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Analytical method development and validation of levosulpride and pantoprazole by using reverse phase HPLC method

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ABSTRACT

A simple and precise method was developed for estimating levosulpride well as pantoprazole. The method was found to be specific and precise. The separation was attained on Hypersil BDS (100x4.6mm ID) 5.0µm column and linearity was achieved in the concentration range of 15µg/ml to 45µg/ml of Levosulpride, 8µg/ml to 24µg/ml of Pantoprazole with correlation coefficient 0.99. The percent recovery from the assay was found to be 99.8% for levosulpride and 99.6% for pantoprazole. Limit of detection and quantitation for levosulpride and pantoprazole were within the acceptable range. From the stability studies, the percentage variation was less than 10.0% which is the desired criteria. Therefore, this method can be adopted to estimate levosulpride as well as pantoprazolein other pharmaceutical formulations.

Keywords: levosulpride, pantoprazole, HPLC, Method development, Validation.

INRODUCTION

Levosulpiride, sold under the brand name Neoprad is a substituted benzamide antipsychotic, reported to be a selective antagonist of dopamine D2 receptor activity on both central and peripheral levels. It is an atypical neuroleptic and a prokinetic agent. Levosulpiride is also claimed to have mood elevating properties. Chemically, it is the (S)-(−)-enantiomer of sulpiride. Levosulpiride is used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome, premature ejaculation.[1-6] In contrast to most other neuroleptics which block both dopamine D1 and D2 receptors, sulpiride is more selective and acts primarily as a dopamine D2 antagonist. Sulpiride appears to lack effects on nor epinephrine, acetylcholine, serotonin, histamine, or gamma-amino butyric acid (GABA) receptors.

Pantoprazole is a first-generation proton pump inhibitor (PPI) used for the management of gastro esophageal reflux disease (GERD), for gastric protection to prevent recurrence of stomach ulcers or gastric damage from chronic use of NSAIDs, and for the treatment of pathological hyper secretary conditions including Zollinger-Ellison (ZE) Syndrome. Proton pump inhibitors such as pantoprazole are substituted benzimidazole derivatives, weak bases, which accumulate in the acidic space of the parietal cell before being converted in the canaliculi (small canal) of the gastric parietal cell, an acidic environment, to active sulfonamide derivatives. This active form then makes disulfide bonds with important cysteines on the gastric acid pump, inhibiting its function. Specifically, pantoprazole binds to the sulfhydryl group of H+, K+-ATPase, which is an enzyme implicated in accelerating the final step in the acid secretion pathway. The enzyme is inactivated, inhibiting gastric acid secretion.[7-12] The inhibition of gastric acid secretion is stronger with proton pump inhibitors such as pantoprazole and lasts longer than with the H(2) antagonists.

From the literature survey, it was revealed that few UV spectro photometric methods were developed but were not economical. Moreover, RP-HPLC[13] and LC-MS[14]and derivative methods were also developed which estimates levosulpride and pantoprazole either individually or in combination. In the present research work, a new method was developed to estimate levosulpride and pantoprazole

simultaneously and validated as per ICH guidelines.[15] (Figure 1 & 2)

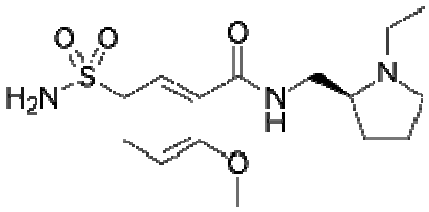

Figure 1: Structure of levosulpride

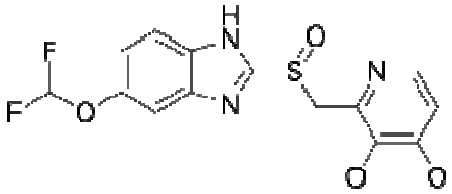

Figure 2: Structure of pantoprazole

MATERIALS AND METHODS

Gift samples of levosulpride and pantoprazole were received from Madras pharmaceuticals, Chennai. Potassium dihydrogen orthophosphate, Di potassium hydrogen orthophosphate was purchased from Final chemicals where as water, Acetonitrile for HPLC and ortho phosphoric acid was purchased from Merck.

