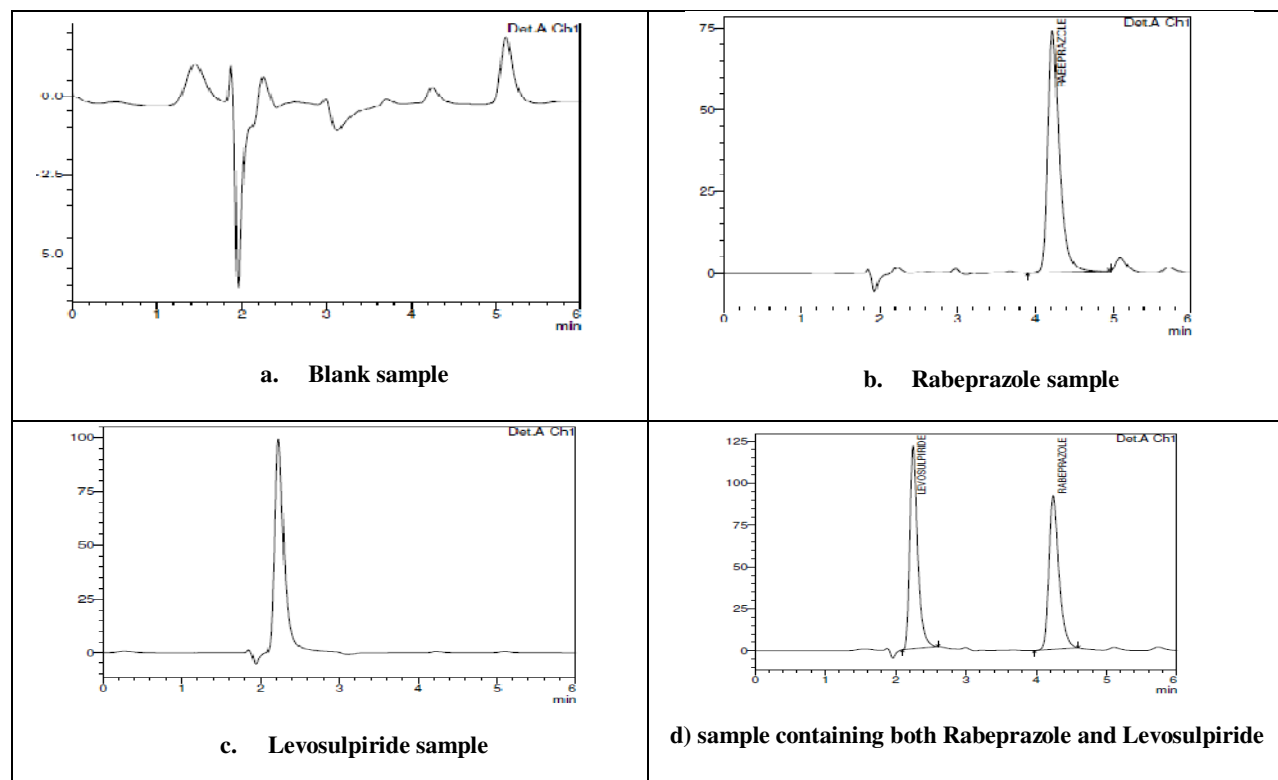


Table 3. Results of best-fit line for triplicate analysis for Levosulpiride and Rabeprazole

Levosulpiride			
Curve	Slope	Intercept	r ²
1	17646.06	-34783.38	0.9909
2	18252.90	-48513.16	0.9948
3	18640.97	-54287.53	0.9953
Mean	18179.97	-137584.07	0.99366

Rabeprazole			
Curve	Slope	Intercept	r ²
1	38506.50	-51390.62	0.9969
2	39467.15	-78151.81	0.9962
3	39738.57	-79858.22	0.9946
Mean	39237.40	-209400.65	0.9959

Table 4. Linearity and Range for Levosulpiride and rabeprazole demonstrating accuracy, carryover effect and specificity of the method (Curve 1).

LEVOSULPIRIDE					
SAMPLE ID	Concentration (Microgram/mL)	Retention Time	Peak Area	Back Calc Concentration	% Accuracy
BLANK	0	NO PEAK	0	NA	NA
CC 1	10.00	2.26	142567	10.05	100.50
CC 2	20.00	2.26	353611	22.01	110.05
CC 3	30.00	2.26	442951	27.07	90.24
CC 4	40.00	2.26	705462	41.95	104.87
CC 5	60.00	2.26	918789	54.04	90.06
CC 6	80.00	2.26	1306797	76.03	95.03
CC 7	90.00	2.26	1629827	94.33	104.82
CC 8	100.00	1.62	1774753	102.55	102.55
CO BLANK	0	NO PEAK	0	NA	NA

