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Simultaneous Determination of Pantoprazole and Levosulpride by RP-HPLC

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ABSTRACT

A rapid, specific reversed phase HPLC method has been developed for simultaneous determination of pantoprazole and levosulpridein their formulations. Chromatographic separation of these two pharmaceuticals was carried out on an Aligent,Zorbax column (250mmx4.6mm, particle size 5µm)with a 600:4000 (v/v/v) mixture of Potassium dihydrogen orthophosphate (pH-3.0; 0.01M) buffer adjusted with Ortho phosphoric acid and methanol as mobile phase. The flow rate 1.0mL.min^{-1} and the analytes are monitored at 230nm.The assay results were linear from 240-720µg/mL for pantoprazole(r^2 >= 0.9999) and 450-1350µg/mL for levosulpride (r^2 >= 0.9999), showed intra- and inter-day precision less than 2.0%, and accuracy of 100%.The LOD was 0.00321 and 0.000549µg.mL^{-1} for pantoprazole and levosulpride respectively. Separation was complete in less than 5 min. Validation of the RP-HPLC method showed to be robust, precise, accurate and linear over the range of analysis.

Keywords: Pantoprazole and levosulpride, RP-HPLC, Validation.

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INTRODUCTION

Pantoprazole[1,2] is chemically, sodium 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy2 -pyridinyl)methyl] sulfinyl]-1H-benzimidazole (Figure 1). It is used for short term treatment of erosion and ulceration of the esophagus caused by gastro-esophageal reflux disease (GERD), peptic ulcer, NSAID-associated ulceration and Zollinger-Ellision syndrome. Levosulpiride[3,4], a purified levo-isomer of sulpirideis chemically 5- (amino sulfonly)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxy benzamide (Figure 2).It is a D_2 dopamine receptor antagonist and indicated in treatment of psychosis, depression, functional dyspepsia as well as used for prokinetic activity and used with some other drugs in combination therapy.

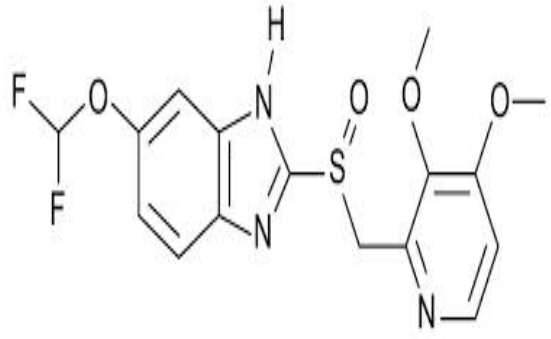

Figure 1:Structureof Pantoprazole

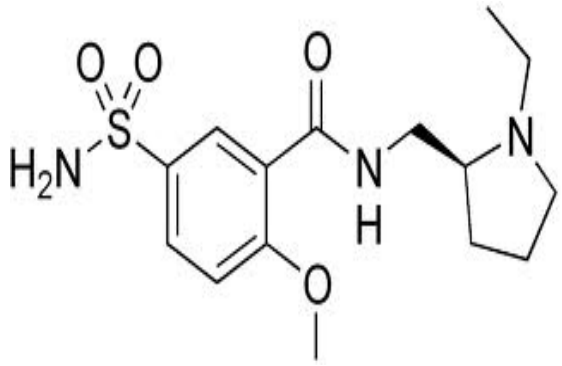

Figure 2:Structure of Levosulpiride

These two drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin. Literature survey revealed that several papers[5-19] have been reported for have been reported for estimation of the above selected drugs in single or in combination forms. In the present paper an attempt have been made to develop a RP-HPLC method for assay of pantoprazole and levosulpride and in combined dosage form and was validated following ICH guidelines.

MATERIALS AND METHOD

Chemicals and Reagents

Reference Standards of pantoprazole sodium and levosulpiride were obtained as gift samples from the ZydusCadila Pharmaceutical Ltd. The drug sample (capsule) PANTO-LEVO manufactured by sun pharma was procured from market. HPLC grade were purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen orthophosphate, ortho phosphoric acid of AR grade from S.D.Fine Chemicals Ltd and methanol of HPLC grade supplied by Fischer Scientific Chemicals were used in the present assay. HPLC grade water was obtained from a Milli-QRO water purification system.

Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, and Waters Empower2 software and Aligent, Zorbax column (250mmx4.6mm, particle size 5µm).

Mobile Phase Preparation

The mobile phase consisted of potassium dihydrogen orthophosphate (pH-3; 0.01M) buffer and methanol in the ratio of 600:400 v/v respectively. The mobile phase was filtered through a 0.45µm membrane filter (Millipore Pvt. Ltd. Bangalore, India) and degassed using an ultrasonic bath.

Diluent Preparation

Mobile phase was used as a diluent in the present assay.

Standard Preparation

Pantoprazole standard stock solution containing 1000µg/mL was prepared in a 25mL volumetric flask by dissolving 100mg of pantoprazole and then diluted to volume with diluent. Further this stock solution is diluted in 10mL volumetric flask and make up to mark with diluent (standard solution of range 240-720µg/mL). Levosulpride standard stock solution containing 1000µg/mL was prepared in a 25mL volumetric flask by dissolving 100mg of levosulpride and then diluted to volume with diluent. Further this stock solution is diluted in 10mL volumetric flask and make up to mark with diluent (this standard solution of 450-1350µg/mL) and sonicated to dissolve.

Sample Preparation

For this twenty capsules were purchased from local pharmacy and their average weight was determined and the capsules were opened and the granules were finely powdered with the help of mortar and pestle. Appropriate quantity of powder from each tablet equivalent to100mg was taken and transferred into a 100mL volumetric flask. About 50mL of diluent was added and sonicated for a minimum 30 minute with intermittent shaking. Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45µm membrane filter. Further take 10mL of this stock solution in 50ml of volumetric flask and make up to mark with diluent. The concentration obtained was 240-720µg/mL of pantoprazole and 450-1350µg/mL of levosulpride.

Chromatographic Conditions

The mobile phase consisting of potassium dihydrogen orthophosphate (pH-3; 0.01M) buffer adjusted with Ortho phosphoric acid and methanol(HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of

600: 400v/v was pumped into the column at a flow rate of 1.0ml/min and the column temperature was 35°C. The detection was monitored at 230nm and the run time was 5min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

RESULT AND DISCUSSION

Development and Optimization Studies:

The analytical conditions for the proposed method were selected, basing on the chemical nature of pantoprazole and levosulpride. Initial spectroscopic analysis of compounds showed that pantoprazole and levosulpride showed a maximum UV absorbance (λmax) at 219nm, 242nm respectively. Therefore, the chromatographic detection was performed at 230nm using a photo diode array detector as both the compounds showed good response at this wavelength. The column selection has been done on the basis of back pressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between pantoprazole and levosulpride peak. After evaluating all these factors, Aligent (Zorbax; 250 mmx4.6 mm I.D; particle size 5µm)was found to be suitable as it gave satisfactory results. The selection of buffer based on chemical nature of both the drugs. Best results were obtained with potassium dihydrogen orthophosphate (pH-3; 0.01M) buffer adjusted with ortho phosphoric acid for pantoprazole and levosulpride. Preliminary trials using different composition of mobile phases consisting of buffer and methanol in the ratio of 500:500 v/v and 550:450v/v, did not give good peak shape for pantoprazole and levosulpride. Finally, the best separation and resolution of pantoprazole and levosulpride is achieved by fixing mobile phase composition consisting of a mixture of potassium dihydrogen orthophosphate (pH-3; 0.01M) buffer and methanol in the ratio of 600:400 v/v achieved. Under these conditions pantoprazole and levosulpride were eluted at 2.174 and 3.768, minutes respectively with a run time of 5 min. Optimized mobile phase proportion provided good resolution between pantoprazole and levosulpride. The chromatogram for simultaneous estimation of pantoprazole and levosulpride standard by using the aforementioned mobile phase from 10µL of the proposed method is represented in Figure: 3. The system suitability results of the method are presented in Table1.

