Gobikrishnan C et al /J. Pharm. Sci. & Res. Vol. 6(9), 2014, 292-296

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Newer Analytical Approach For Validation Of Levosulpiride And Rabeprazole Raw Drug And In Their Capsule Dosage Form By RP-UPLC

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Abstract
A new simple, precise, accurate, sensitive, less time consuming isocratic Reverse Phase Ultra Performance Liquid Chromatography (RP-UPLC) method was developed and validated for the determination of Levosulpiride (LEVO) and Rabeprazole (RABE) in their capsule dosage form. This method employs, Thermo scientific hypersil ODS C-18 column (50×2.1 mm, particle size of 1.9 µ) and flow rate 1mL/min with a load of 10µL. Water, Acetonitrile, Methanol and Acetic acid (25:55:20:0.3) composition was used as a mobile phase. pH 5.5 adjusted with tri ethyl amine. The detection was carried out at 284 nm for RABE & 290 nm for LEVO. Retention time of LEVO and RABE were found to be 1.709 minutes and 2.414 minutes respectively. Linearity range for LEVO and RABE were 16-24µg/mL and 60-90 µg/mL respectively. Percent recovery study of LEVO and RABE were performed at three different levels. This newly developed method was successfully utilized for the routine analysis of LEVO and RABE in bulk and capsule dosage forms. This method was validated for accuracy, precision, linearity, system suitability parameters and inters day precision as per ICH guidelines.

Keywords
RP- UPLC, Levosulpiride, Rabeprazole, Validation, Simultaneous method.

INTRODUCTION

Levosulpiride is chemically N-[(1-ethyl pyrolidine-2-yl) methyl]-2-methoxy-5 sulfamoyl benzamide. It is a substituted benzamide anti psychotic, reported to be a selective antagonist of central dopamine (D-2, D-3, D-4) receptors. It is used to treat psychoses, particularly negative symptoms of schizophrenia, anxiety, vertigo, dyspepsia, irritable bowel syndrome and premature ejaculation. (Figure-1)

Rabeprazole is chemically 2-[(4-(3-methoxy propoxy)-3-methyl pyridine-2-yl) methyl sulfinyl]-1H-benzo imidazole. It is a substituted benzimidazole. that inhibit gastric acid secretion and primarily used to treat ulcerative colitis, GERD.It belongs to a class of anti secretary compound that suppress gastric acid secretion by inhibiting the gastric H^+,K^+ ATPase at the secretary surface of the gastric parietal cells. It is also charectarized as a proton pump inhibitor. It blocks the final step of gastric secretion in parietal cells[1]. (Figure-2)

Chemical structure

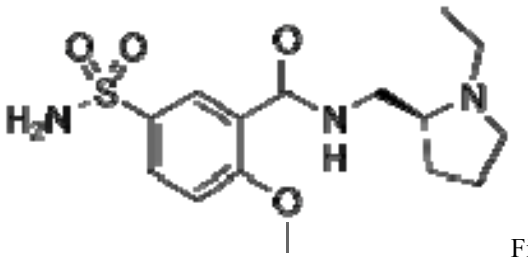

Figure- 1: Chemical structure of Levosulpiride

Figure- 2: Chemical structure of Rabeprazole

MATERIALS AND METHODS

Instruments employed

An Accela RP- UPLC system with PDA detector, hypercil ODS C-18 column (50×2.1 mm, particle size of 1.9 µ) and Chromequese Software was used for this method of analysis. Shimadzu- Libror AGE -220 balances used.

Reagents and Standards

Levosulpiride and Rabeprazole raw drug were received from Sunglow Pharmaceuticals at puducherry. UPLC grade Water, Acetonitrile, Methanol, Acetic acid reagents were used. LEVO (75mg) + RABE (20mg) combination capsule (BRAND-A) were purchased from local pharmacy.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Mobile phase : Water : Acetonitrile : Methanol : Acetic acid (25:55:20:0.3)
pH :5.5 adjusted with Tri ethyl amine
Flow rate : 1mL/min

Column :Thermoscientific C-18(ODS) hypercil gold, particle size 1.9µ, 50×2.1mm(length & diameter)
Detector : PDA
Injection volume: 10µL

PREPARATION OF STANDARD STOCK SOLUTION

LEVO:

Weigh accurately about 47.15 mg of Levosulpiride raw drug transferred in to a 25mL standard flask make up with methanol up to the volume. The concentration of drug is 1.88mg/mL.

RABE:

Weigh accurately about 14.28 mg of Rabeprazole raw drug transferred in to a 25mL standard flask make up with methanol up to the volume. The concentration of drug is 0.5712mg/mL.

