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A New RP-HPLC Method Development and Validation of Levosulpiride and Rabeprazole

Alekhya Somepalli[1], Nanda Gopala Krishna Gona[2], Sri lakshmi Avutu[3], Naga Sailaja Chandolu[4] , Valli Padma Chilaka[5], Sony Priyanka Arava[6]
[1, 5]*Department of Pharmaceutical chemistry*
[2, 4]*Department of Pharmaceutical Analysis*
[3]*Department of Pharmacy Practice,* [6]*Department of Pharmacology*

Abstract: ***The Present study was conducted to obtain a new, affordable, cost-effective and convenient method for RP- HPLC determination of Rabeprazole and Levosulpiride in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2002.The method was validated for the parameters like system suitability , selectivity, linearity, accuracy, precision ,LOD, LOQ, and robustness. 20mg Rabeprazole and 75mg Levosulpiride was dissolve in 100 ml of Diluent (1:1,Methanol:Na_2HPO_4) and was further diluted to get stock solution of Rabeprazole and Levosulpiride (47.5 µg/ml). This is taken as a 100% concentration. Solution containing mixture of Rabeprazole and Levosulpiride of five different concentrations (50%,75%, 100% 125%, and 150% of target concentration) were prepared in the same way. System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Rabeprazole and Levosulpiride. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters. . % Recovery was 98.6% for Levosulpiride and 98.7% for Rabeprazole. All the results indicate that the method is highly accurate, retention time for standard sample and commercial product of Rabeprazole and Levosulpiride are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. The method is highly precise as % RSD of peak area was less than 2% in all tests.***

Keywords: RP-HPLC, Chromatography, Mobile phase, Column, Levosulperide, Rabeprazole.

I. INTRODUCTION

Analytical techniques play an important role in Production and evaluation of new drugs in bulk and formulation and also estimation from biological fluids, Detection and quantification of impurities and metabolites, Accelerated stability studies, Invitro dissolution studies, Pharmacokinetic studies and drug metabolism studies, Determination of bioavailability of two or more formulation.

A. Rabeprazole

It is a Proton pump inhibitor. Proton-pump inhibitors (PPIs) are a group of drugs whose main action is a pronounced and long-lasting reduction of gastric acid production. They are the most potent inhibitors of acid secretion available today. The group followed and has largely superseded another group of pharmaceuticals with similar effects, but different mode-of-action, called H_2-receptor antagonists. These drugs are among the most widely-selling drugs in the world and are generally considered effective. The vast majority of these drugs are benzimidazole derivatives; however, promising new research indicates that imidazopyridine derivatives may be a more effective means of treatment. High dose or long-term use of PPIs carry a possible increased risk of bone fracture.[2]

B. Levosulpiride

An antipsychotic (or Neuroleptic) is a tranquilizing psychiatric medication primarily used to manage psychosis (including delusio ns or hallucinations,aswell as disordered thought), particularly in schizophrenia and bipolar disorder, and is increasingly being used in the management of non-psychotic disorders. Reverse phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}.

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With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily. The new RP-HPLC method developed and validated for simultaneous determination of Levosulpiride and Rabeprazole pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form. This method can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.[4]

II. MATERIALS AND METHOD

HPLC system connected with PDA Detector 2998 and Empower2 Software, Electronic balance, Sonicator, 0.45µ membrane filter.

A. Reagents and Chemicals

Rabeprazole and Levosulpiride	:	Pharmaceutical grade
Methanol	:	HPLC grade
Disodium hydrogen orthophosphate	:	analytical reagent
Water	:	HPLC grade

B. Selection and Preparation of Mobile Phase

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of Ammonium Acetate buffer pH 7.0 and Methanol were studied for simultaneous separation of both the drugs. The optimal composition of mobile phase determined to be Buffer:Methanol (50:50 v/v) and filtered through 0.45µ membrane filter.[9][10][11]

C. Preparation of Standard Solution:

20mg Rabeprazole and 75mg Levosulpiride was dissolve in 100 ml of Diluent (1:1,Methanol:Na_2HPO_4) and was further diluted to get stock solution of Rabeprazole and Levosulpiride (47.5µg/ml). This is taken as a 100% concentration. Solution containing mixture of Rabeprazole and Levosulpiride of five different concentrations (50%,75%, 100% 125%, and 150% of target concentration) were prepared in the same way. [9][10][11]

D. Preparation of Sample Solution

Sample solution containing both the drugs was prepared by dissolving tablet powder into Diluent(1:1,Methanol:Na2HPO4) Ten tablets were weighed separately. Their average weights were determined. Powder of tablets equivalent to one tablet weight were weighed and taken in a 100 ml volumetric flask, dissolved in diluent and shaken and sonicated for about 10 minutes then filtered through 0.45µ membrane filter . The filtered solution was further diluted in the diluent to make the final concentration of working sample equivalent to 100% of target concentration. [9][10][11]

E. Wave length Selection

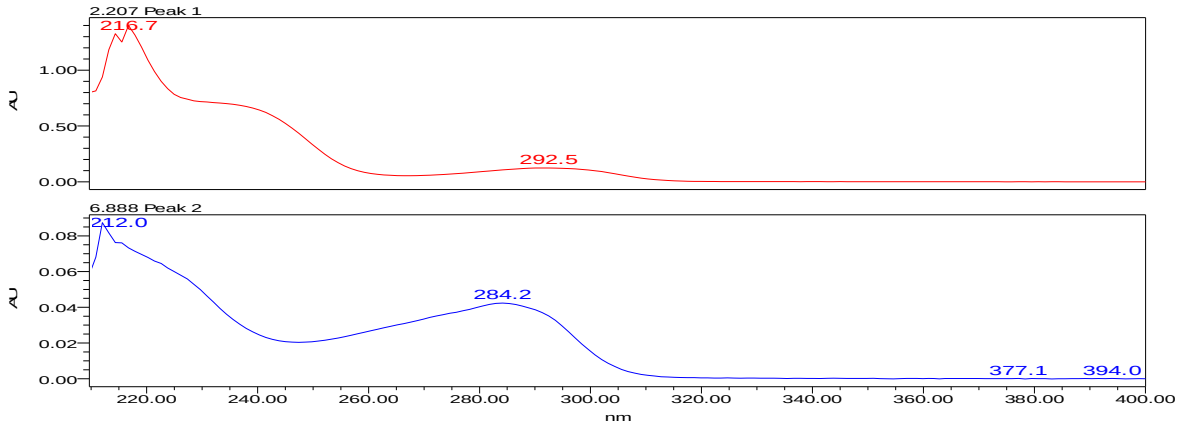


fig 1: Wavelength Selection of Levosulpiride and Rabeprazole

F. Optimized Chromatographic Conditions:

Column	:	A Thermo Hypersil ODS (C18; 5µ, 250 x 4.6mm) Isocratic mode
Mobile phase	:	0.1M Ammonium Acetate : methanol (50:50 %v/v).
PH	:	7.0
Flow rate	:	1.5ml/min
Wavelength	:	290nm.
Retention time	:	Levosulpiride - 2.2min Rabeprazole - 6.8min
Column temperature	:	50^0c
Injection volume	:	50µl
Diluent	:	Disodium hydrogen phosphate +Methanol

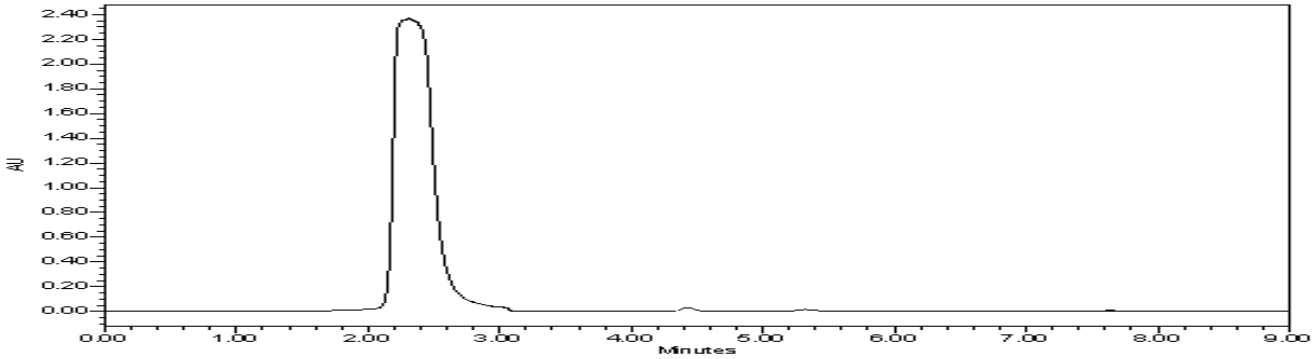


fig 2: Standard Graph of Levosulpiride

Observation: With reference to the standard graph of Levosulpiride the first peak which is eluted at the retention time of 2.2 was found to be Levosulpiride.

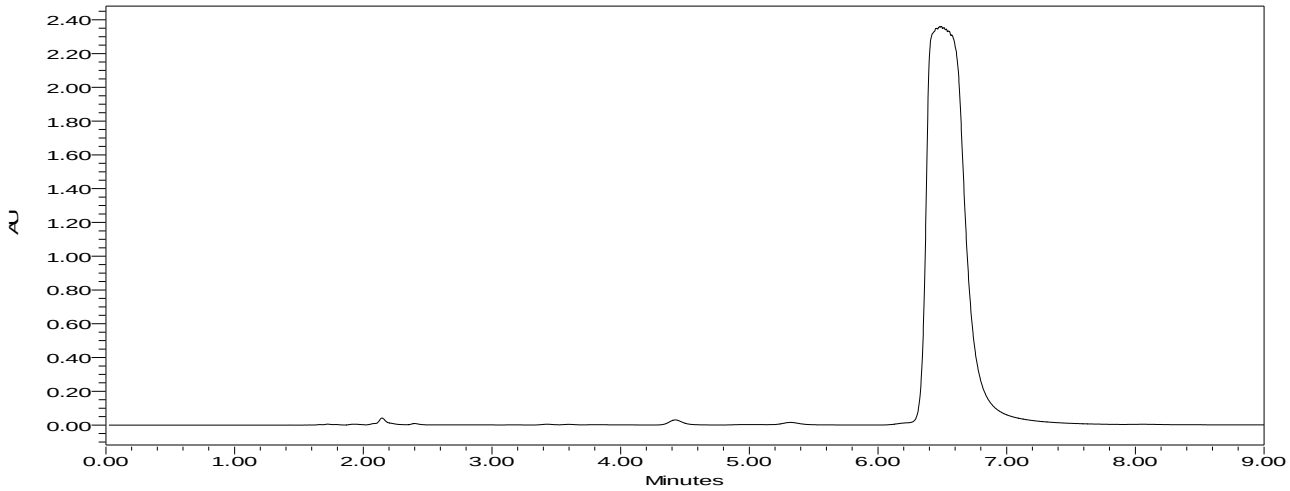


Fig 3: Standard Graph of Rabeprazole

Observation: With reference to the standard graph of Rabeprazole the second peak which is eluted at the retention time of 6.8 was found to be Rabeprazole.