- NA - Not applicable

RABEPRAZOLE					
SAMPLE ID	Concentration (Microgram/mL)	Retention Time	Peak Area	Back Calc Concentration	% Accuracy
BLANK	0.00	NO PEAK	0	NA	NA
CC 1	5.00	4.42	138752	4.94	98.76
CC 2	10.00	4.44	334571	10.02	100.23
CC 3	15.00	4.44	493687	14.16	94.37
CC 4	20.00	4.47	722196	20.09	100.45
CC 5	30.00	4.46	1023915	27.92	93.08
CC 6	40.00	4.46	1483126	39.85	99.63
CC 7	45.00	4.46	1719781	46.00	102.21
CC 8	50.00	4.48	1900356	50.69	101.37
CO BLANK	0	NO PEAK	0	NA	NA

NA - Not applicable

Table 5a. Results of inter and intra-day accuracy & precision for levosulpiride

	Nominal Concentration ($\mu\text{g/mL}$)		
	25.00	50.00	75.00
<u>DAY 1</u>			
MEAN (n=6)	26.37	52.50	77.09
SD	1.7331	1.6663	2.9448
% CV	6.573	3.174	3.820
<u>DAY 2</u>			
MEAN (n=6)	26.17	52.82	77.65
SD	1.0087	1.6410	2.6389
% CV	3.855	3.106	3.398
<u>DAY 3</u>			
MEAN (n=6)	25.65	52.71	77.51
SD	0.7023	1.246	3.218
% CV	2.7376	2.3645	4.129

Table 5b. Results of inter and intra-day accuracy & precision for Rabeprazole

	Nominal Concentration ($\mu\text{g/mL}$)		
	25.00	50.00	75.00
<u>DAY 1</u>			
MEAN (n=6)	12.92	26.22	39.70
SD	0.494	0.539	1.068
% CV	3.824	2.055	2.691
<u>DAY 2</u>			
MEAN (n=6)	12.73	26.13	39.57
SD	0.384	0.446	1.104
% CV	3.019	1.709	2.790
<u>DAY 3</u>			
MEAN (n=6)	12.54	26.04	39.44
SD	0.317	0.408	1.198
% CV	2.531	1.569	3.039

Fig-5a: Overlay Chromatogram showing the influence of various stress conditions on Rabeprazole; Data 1-Acid Stress, Data 2 – Oxidative Stress; Data 3 –Photolytic Stress; Data 4 – Alkaline Stress. Data 4 clearly indicates the spectral degradation of Rabeprazole due to alkaline instability.

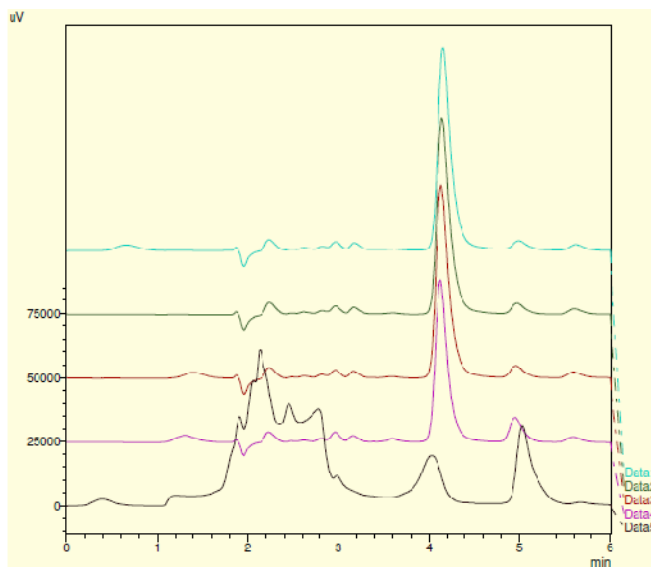


Fig-5b: Overlay Chromatogram showing the influence of various stress conditions on Levosulpiride; Data 1- Acid Stress, Data 2 – Oxidative Stress; Data 3 –Photolytic Stress; Data 4 – Alkaline Stress.. Data 3 shows the Photolytic degradation product of Levosulpiride Data 4 clearly indicates the spectral degradation of Levosulpiride due to alkaline instability.