Instrumentation: Waters HPLC was used for the separation of levosulpride and pantoprazole. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A. ‘

Method development

Preparation of Phosphate buffer: Prepare 800 mL of distilled water in a suitable container. Add 20.214 g of Na_2HPO_4-$7H_2O$ to the solution. Add 3.394 g of $NaH_2PO_4H_2O$ to the solution. Adjust solution to final desired pH of 7.4 using NaOH. It was then subjected to filtration through membrane filter followed by sonication for 10mins.

Mobile phase preparation: From the above prepared buffer, 500ml is mixed with 500ml of HPLC grade Acetonitrile, mixed, degassed, sonicated for 10min followed by filtration via vacuum filter.

Standard solution preparation: Around 100 mg of Levosulpride and 5mg of Pantoprazole were weighed into a volumetric jar of 100 mL, to this portable stage is included approximately 70mL, and sonicated. 5 mL of the arrangement is pipette out in to volumetric jar of 50 mL and volume is making up by utilizing portable stage.

Sample solution preparation: Almost 100mg of identical powder of Levosulpride and 5mg of Pantoprazole is weighed and taken in volumetric jar of 100 mL and to this portable stage is included around 70mL and sonicated. 5 mL of the arrangement is pipette out in volumetric carafe of 50 mL and volume is making up by utilizing versatile stage. Record the chromatogram.

Procedure: Mixture of buffer (pH 7.4) and acetonitrile in the ratio 50:50% v/v was used as mobile phase which was injected into the system for 30 minutes prior to injecting the prepared solutions of standard as well as sample. Detection of the drug was achieved at the wavelength of 274nm at 25°C. After several trials, method was optimized followed by validation of the method considering various validation parameters.

RESULTS AND DISCUSSION

Method development was achieved using Hypersil BDS (100x4.6mm ID) 5.0µm column. Mobile phase was mixture of Phosphate buffer and acetonitrile (50:50% v/v). Flow rate (1ml/min) and injection volume (20µl) was set. The peaks obtained had good resolution with the retention time 3.386 and 4.433 for levosulpride and pantoprazole respectively. Chromatogram of optimized trial is shown in figure 3.

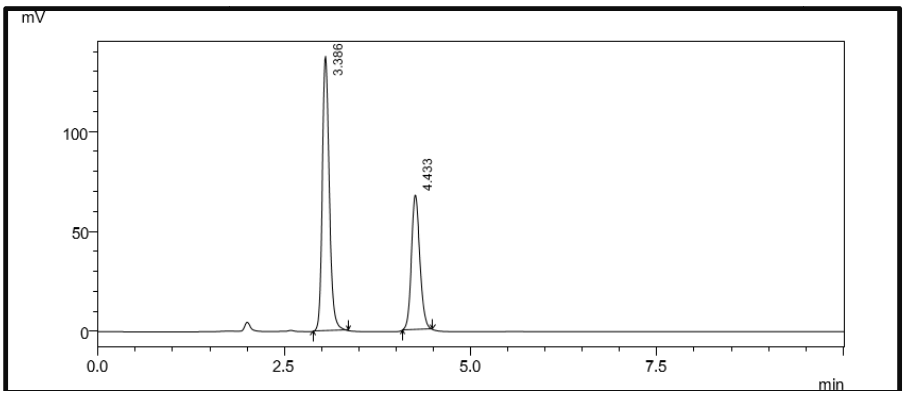

Figure 3: Chromatogram of optimized trial

System suitability: All the parameters were evaluated by performing system suitability studies. The recorded responses for suitability studies are depicted in table 1.

Table 1: results of system suitability parameters

Peak	Retention Time	Area	Height	Theoretical plates/Mt	Area%	Tailing Factor
1	3.386	920431	135380	30454.854	62.727	1.261
2	4.433	546931	62870	44763.294	37.273	1.144
Total		1468363	198249		100.000	

Method validation: Validation of the method was evaluated for various parameters which include linearity, specificity, robustness and stability. The method was also evaluated for specificity of the method and was found to be specific as there were no interactions found. Linearity obtained was shown to have good correlation as shown in table 2.

Table 2: Results of assay

Drug	Label claim(mg)	Amount found(mg)	% Assay
Levosulpride	75	74.85	99.8
Pantoprazole	40	39.84	99.6

Linearity: The linearity range was observed from 15µg/ml to 45µg/ml of Levosulpride, 8µg/ml to 24µg/ml of Pantoprazole. The respective absorbance values are depicted in table 3. The linearity graph plotted is presented in figure 4 and 5 for Levosulpride as well as Pantoprazole respectively.