G. System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Rabeprazole and Levosulpiride. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

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H. Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of Rabeprazole and Levosulpiride were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.[8][12]

I. LINEARITY

1) *Procedure:* Standard solutions of Rabeprazole and Levosulpiride of different concentrations level (50%, 75%, 100%,125%, and 150%) were used for this purpose. Inject each concentration into the chromatographic system and measure the peak area. A calibration curve was plotted for concentration v/s peak area and calculate the correlation coefficient. [8][12]
2) *Preparation of stock solution:* 20mg of Rabeprazole and 75mg of Levosulpiride was dissolve in 100 ml of Diluent (1:1,Methanol:Na_2HPO_4).
3) *Preparation of 50% concentration :* Take 3.1ml of Stock solution and diluted in 100ml of diluent to get 50% concentration solution of Rabeprazol(6.3µg/ml) and Levosulpiride(23.7µg/ml) .
4) *Preparation of 75% concentration :* Take 4.7ml of Stock solution and diluted in 100ml of diluent to get 75% concentration solution of Rabeprazole(9.45µg/ml) and Levosulpiride(35.6µg/ml) .
5) *Preparation of 100% concentration :* Take 6.3ml of Stock solution and diluted in 100ml of diluent to get 100% concentration solution of Rabeprazole(12.6µg/ml) and Levosulpiride(47.5µg/ml) .
6) *Preparation of 125% concentration :* Take 7.5ml of Stock solution and diluted in 100ml of diluent to get 125% concentration solution of Rabeprazole(15.7µg/ml) and Levosulpiride(59.3µg/ml) .
7) *Preparation of 150% concentration :* Take 9.4ml of Stock solution and diluted in 100ml of diluent to get 150% concentration solution of Rabeprazole(18.9µg/ml) and Levosulpiride(71.16µg/ml).

J. Accuracy (Recovery Studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Rabeprazole and Levosulpiride were added to pre-analyzed samples and were subjected to the proposed HPLC method. [8][12]

K. Precision

1) *Preparation of Stock Solution:* Accurately weighed and taken in a 100 ml volumetric flask, dissolved in diluent and shaken and sonicated for about 10 minutes then filtered through 0.45µ membrane filter . The filtered solution was further diluted in the diluent to make the final concentration of working sample equivalent to 100% of target concentration. Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2%[8][12] for within a day and day to day variations, which proves that method is precise.

$$\text{Standard Deviation}(\sigma) = \frac{\Sigma(x-x_i)^2}{n-1}$$

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Average}} \times 100$$

Acceptance criteria:

Percentage relative standard deviation (%RSD) for the area of six injections results should not be more than 2%.

L. Robustness Of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºc change in temperature. [8][12]

1) *LOD:* Based on standard deviation of the response and slope The limit of detection (LOD) may be expressed as

$$LOD = 3.3\ \sigma/S$$

Where σ = the standard deviation of the response S = slope of calibration curve of analyte

2) *LOQ:* Based on standard deviation of the response and slope The limit of Quantification (LOQ) may be expressed as

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LOQ = 10 σ/S

Where σ = the standard deviation of the response S = slope of calibration curve of analyte

III. RESULTS AND DISCUSSION

A. *System Suitability*

Table I
System suitability of Levosulpiride

S.NO	Number of Injections	Retention Time	Area	USP Tailing	USP Plate count
1	Injection-1	2.2	550230	1.46	6141
2	Injection-2	2.2	551141	1.47	6001
3	Injection-3	2.2	551102	1.45	6109
4	Injection-4	2.2	550725	1.46	6040
5	Injection-5	2.2	551677	1.47	6002
6	Injection-6	2.2	551520	1.45	6110
	AVG			1.45	6389

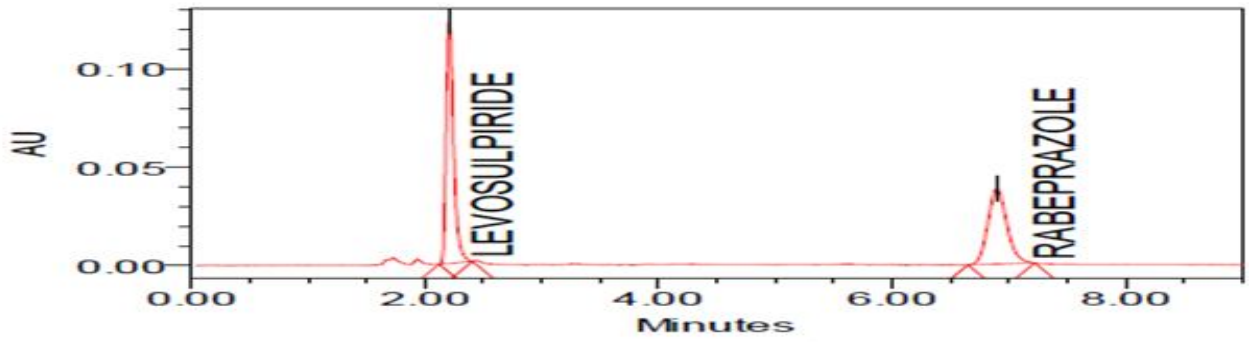
Table II
System suitability of Rabeprazole

S.NO	Number of Injections	Retention Time	Area	USP Tailing	USP Plate count
1	Injection-1	6.8	432553	1.15	8791
2	Injection-2	6.8	431551	1.14	8696
3	Injection-3	6.8	424472	1.11	9063
4	Injection-4	6.8	422931	1.11	8885
5	Injection-5	6.8	426082	1.12	8710
6	Injection-6	6.8	428710	1.15	9052
	AVG			1.14	8866

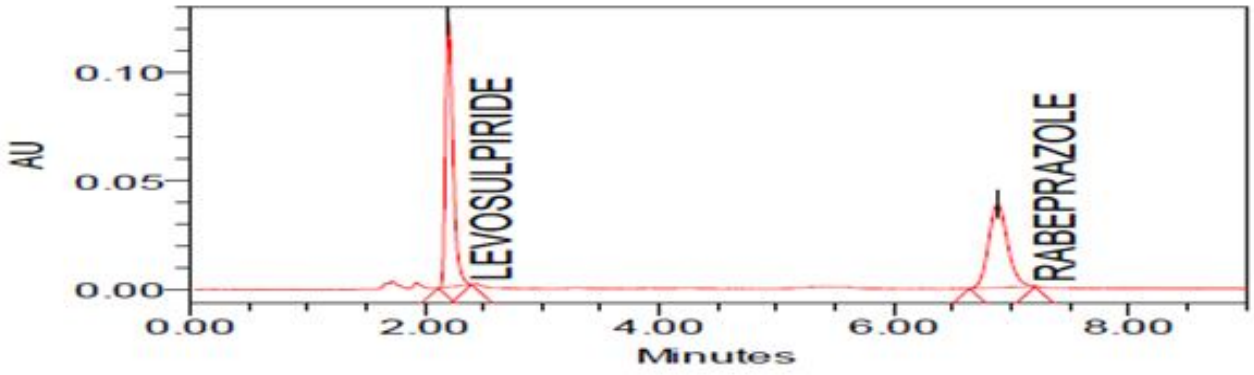
Table III
System suitability of Levosulpiride and Rabeprazole

Drug name	USP Tailing	USP Plate count	Resolution
Levosulpiride	1.45	6389	
Rabeprazole	1.14	8866	22.62
Acceptance criteria	NMT 2.0	Above 2000	Above 2

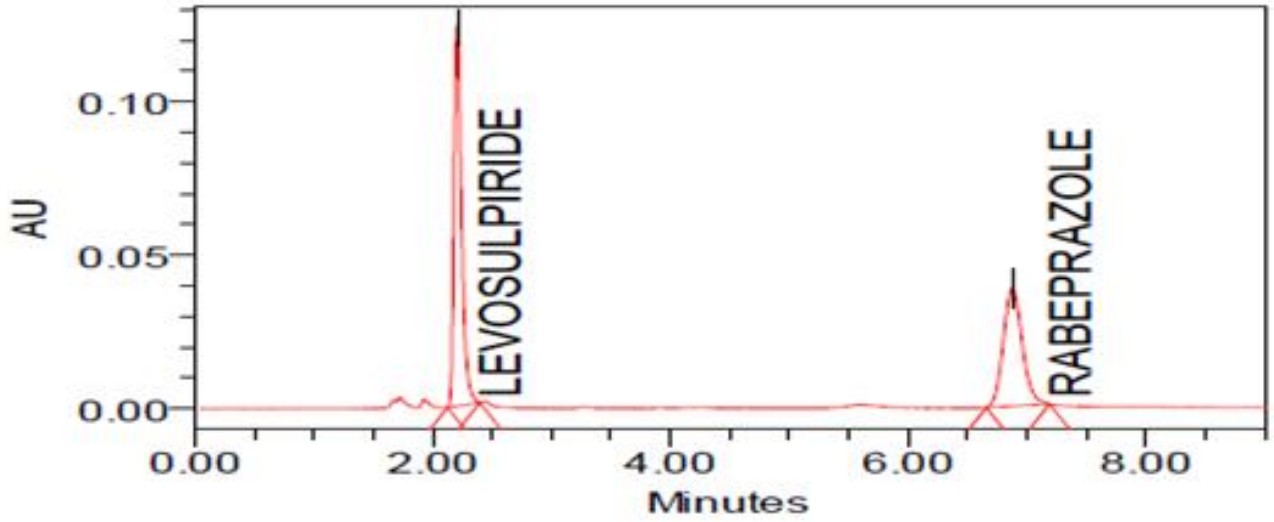
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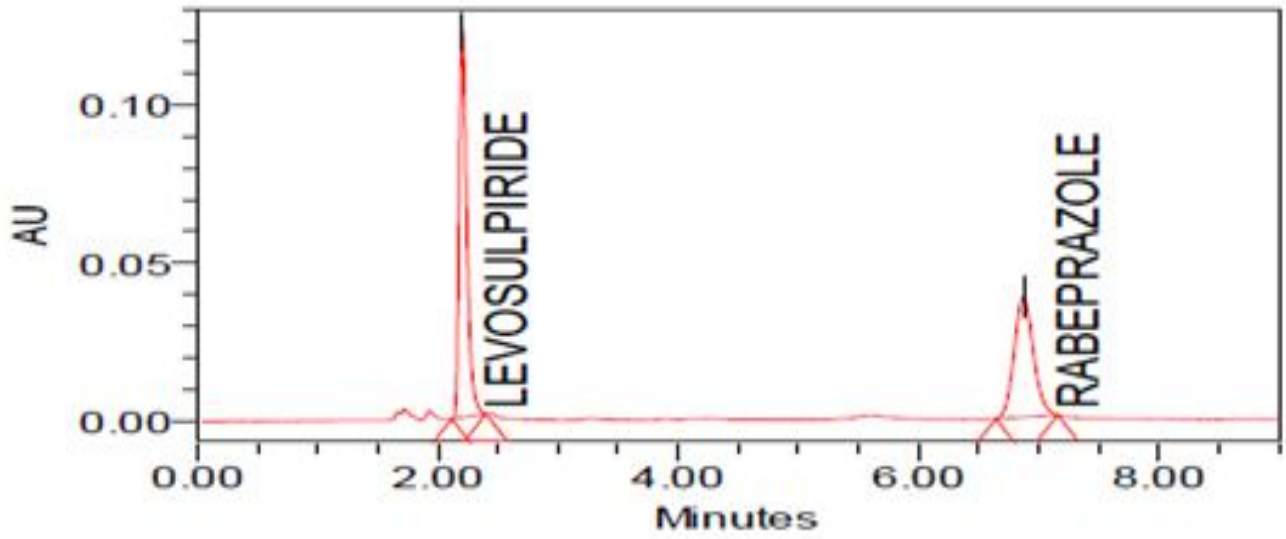
Injection-2