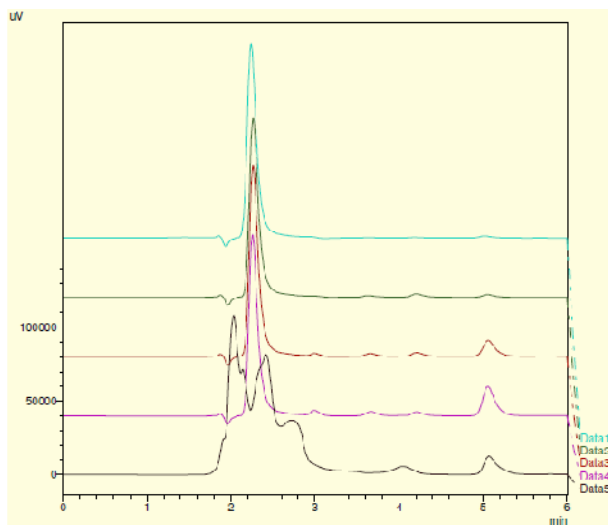


Table 6a. Room Temperature Stability of Rabeprazole (n = 6).

RABEPRAZOLE				
FRESH SAMPLE			DRUG	
SR NO	SAMPLE ID	CONC (µg/mL)	Rt	PEAK AREA
1	FRESH	250.00	6.06	1152175
2	FRESH	250.00	6.07	1145988
3	FRESH	250.00	6.08	1131650
4	FRESH	250.00	6.05	1144029
5	FRESH	250.00	6.04	1141886
6	FRESH	250.00	6.05	1149332
Mean				1144176.67
Stdev				7155.67
%				0.63

STABILITY SAMPLE				
SR NO	SAMPLE ID	CONC (µg/mL)	Rt	PEAK AREA
1	STABILITY	250.00	6.10	1122750
2	STABILITY	250.00	6.12	1165872
3	STABILITY	250.00	6.14	1152038
4	STABILITY	250.00	6.16	1187206
5	STABILITY	250.00	6.28	1106106
6	STABILITY	250.00	6.15	1195974
Mean				1154991.00
Stdev				35422.46
% Cv				3.07

% Stability **100.95**

Table 6b. Room Temperature Stability of levosulpiride (n = 6).

LEVOSULPIRIDE				
FRESH SAMPLE			DRUG	
SR NO	SAMPL E ID	CONC (µg/mL)	Rt	PEAK AREA
1	FRESH	50.03	8.94	3754020
2	FRESH	50.03	8.97	3745764
3	FRESH	50.03	8.97	3733460
4	FRESH	50.03	8.93	3731874
5	FRESH	50.03	8.93	3717159
6	FRESH	50.03	8.95	3726962
MEAN				3734873.17
STDE				13210.46
% CV				0.35

STABILITY SAMPLE				
SR NO	SAMPLE ID	CONC (µg/mL)	DRUG	PEAK AREA
1	STABILITY	50.03	9.08	3712110
2	STABILITY	50.03	9.14	3717038
3	STABILITY	50.03	9.19	3670043
4	STABILITY	50.03	9.22	3734664
5	STABILITY	50.03	9.32	3705677
6	STABILITY	50.03	9.20	3739258
MEAN				3713131.67
STDE				24798.13
% CV				0.67

% Stability **99.42**

References:

1. Mucci A, Nolf G and Maj M: Levosulpiride A review of its clinical use in psychiatry. *Pharmacology and Respiratory* 1995; 31: 95-101
2. Sabnis SS, Dhavale ND, Jadhav VY, Gandhi SV, Spectrophotometric simultaneous determination of Rabepazole Sodium and Itopride Hydrochloride in capsule dosage form. *Spectrochimica Acta*, 2008; 69: 849-852
3. 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.
4. 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.
5. The Martindale 35th ed: The complete drug reference, published pharmaceutical press, lambeta high street, london SE1 75M, UK. 2006.
6. Rena S, Park M, Sah H, and Lee B, Effect of pharmaceutical excipients on aqueous stability of rabepazole sodium. *International J. Pharmaceutics*, 2008; 350: 197-204.
7. Ramakrishna NVS, Vishwottam KN, Wishu S, Koteswara M, Suresh Kumar S, High performance liquid chromatography method for the quantification of rabepazole in human plasma using solidphase extraction. *J. of Chromatography B*, 2005; 816: 209-214.
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 rabepazole and Levosulpiride in bulk and pharmaceutical dosage forms, *int. res j pharm. app sci.*, 2012; 2(2):99-106.
12. N.B. Dobarra, N. H. Vadia and S. J. Rajput, Simultaneous Spectrophotometric Estimation of Rabepazole 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 Rabepazole 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 rabepazole 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 Rabepazole 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 Rabepazole sodium in Pharmaceutical formulations and Human Plasma, *Asian J. Research Chem.* 2(1):49-51, Jan.-March, 2009.
17. Vaithyanathan Sree Janardhanan*, Rajappan Manavalan and Kannappan Valliappan, Stability-indicating HPLC method for the simultaneous determination of pantoprazole, rabepazole, lansoprazole and domperidone from their combination dosage forms, *Int. J. Drug Dev. & Res.*, Oct-Dec 2011, 3 (4): 323-335.