PREPARATION OF SAMPLE STOCK SOLUTION

LEVO:

Weigh accurately about 137.56 mg of Levosulpiride formulation transferred in to a 25mL standard flask make up with methanol up to the volume. The concentration of drug per mL is 5.5024mg/mL.

RABE:

Weigh accurately about 91.79 mg of Rabeprazole formulation transferred in to a 25mL standard flask make up with methanol up to the volume. The concentration of drug is 3.6716mg/mL.

ASSAY PROCEDURE

Take 1mL from the standard and sample stock solution transferred into 25mL standard flask make up with methanol up to the volume. Inject 10µL of the 2nd dilution of standard & sample solution in to the chromatographic system. Perform the analysis for 2 different days measure the area of the corresponding peaks (Fig-3&4). The amount of Levosulpiride and Rabeprazole present in the commercial capsule was calculated with the help of standard and sample peak area. The amount of Levosulpiride & Rabeprazole was found to be in first day 75.15mg and 20.07mg, in second day is 75.31mg and 20.03mg against the label claim of 75mg & 20mg.(Figure-3&4),(Table-1&2)

VALIDATION OF ANALYTICAL METHOD

This method is validated for accuracy, precision, linearity, system suitability parameters, inter day precision as per ICH guidelines [2-7].

LINEARITY:

Aliquots of standard solution of LEVO & RABE is 60-90 µg/mL and 16-24 µg/mL respectively was prepared from working standard solution and injected to chromatographic system. The peak area obtained versus respective concentration was plotted. The mean area with its Standard Deviation and % Relative Standard Deviation of peak were calculated. (Figure-5&6),(Table-3,4,5&6)

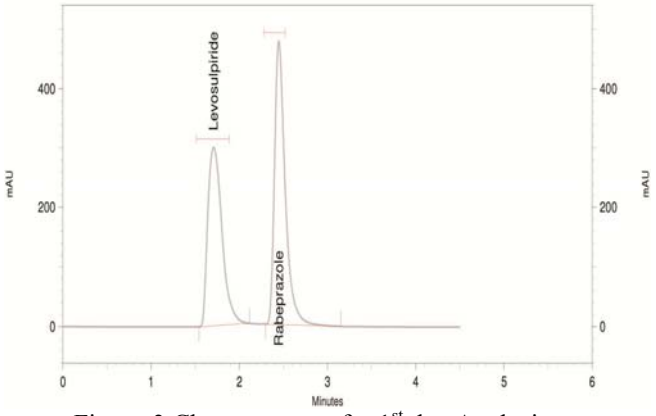

Figure-3 Chromatogram for 1st day Analysis

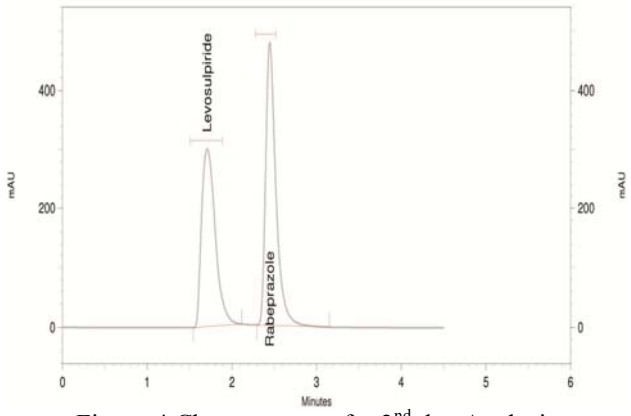

Figure-4 Chromatogram for 2nd day Analysis

Table-1 Assay datas for First day Analysis

Drug	Label claim (mg)	Amount present (mg)	Standard area	Sample area	Percentage
Levosulpiride	75	75.15	3340641	3331327	100.20
Rabeprazole	20	20.07	3962032.2	3977844	100.37

Table-2 Assay datas for second day Analysis

Drug	Label claim (mg)	Amount present (mg)	Standard area	Sample area	Percentage
Levosulpiride	75	75.31	3300155	3298172	100.42
Rabeprazole	20	20.03	3942685	3950057	100.16

LINEARITY FOR LEVO

Table-3

Concentration in %	79.95	89.92	100.2	109.81	119.42
Area	2693048	3029675	3369122	3703041	4039765

Figure-5 LEVO Linearity graph:

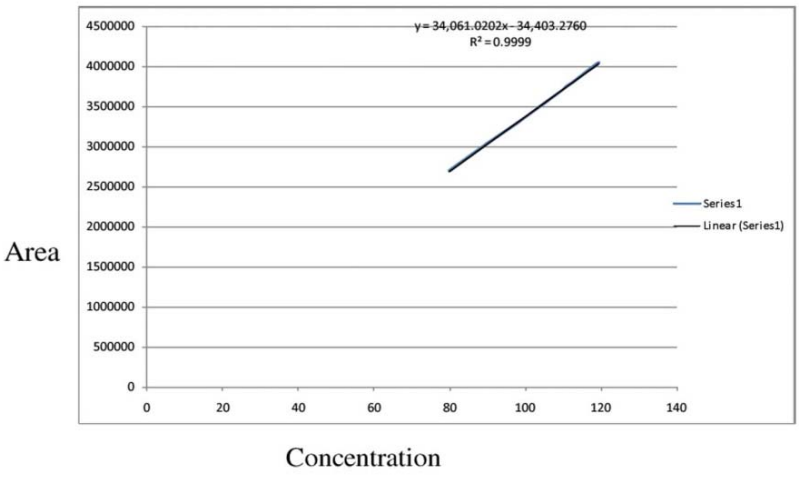

Table-4

Y=mX+c	
Intercept(c)	-34403.27
Slope(m)	34061.02

LINEARITY FOR RABE

Table-5

Concentration in %	79.94	89.9	100.32	109.08	119.5
Area	3196804	3596667	3996159	4395583	4795647

Figure-6 RABE Linearity graph:

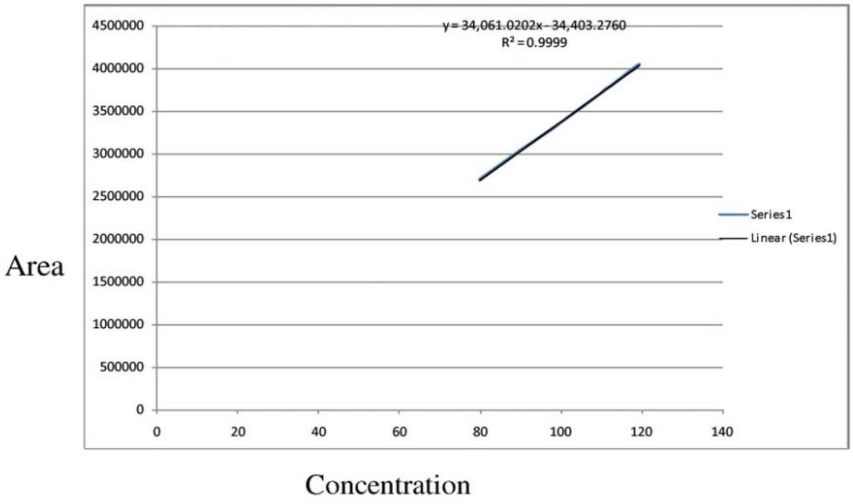

Table-6

Y=mX+c	
Intercept(c)	-25235.19
Slope(m)	40234.99

PRECISION:
Precision studies were carried out for 6 samples from 6 various batches of the formulations. Injected the mixed standards of LEVO & RABE and calculated the SD and %RSD as compared with the formulations.

INTERDAY PRECISION
75.152µg/mL of Levosulpiride and 22.672 µg/mL of Rabeprazole standard solutions were prepared from the Standard stock solution. Prepared 6 different concentration of sample solution of LEVO& RABE from the sample stock solution then injected 6 times into the chromatographic system and analysed for 2 days, calculated the average area of peak, SD & %RSD. Compared the results of 2 days analysis. Prepared 6 different concentration of 6 various batches of this formulations and injected into the chromatographic system and analysed in first day & second day. Calculated the area of peak, SD, %RSD and compared the results of 2 days analysis. With the help of standard & sample area we can calculate the amount of tablet present in the capsule.(Table-7)

RECOVERY STUDY
Accuracy may expressed as percentage recovery. The accuracy was determined by standard addition method. Prepare 75.152 µg/mL of standard solution of Levosulpiride & 22.672 µg/mL of standard solution of Rabeprazole and calculate the average peak area of 5 injections. Prepare the known concentration (361.84 µg/mL) of sample solution of Levosulpiride & Rabeprazole from the sample stock solution then add 10% & 20% of standard solution to it and perform recovery study. Measure the peak area of 100%, 110%, 120% and compare with standard peak area from this we can calculate the accuracy or recovery of added standard.(Table-8&9)

Table-7 Inter day precision data

LEVOSULPIRIDE					RABEPRAZOLE				
DAY-1			DAY-2		DAY-1			DAY-2	
Batches	**Area Mean ± SD**	**%RSD**	**Area Mean ± SD**	**%RSD**	**Batches**	**Area Mean ± SD**	**%RSD**	**Area Mean ± SD**	**%RSD**
B-1	**3377635± 0.4**	**0.35**	**3377655+0.4**	**0.37**	**B-1**	**4009021±0.4**	**0.34**	**4010089±0.4**	**0.32**
B-2	**3326218± 0.4**		**3326745+0.4**		**B-2**	**3984161±0.4**		**3978658±0.4**	
B-3	**3385893± 0.4**		**3377593+0.4**		**B-3**	**4019330±0.4**		**4070223±0.4**	
B-4	**3370362± 0.4**		**3365489+0.4**		**B-4**	**3994155±0.4**		**3988431±0.4**	
B-5	**3380041± 0.4**		**3375891+0.4**		**B-5**	**4012247±0.4**		**4025789±0.4**	
B-6	**3320580± 0.4**		**3336874+0.4**		**B-6**	**3960510±0.4**		**3996851±0.4**	