Injection-3

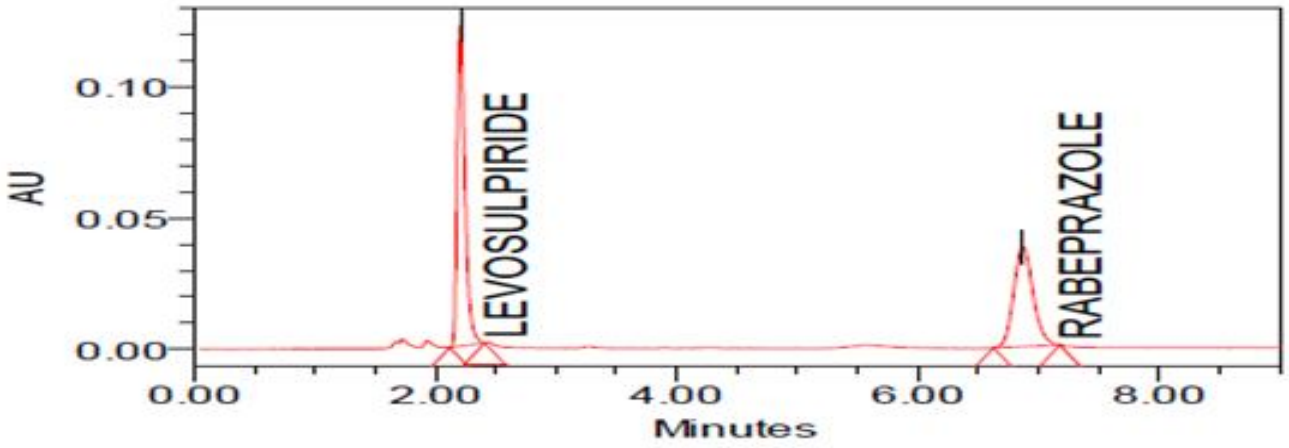


Injection-4



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Injection-5



Injection-6

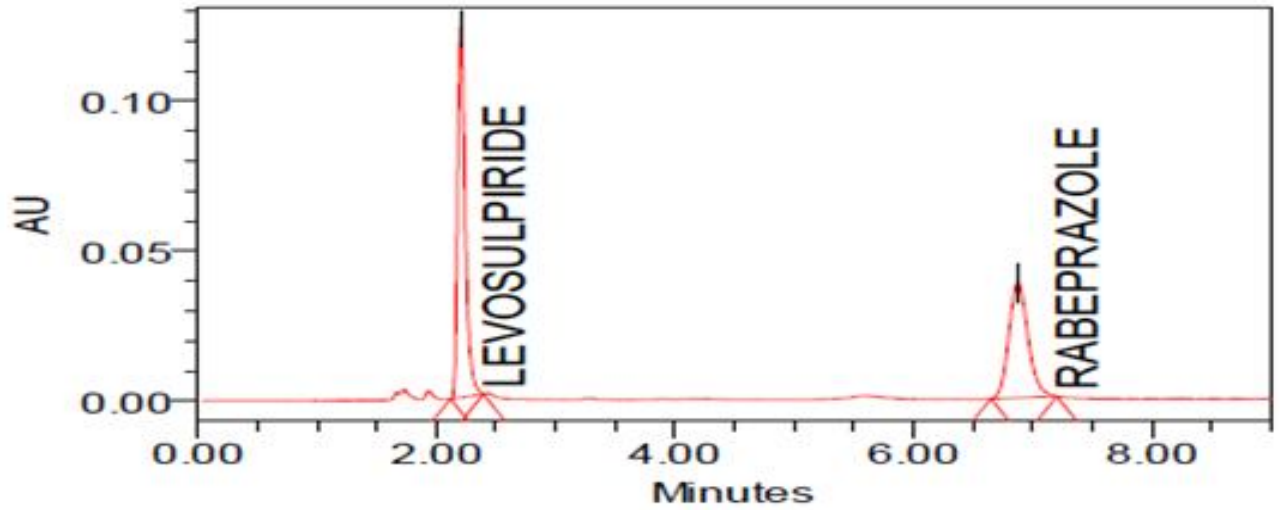


Fig 4: Chromatograms Indicating System Suitability

Table IV
Result of system suitability tests of Rabeprazole and Levosulpiride

Parameters	Levosulpiride	Rabeprazole
Linearity range	5-15 µg/mL	18.75-56.25 µg/Ml
Correlation coefficient	0.999	0.999
Slope	5545.7x-3449.1	4365.4x-6285
Retention time	2.2	6.8
Resolution Factor		22.62
USP plate count	6389	8745
Tailing factor*	1.45	1.14
Limit of Detection(LOD)	1 µg/mL	1 µg/Ml
Limit of quantification(LOQ)	6 µg/Ml	5 µg/Ml

Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis

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B. Precision

Table V
System Precision of Levosulpiride and Rabeprazole

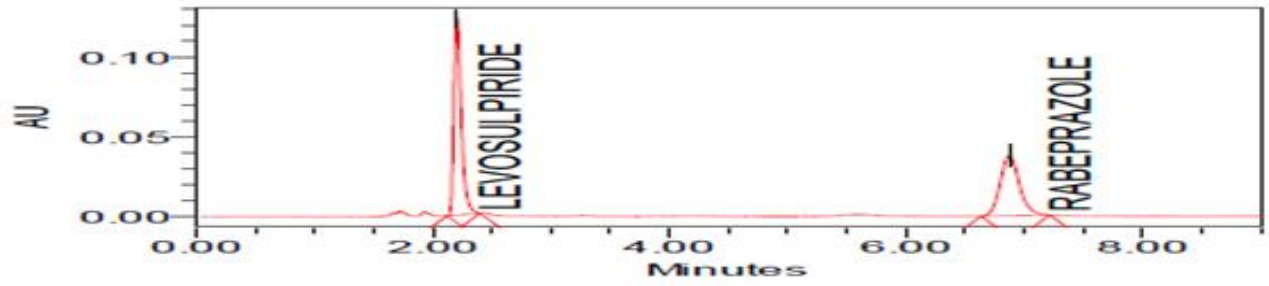
S.NO	Number of Injections	Retention Time of Levosulpiride	Retention Time of Rabeprazole	Area of Levosulpiride	Area of Rabeprazole
1	Injection-1	2.2	6.8	541504	424015
2	Injection-2	2.2	6.8	539909	412798
3	Injection-3	2.2	6.8	540608	415366
4	Injection-4	2.2	6.8	539693	413216
5	Injection-5	2.2	6.8	539310	411184
6	Injection-6	2.2	6.8	540419	412332
	AVG			540240	414818
	STD			779.593	4709.948
	%RSD			0.14	1.13

Table VI
Intra day and inter day Precision of Levosulpiride and Rabeprazole

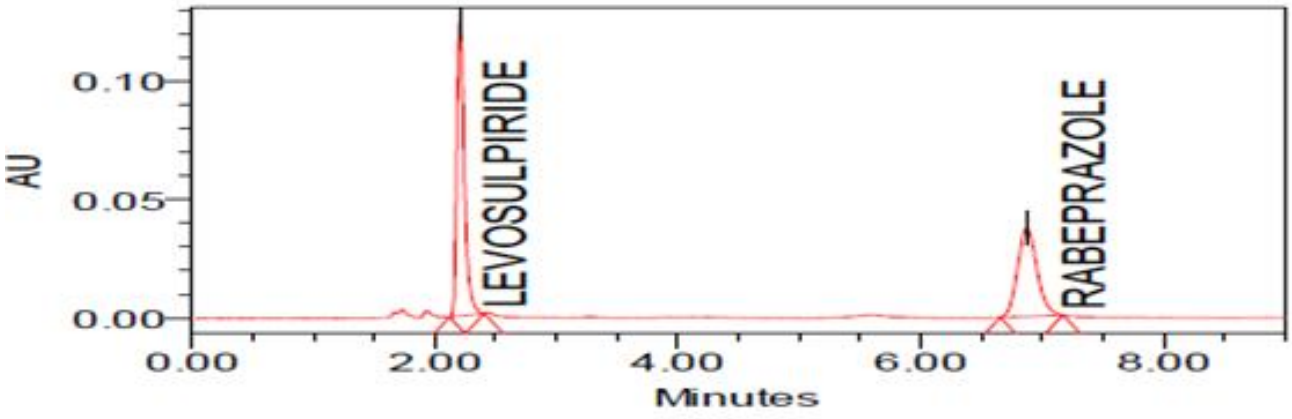
Drug	%RSD (intra day)	%RSD (inter day)
Levosulpiride	0.14	0.4
Rabeprazole	1.12	1.5

Results of Intra day and inter day variability were summarized in table 6. Intra day variability was done from 9.00 am to 6.00 pm on the same day. % RSD of peak areas was calculated for various run .The method is highly precise as % RSD of peak area was less than 2% in all tests.