Table 3: linearity results

S. No.	Levosulpride		Pantoprazole	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	15	454530	8	264727
2	22.5	733496	12	443366
3	30	883374	16	524516
4	37.5	1200148	20	717888
5	45	1388503	24	842245

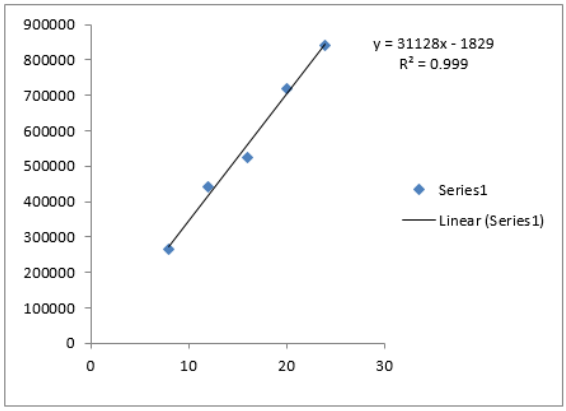

Figure 4: Linearity graph for Levosulpride

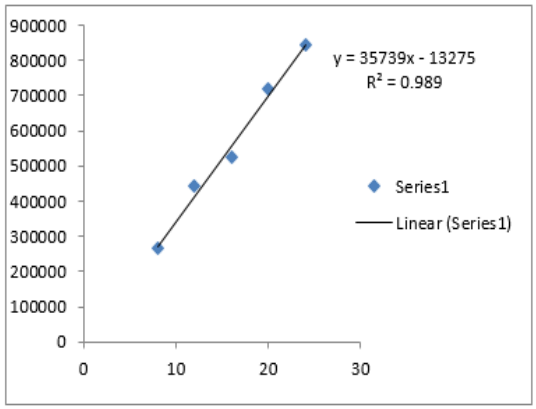

Figure 5: Linearity graph for Pantoprazole

Accuracy: Percent recovery of sample solutions at different concentrations (50%, 100%, and 150%) was calculated. The Percent recovery of levosulpride and pantoprazole are depicted in table 4 and 5 respectively.

Table 4: Accuracy (recovery) data for Levosulpride

% Recovery	Standard Weight in mg	Area	Concentration Added	Concentration Recovered	%Recovery	Average
50% -1	37.5	461018	15	15.03	100.2	99.3
50% -2	37.5	456169	15	14.865	99.1	
50% -3	37.5	454159	15	14.805	98.7	
100% -1	75	907152	30	29.55	98.5	
100% -2	75	909748	30	29.64	98.8	
100% -3	75	899426	30	29.31	97.7	

150% -1	112.5	1398530	45	45.585	101.3
150% -2	112.5	1348013	45	43.92	97.6
150% -3	112.5	1400913	45	45.63	101.4

Table 5: Accuracy (recovery) data for Pantoprazole

% Recovery	Standard Weight in mg	Area	Concentration Added	Concentration Recovered	%Recovery	Average
50% -1	20	277733	8	7.76	97.1	
50% -2	20	271508	8	7.9	99.9	
50% -3	20	261903	8	7.888	98.6	
100% -1	40	60846	16	16.096	100.6	
100% -2	40	554904	16	15.68	98.0	101.1
100% -3	40	899426	16	18.752	117.2	
150% -1	60	855433	24	23.9	99.7	
150% -2	60	857891	24	24	100.0	
150% -3	60	847447	24	23.712	98.8	

Precision: Precision of the method was performed for both sample solutions as described under experimental work. The results are depicted in the table 6.

Table 6: Results of precision

Levosulpride			Pantoprazole		
S.No.	**Retention time**	**Area**	**S.No.**	**Retention time**	**Area**
1	3.379	909854	**1**	4.439	552029
2	3.399	897813	**2**	4.426	555619
3	3.383	903496	**3**	4.465	546873
4	3.378	900330	**4**	4.439	544088
5	3.341	894921	**5**	4.462	541606
6	3.326	867809	**6**	4.483	525570
Average	3.367	895704	**Average**	4.452	544298
Standard deviation	0.009	14598	**Standard deviation**	0.036	10513
%RSD	0.3	1.6	**%RSD**	0.8	1.8

Limit of Detection and Quantitation:

The constrain of location of both drugs were decided by calculating the signal-to-noise(S/N) proportion of 3:1 individually concurring to rules.