Table-8: Recovery data for Levosulpiride

Level of recovery	Conc of sample (µg/mL)	Conc of std added (µg/mL)	Total conc (µg/mL)	Standard area	Sample area	Observed mg/tab	%Recovery	Mean recovery	%RSD
100%	361.84	0	361.84	3340641	3331327	75.12	100.37	100.25	0.701
100%	361.84	0	361.84	3340641	3330123	75.08	100.01		
100%	361.84	0	361.84	3340641	3334561	75.18	100.39		
110%	361.84	1.71	363.55	3340641	3670399	82.41	109.94	109.95	0.681
110%	361.84	1.72	363.56	3340641	3669987	82.38	109.90		
110%	361.84	1.73	363.57	3340641	3689876	82.43	110.01		
120%	361.84	3.12	364.96	3340641	4024898	90.02	119.95	120.02	1.032
120%	361.84	3.14	364.99	3340641	4036586	90.11	120.02		
120%	361.84	3.15	365.02	3340641	4037454	90.06	120.09		

Table-9: Recovery data for Rabeprazole

Level of recovery	Conc of sample (µg/mL)	Conc of std added (µg/mL)	Total conc (µg/mL)	Standard area	Sample area	Observed mg/tab	%Recovery	Mean recovery	%RSD
100%	361.84	0	361.84	3962032	3977844	20.07	100.35	100.28	0.701
100%	361.84	0	361.84	3962032	3976998	20.01	100.05		
100%	361.84	0	361.84	3962032	3978049	20.09	100.45		
110%	361.84	1.71	363.55	3962032	4377575	21.99	109.95	109.95	0.681
110%	361.84	1.72	363.56	3962032	4377561	21.98	109.90		
110%	361.84	1.73	363.57	3962032	4381121	22.09	110.01		
120%	361.84	3.12	364.96	3962032	4794759	23.99	119.95	120.02	1.032
120%	361.84	3.14	364.99	3962032	4796657	24.01	120.02		
120%	361.84	3.15	365.02	3962032	4796701	24.09	120.09		

Table-10 System suitability parameters for Levosulpiride

No of injection	Area	Avg Area	SD	%RSD	No of theoretical plates	Retention time	asymmetry
1	3366439	3340641.2	22414	0.67	2572	1.708	1.537
2	3344025				2580	1.702	1.521
3	3305584				2576	1.705	1.524
4	3350147				2594	1.707	1.521
5	3337011				2587	1.709	1.521

Table-11 System suitability parameters for Rabeprazole:

No of injection	Area	Avg Area	SD	%RSD	No of theoretical plates	Retention time	asymmetry
1	3996425	3962032.4	27823.8	0.70	4123	2.418	1.557
2	3969540				4152	2.412	1.579
3	3925324				4045	2.413	1.568
4	3975191				4086	2.412	1.572
5	3943681				4108	2.413	1.575

SYSTEM SUITABILITY

Prepared 75.152 µg/mL of LEVO & 22.672 µg/mL of RABE standard solutions from the standard stock solution and injected 5 times in to the chromatographic system . From this chromatogram measure the Area, SD, %RSD. The %RSD was 0.67 for LEVO and 0.70 for RABE so the system is well suitable for the analysis of this combination drug. (Table-10&11)

CONCLUSION

A simple, rapid, accurate, precise & less time consuming RP-UPLC method was developed and validated for estimation of LEVO & RABE in combined capsule dosage form.

RESULTS & DISCUSSION

The present study was carried out to develop a simple, specific, sensitive, precise, highly accurate & less time consuming RP-UPLC method for analysis of this combination capsule formulations as well as raw drug. The retention time of LEVO & RABE is 1.709min & 2.414min respectively. A good linearity relationship (r=0.999) was observed between the concentration and respective peak areas. The %RSD below 1 i.e (0.67 for LEVO and 0.70 for RABE) shows that the proposed RP-UPLC method was highly precise. The absence of additional peaks indicates no interference of excipients in used capsule formulations.

ACKNOWLEDGEMENT

The authors are greatful to the Founder Chairperson of Periyar College of Pharmaceutical Sciences, Trichy-21, & Ideal analytical lab pudhucherry for extending laboratory facilities to carry out this work and Sunglow pharma for providing gift sample .

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