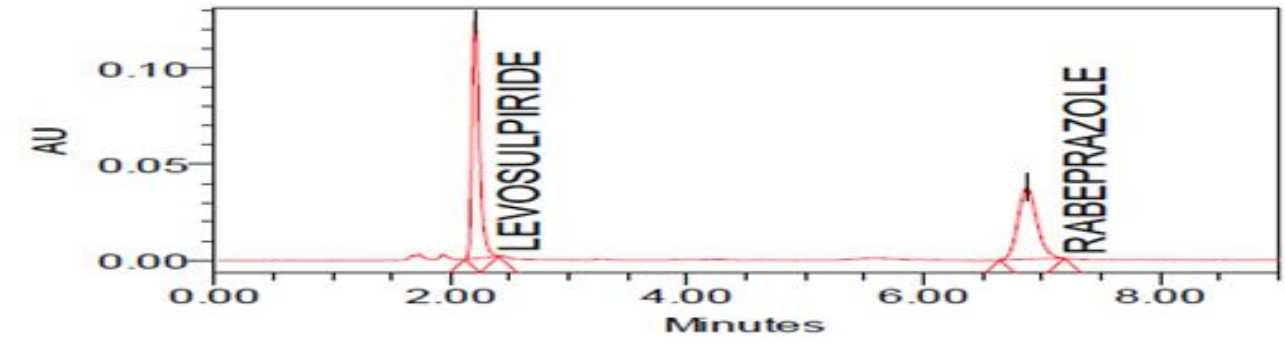
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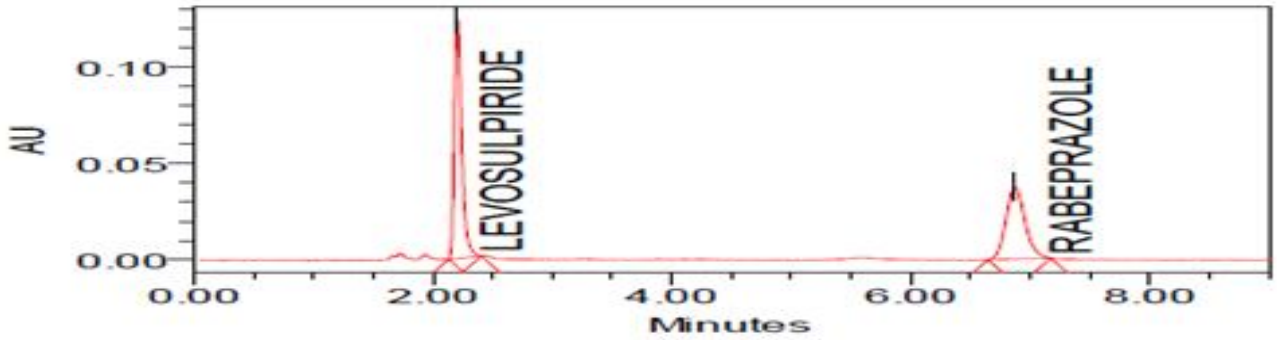
Injection-2



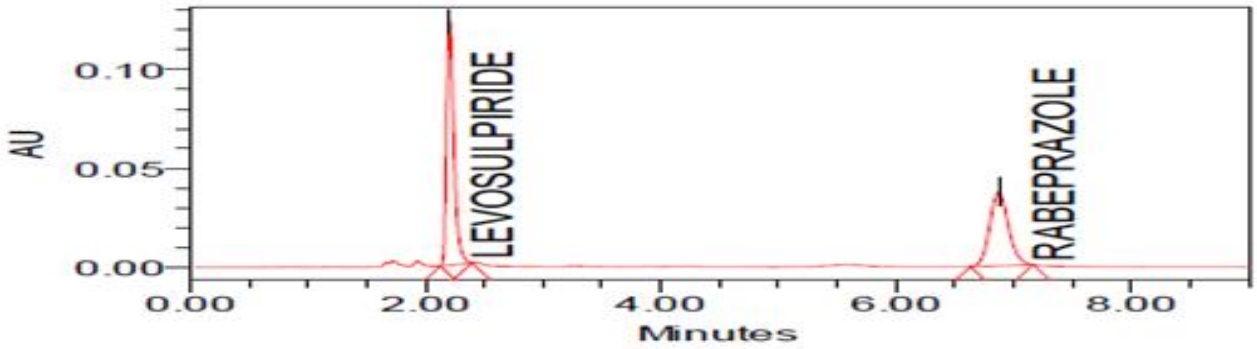
Injection-3



Injection-4



Injection-5



Injection-6

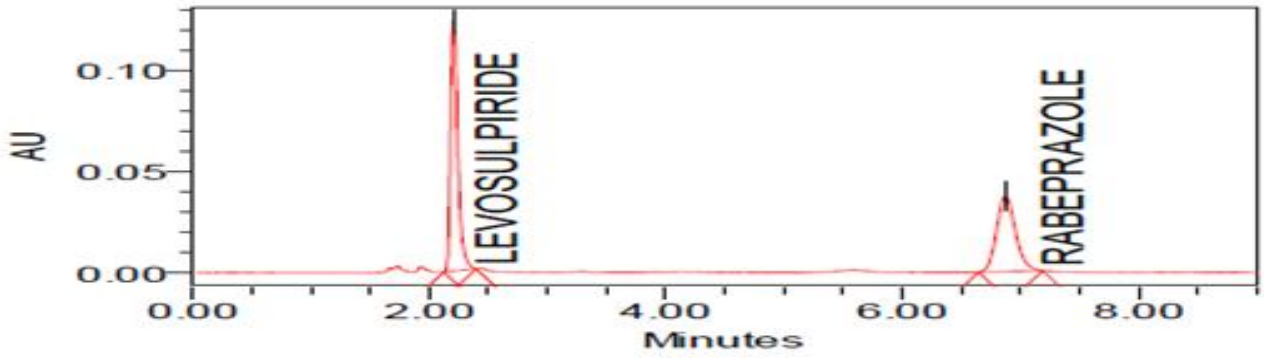


Fig :5 Chromatograms indicating precision

C. Accuracy

Table VII
Accuracy Data of Levosulpiride

Concentration	Area	Spiked amount	Recovered amount	% Recovered	% Average recovery
50	269222	37.5	36.75	98	98.6%
100	544590	75	74.25	99	
150	829958	112.5	111.3	99	

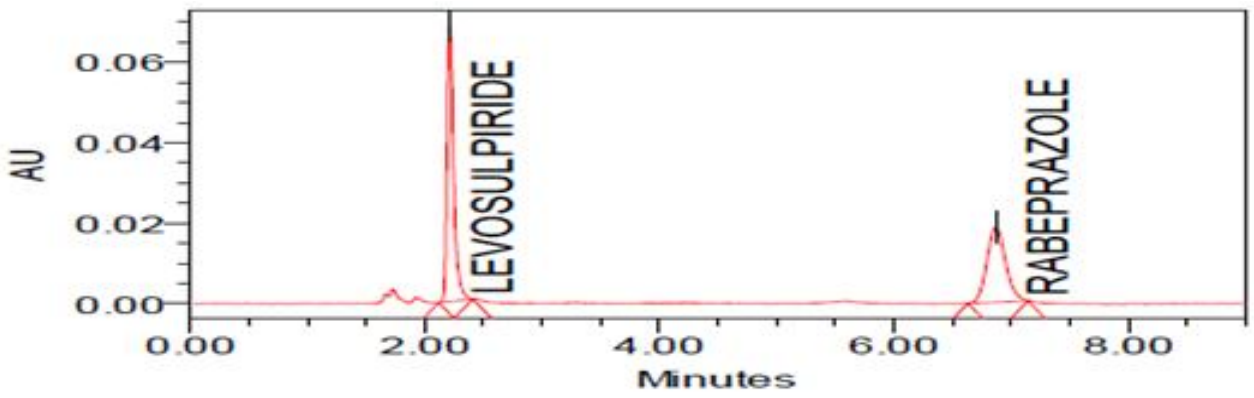
Table VIII
Accuracy Data of Rabeprazole

Concentration	Area	Spiked amount	Recovered amount	% Recovered	% Average recovery
50	200153	10	9.85	98.5	98.7%
100	407931	20	19.6	98	
150	665617	30	29.91	99.7	

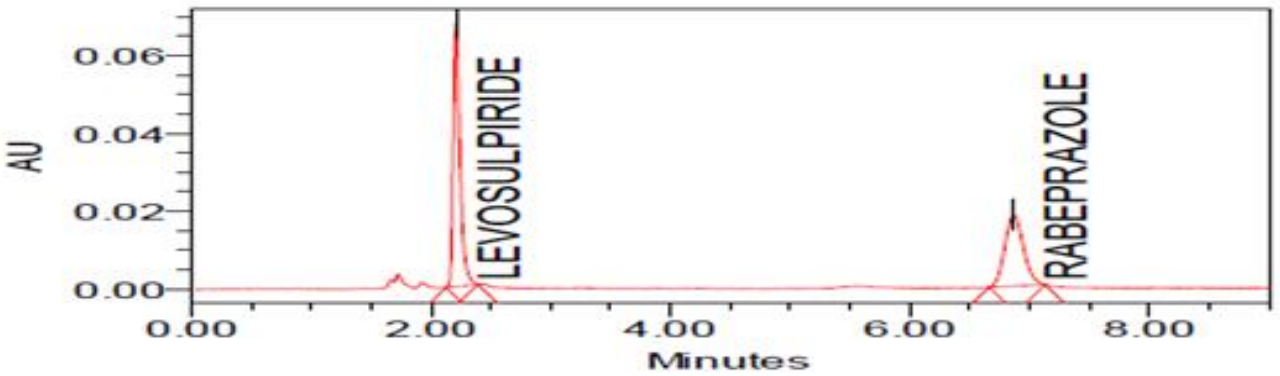
Results of accuracy study are presented in table 7&8. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 98.6% for Levosulpiride and 98.7% for Rabeprazole. All the results indicate that the method is highly accurate.