LOD = 3.3 α / S

= (3.3) * (2283.05)/9315

= 0.80µg/ml (Levosulpride)

= (3.3) * (3547.5)/11988 = 0.97µg/ml (Pantoprazole)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The constrain of evaluation of Levosulpride and Pantoprazole were decided by calculating the signal-to-noise(S/N) proportion of 10:1 individually concurring to Universal Conference on Harmonization rules.

LOQ = 10 α / S

= (10) * (2283.05)/9315

= 2.45µg/ml (Levosulpride)

Robustness: The standard and samples of both drugs were injected by changing the conditions of chromatography. There was no change observed in the parameters like tailing factor, resolution, plate count and asymmetric factor. Chromatograms for variation in flow rate are presented in figure 6 and 7where as chromatograms for variation in wavelength are presented in figure 8 and 9. Their respective results are depicted in table7.

Variation in flow

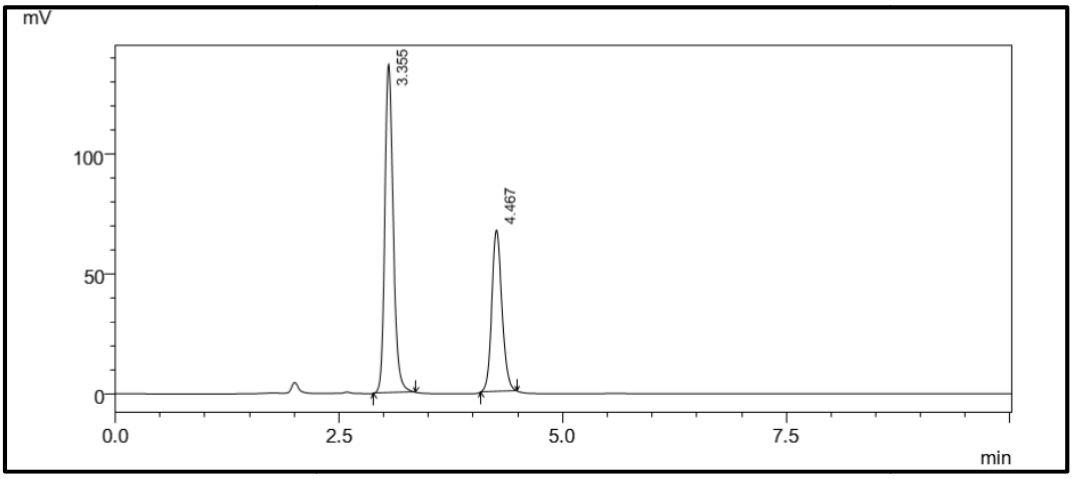

Figure 6: Chromatogram showing less flow

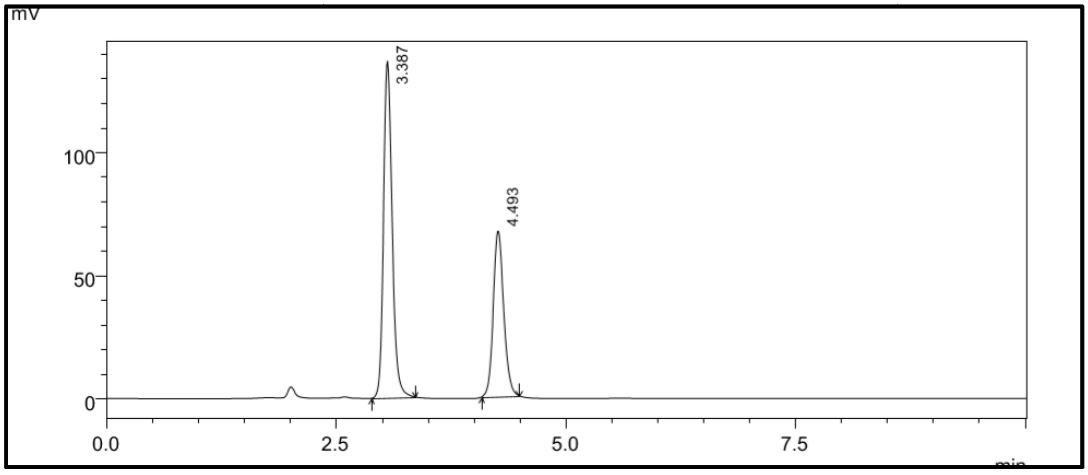

Figure 7: Chromatogram showing more flow

Variation of mobile phase organic composition

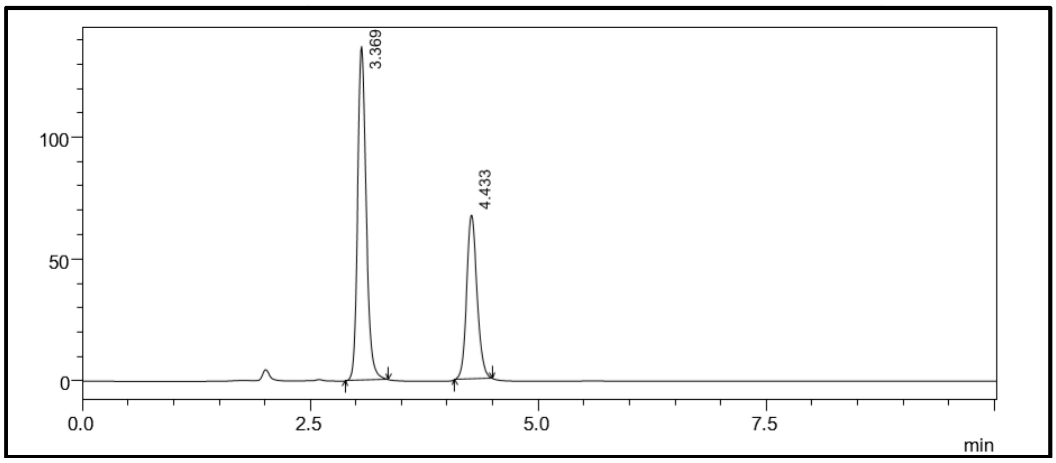

Figure 8: Chromatogram with less organic composition

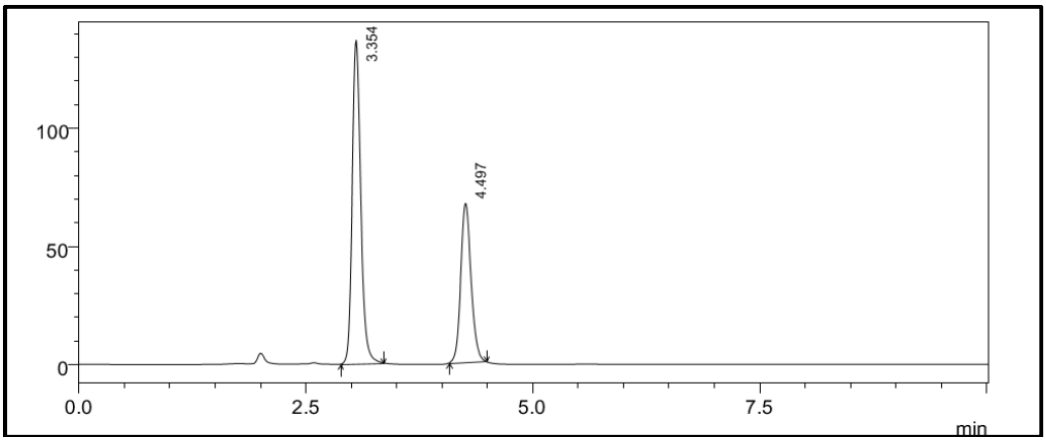

Figure 9: Chromatogram with more organic composition

Table 7: Results for Robustness

Chromatographic changes		Theoretical Plates		Tailing factor	
		Levosulpride	Pantoprazole	Levosulpride	Pantoprazole
Flow rate(mL/min)	0.8	34208	47021	1.238	1.198
	1.2	27281	37074	1.254	1.166
Wave length (nm)	272	31000	41822	1.247	1.168
	276	30697	41839	1.248	1.205

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Levosulpride and Pantoprazole was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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