1) 50% Concentration

Injection-1



Injection-2



Injection-3

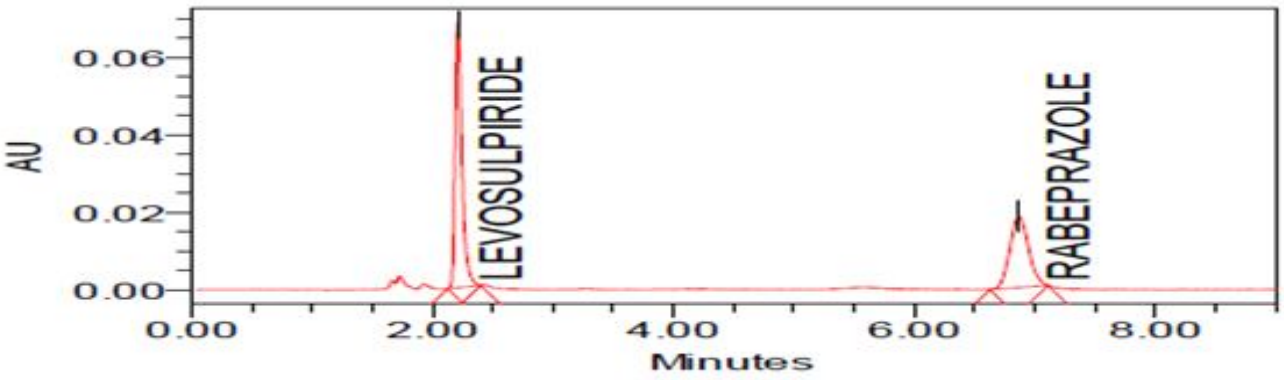
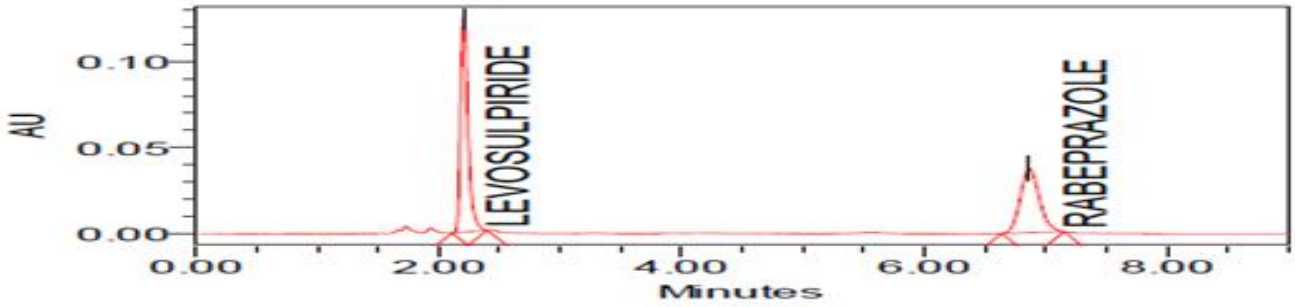


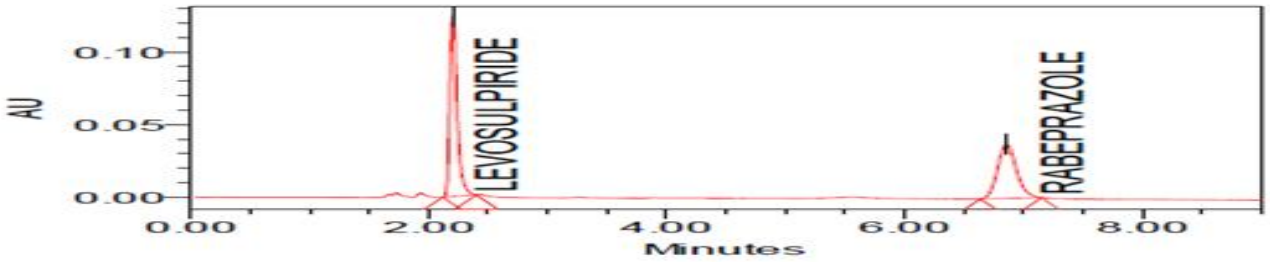
Fig : 6 Chromatograms Indicating Accuracy

2) 100% concentration

Injection-1



Injection-2



Injection-3

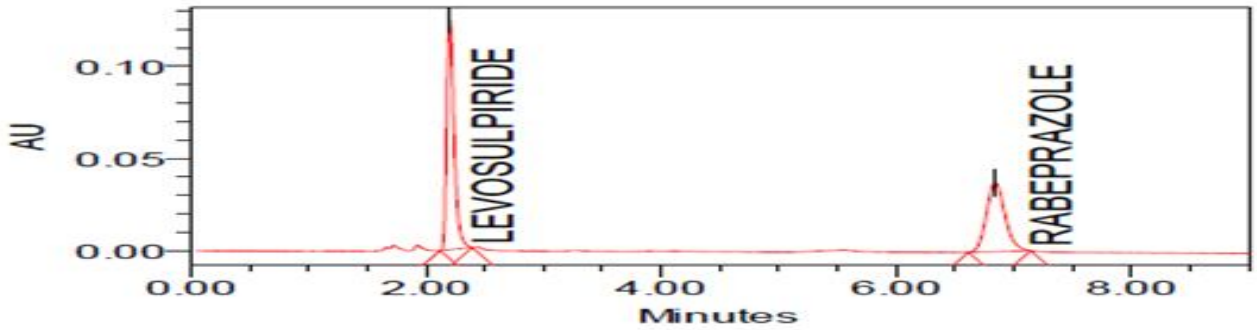
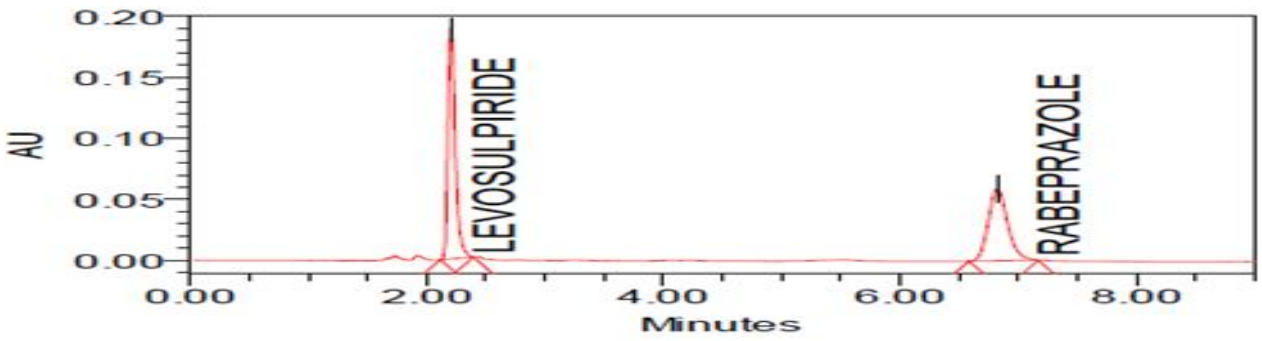


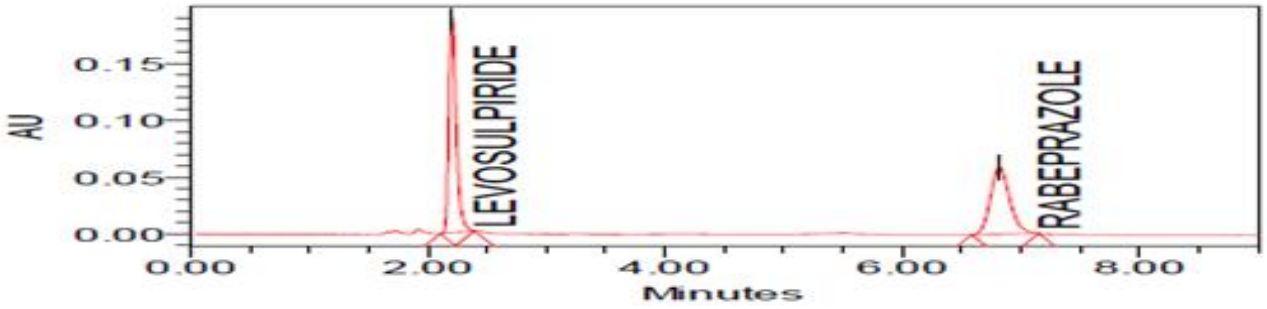
Fig:7 Chromatograms Indicating Accuracy

3) 150% concentration

Injection-1



Injection-2



Injection-3

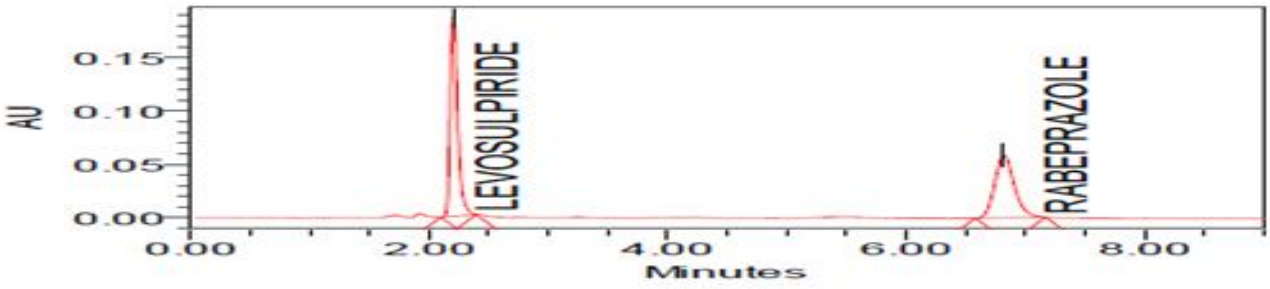


Fig : 8 Chromatograms Indicating Accuracy

D. Linearity

Table IX
Linearity Data of Levosulpiride

S.No	Linearity level	Concentration	Area
1	I	50ppm	270912
2	II	75ppm	409802
3	III	100ppm	551020
4	IV	125ppm	689032
5	V	150ppm	831414
Standard Deviation			221398.6
Correlation Coefficient			0.999

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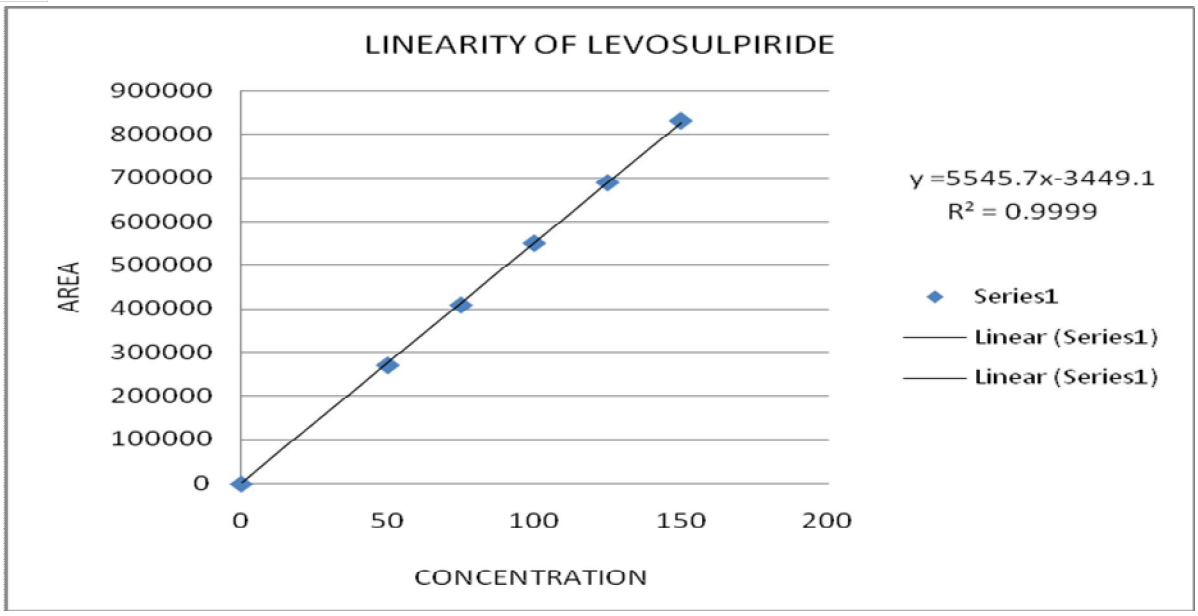


Fig : 9 Calibaration Curve For Linearity of Levosulpiride

Table X
Linearity Data of Rabeprazole

S.No	Linearity level	Concentration	Area
1	I	50	208347
2	II	75	318099
3	III	100	419247
4	IV	125	543661
5	V	150	654079
Standard Deviation			176698.2
Correlation Coefficient			0.999

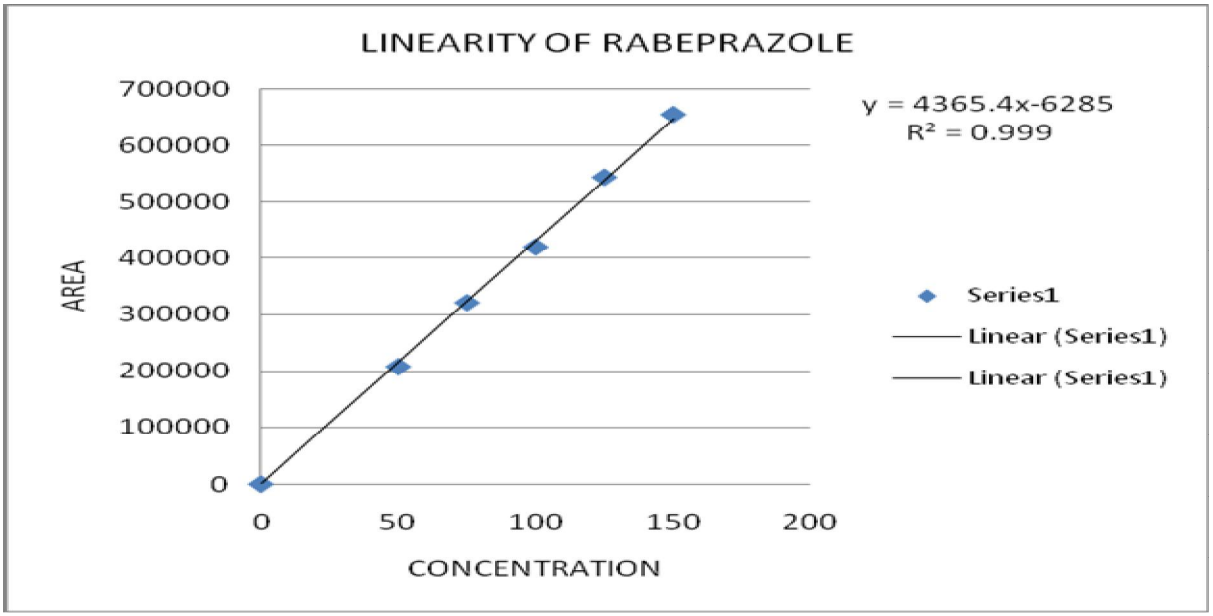
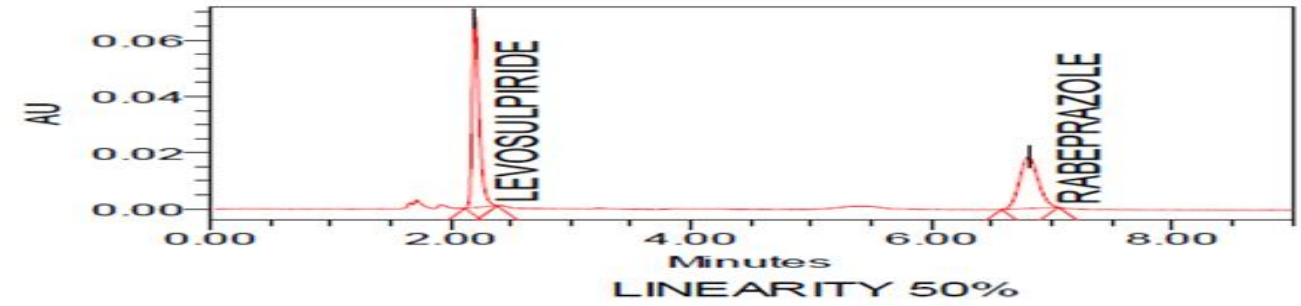


Fig : 10 Calibaration Curve for Linearity of Rabeprazole

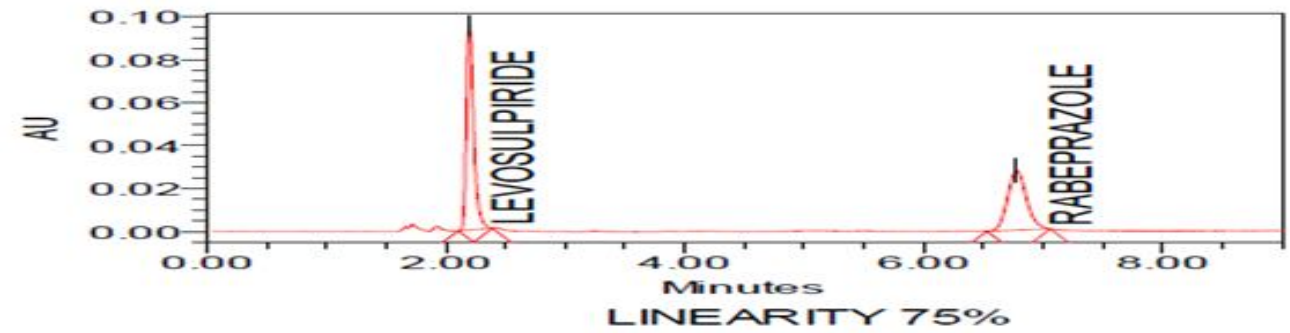
Table XI
Linearity Data of Levosulpiride and Rabeprazole

Parameters	Results observed Levosulpiride	Results observed Rabeprazole
Slope	5545.7	4365.4
Intercept	-3449.1	-6285
Correlation	0.999	0.999

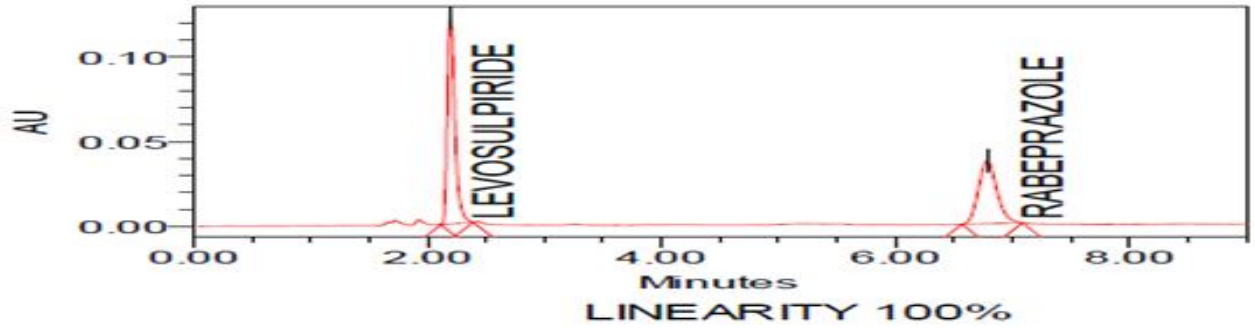
A linear relationship between peak areas versus concentrations was observed for Rabeprazole and Levosulpiride in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both the drugs which prove that the method is linear.



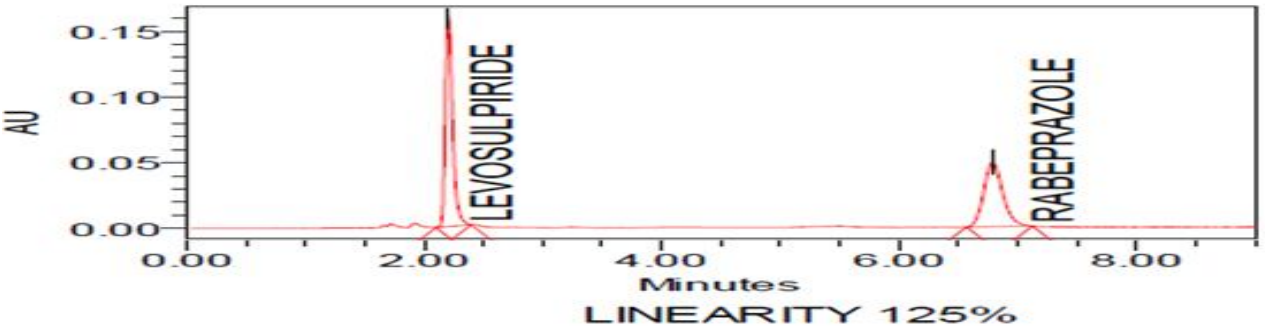
LINEARITY 50%



LINEARITY 75%



LINEARITY 100%



LINEARITY 125%

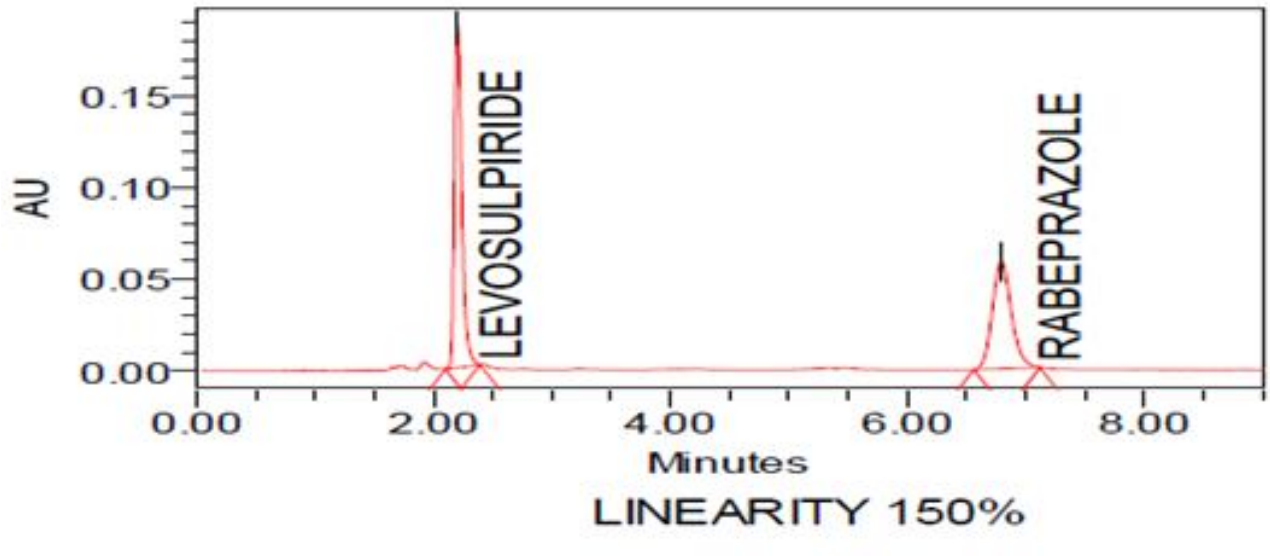


Fig : 11 Chromatograms Indicating Linearity

E. LOD

1) Levosulpiride: LOD = $\frac{3.3 \times \text{Standard Deviation}}{\text{Slope}}$

$$\frac{3.3 \times 221398.6}{5545.7} = 131.744$$

2) Rabeprazole: LOD = $\frac{3.3 \times \text{Standard Deviation}}{\text{Slope}}$

$$\frac{3.3 \times 176698.2}{4365.4} = 133.574$$

F. LOQ

1) Levosulpiride: LOQ = $\frac{10 \times \text{Standard Deviation}}{\text{Slope}}$

$$\frac{10 \times 221398.6}{5545.7} = 399.22$$

2) Rabeprazole: LOQ = $\frac{10 \times \text{Standard Deviation}}{\text{Slope}}$

$$\frac{10 \times 176698.2}{4365.4} = 404.769$$

G. Selectivity

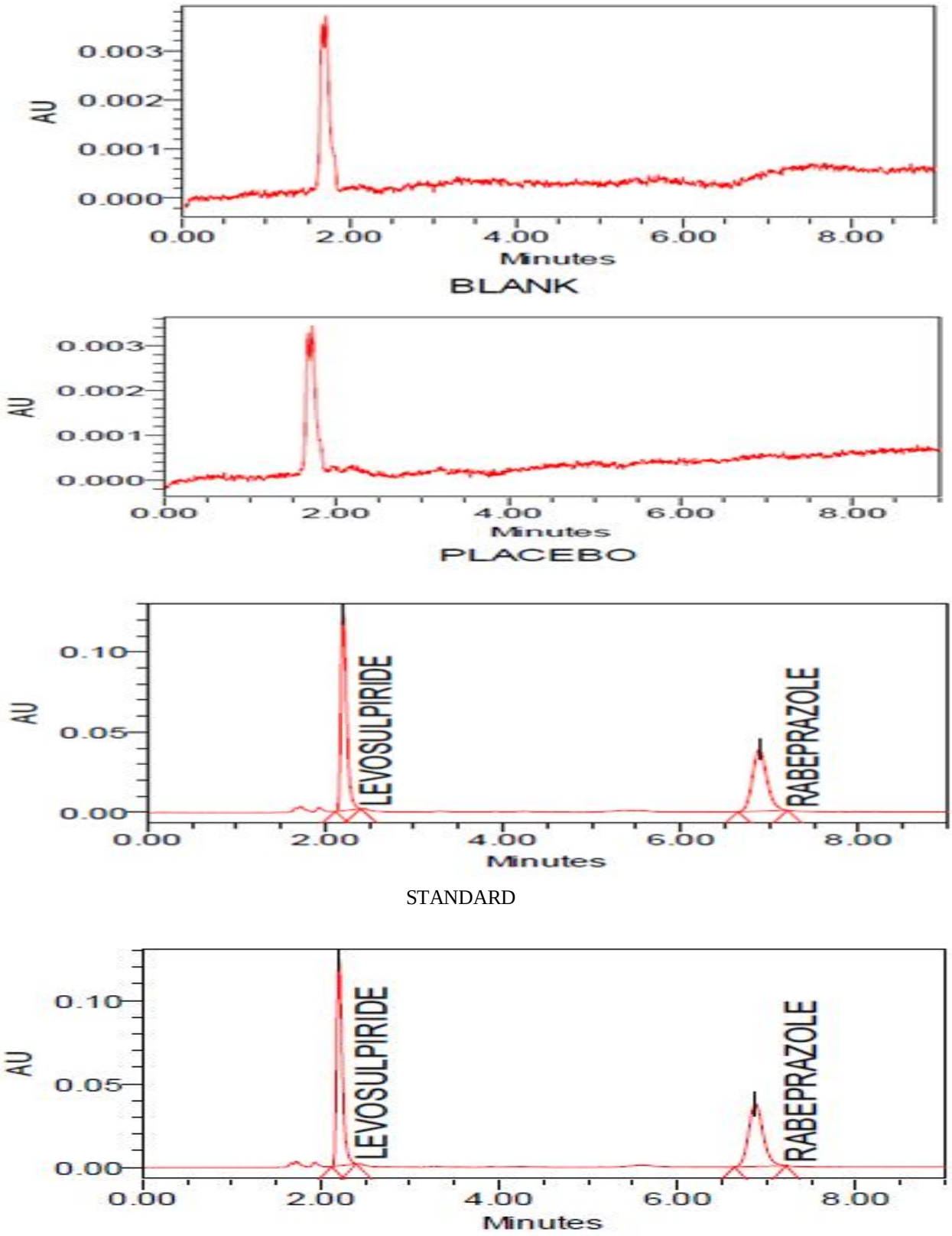


Fig : 12 Chromatograms Indicating Selectivity

Table XII
Selectivity of Levosulpiride and Rabeprazole

	Drug		Retention Time	Area	USP Tailing	USP Platecount
1	LEVOSULPIRIDE	Standard	2.20	551141	1.47	6001
		Sample	2.20	541504	1.45	6389
2	RABEPRAZOLE	Standard	6.88	431551	1.14	8696
		Sample	6.87	424015	1.14	8745

Chromatograms shown in figure 12 explain that retention time for standard sample and commercial product of Rabeprazole and Levosulpiride are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

H. Robustness

Table XIII
Robustness of Levosulpiride

Parameters	Changes	RT	USP Tailing	USP Plate count
Variation in Flowrate(ml/min)	1	2.5	1.44	6059
	1.4	1.9	1.42	6017
Variation in Temperature	45^0c	1.9	1.42	5751
	55^0c	1.95	1.40	6001

Table XIV
Robustness of Rabeprazole

Parameters	Changes	RT	USP Tailing	USP Plate count
Variation in Flowrate(ml/min)	1	7.8	1.13	9996
	1.4	6.0	1.11	8033
Variation in Temperature	45^0c	6.2	1.09	8221
	55^0c	6.01	1.13	10184

The results of robustness of the present method showed that small changes were made in the flow rate and temperature did not produce significant changes in analytical results which are presented in Table 13&14. As the changes are not significant we can say that the method is robust

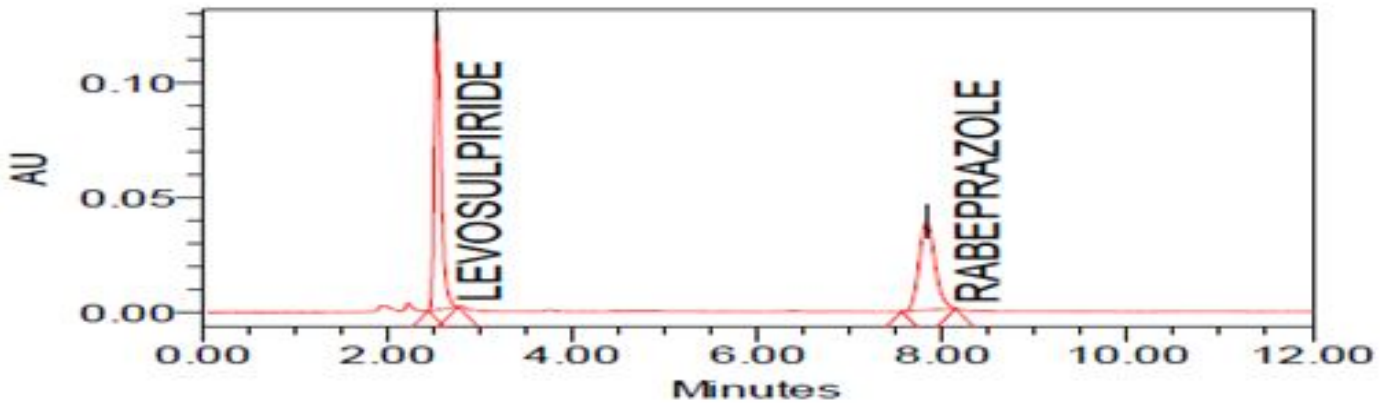


Fig : 13 Chromatogram Indicating Robustness (Less Flow Rate)

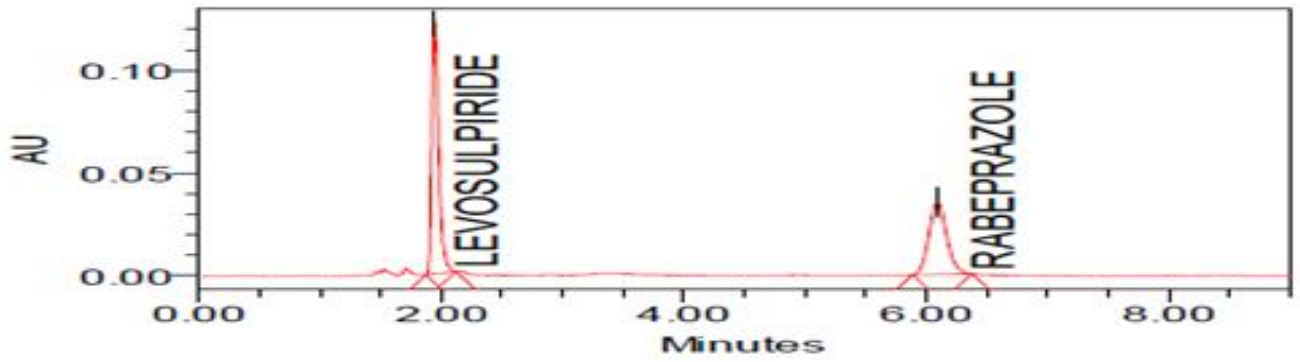


Fig : 14 Chromatogram indicating Robustness (More flow rate)

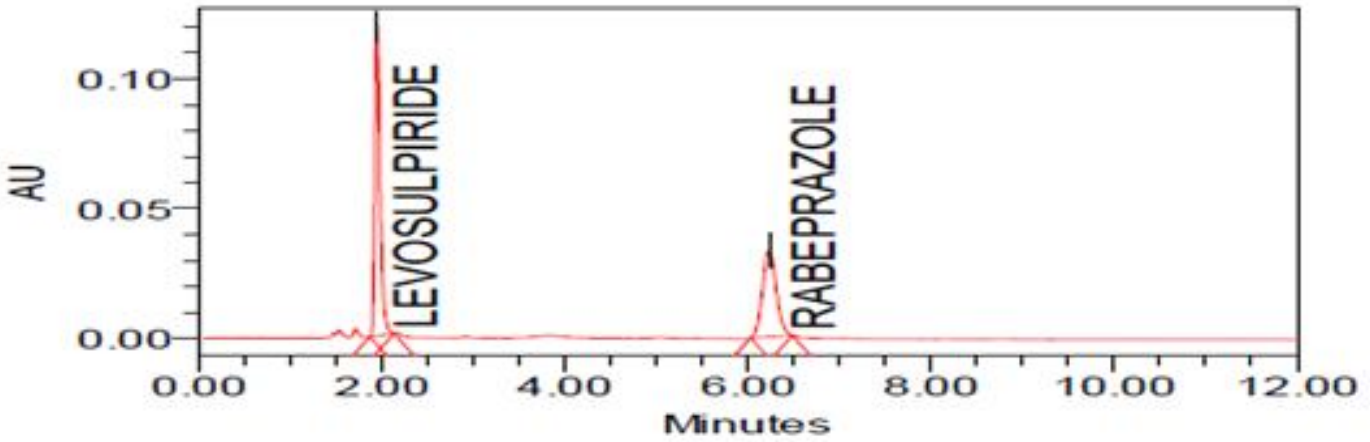


Fig : 15 Chromatogram Indicating Robustness (Less Temperature)

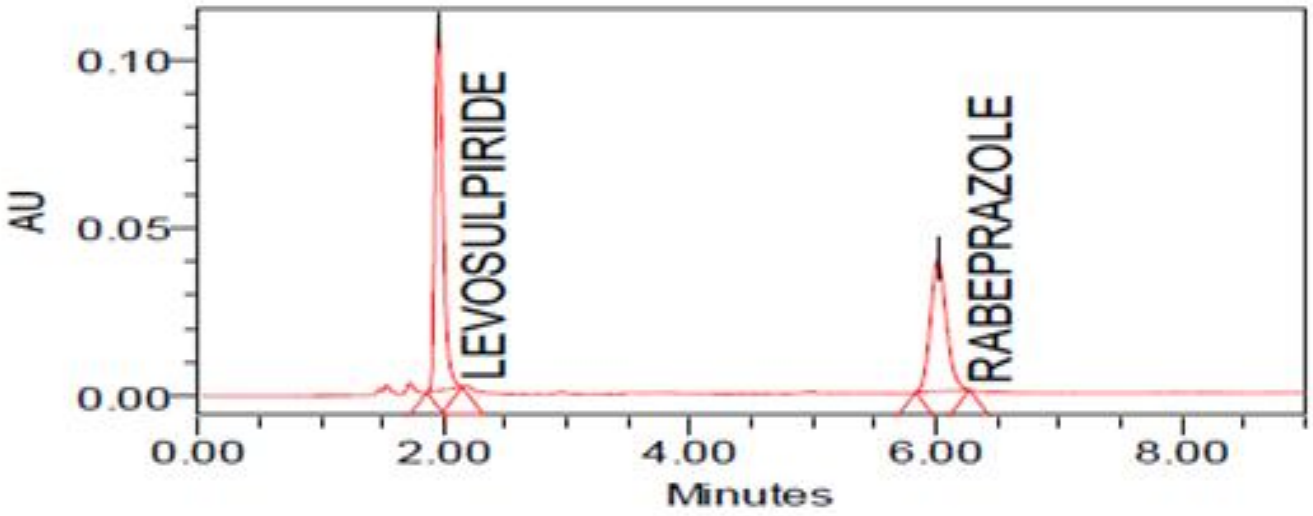


Fig : 16 Chromatogram Indicating Robustness (More Temperature)

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IV. CONCLUSIONS

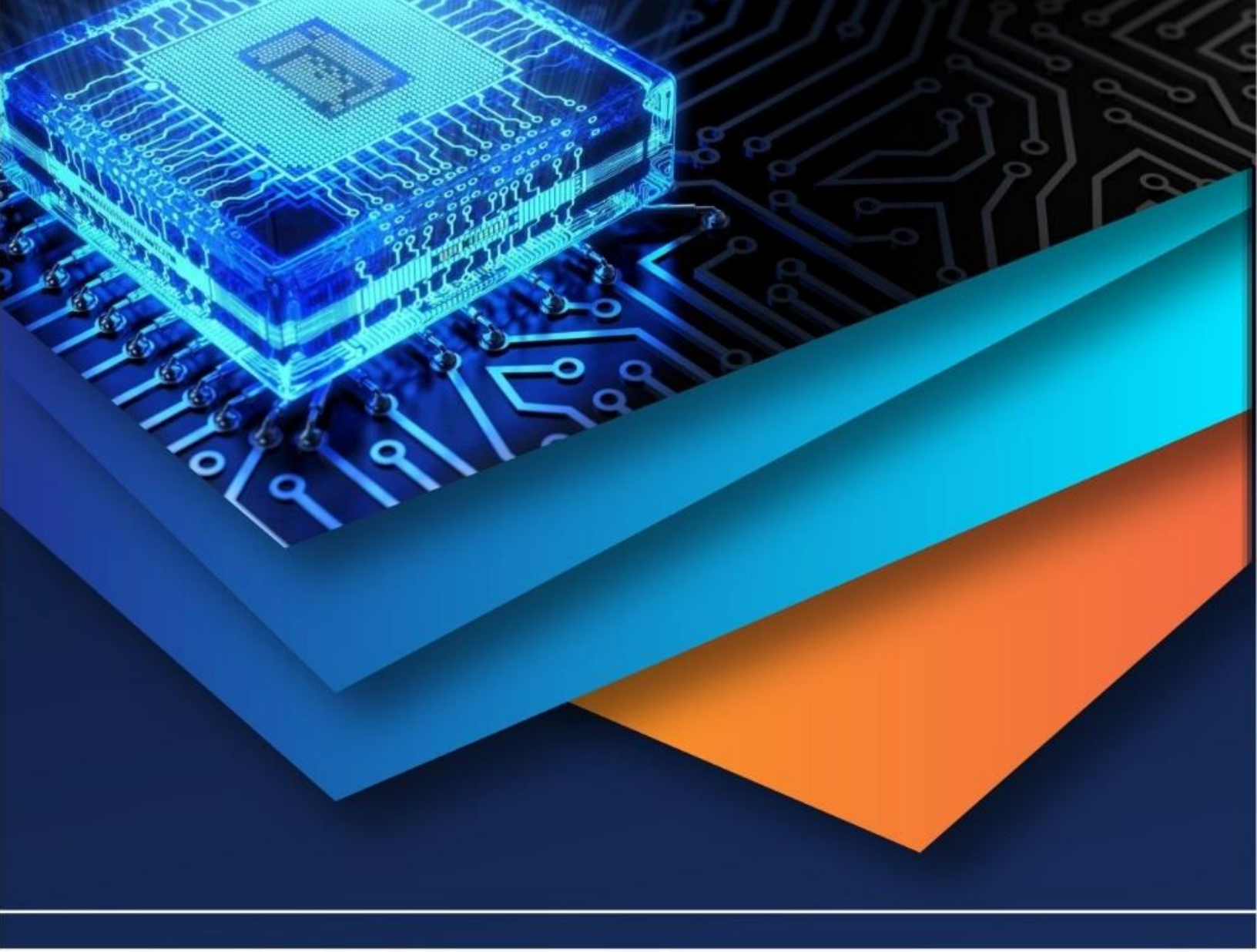
A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Rabeprazole and Levosulpiride in Tablet dosage form.

The new RHPLC method developed and validated for simultaneous determination of Levosulpiride and Rabeprazole pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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