Table 1:System Suitability Parameters of Pantoprazole and Levosulpride

Name of the compound	Retention time	Theoretical plates	Tailing factor	Usp resolution
Pantoprazole	2.174	4732	1.645	-
Levosulpride	3.768	8492	1.461	10.823

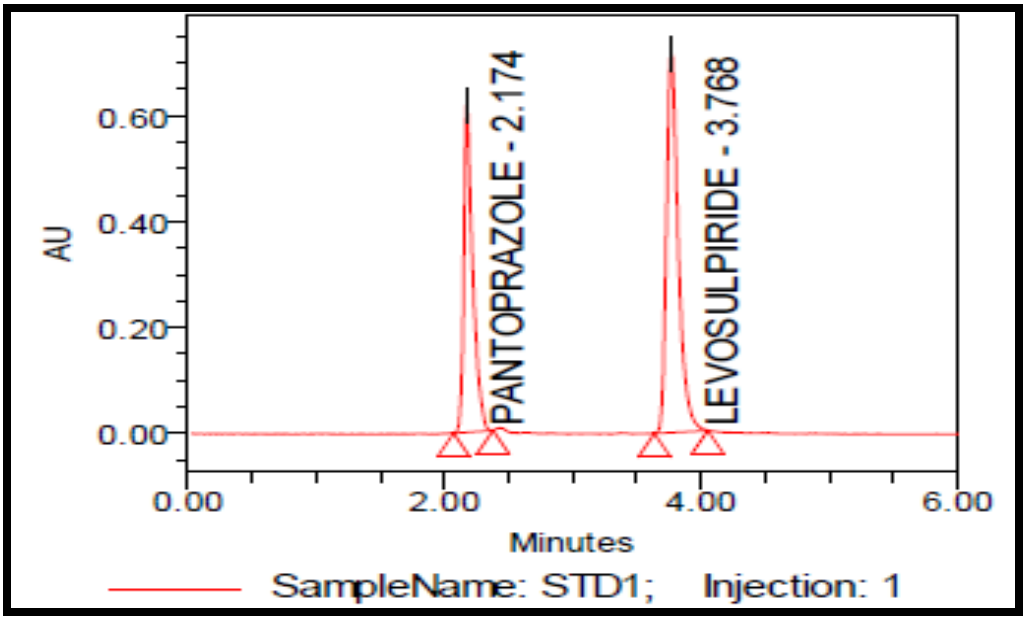

Figure: 3 Typical HPLC Chromatogram Showing the Peaks of Pantoprazole and Levosulpride

METHOD VALIDATION

System Suitability:

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were calculated for pantoprazole and levosulpride. It was observed that all the values are within the limits (Table 1).

Blank and Placebo interference:

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution showed no peaks at the retention time of pantoprazole and levosulpride peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of pantoprazole and levosulpride in its formulations. Similarly Chromatogram of Placebo solution showed no peaks at the retention time of pantoprazole and levosulpride peak revealing that the placebo used in sample preparation do not interfere in estimation of pantoprazole and levosulpride in formulations (tablets).

Linearity:

The linearity of the method was determined at five concentration levels ranging from 240 to 720µg/ml for pantoprazole and 450 to 1350µg/ml for levosulpride. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept

value for calibration curve was y = 6280.61.x - 8509 (R2 = 0.999) for pantoprazole and y = 5207.34.x - 3082 (R2= 0.999) for levosulpride. Then results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above (**Table2**).

Table 2: Linearity Studies of Pantoprazole by the Proposed Method

Linearity study for pantoprazole			**Linearity study for levosulpride**		
% Level (Approx.)	**Conc. µg/mL**	**Area**	**% Level (Approx.)**	**Conc. µg/mL**	**Area**
50	240	1500539	50	450	2340729
75	360.00	2253356	75	675	3510400
100	480.00	3004174	100	900	4684803
125	600	3754537	125	1125	5855001
150	720	4518315	150	1350.00	7026686
Slope		6280.61	Slope		5207.34
RSQ(r2)		0.9999	RSQ(r2)		0.9999
LOD (µg/mL)		0.00321	LOD (µg/mL)		0.000549

LOD and LOQ:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal-to-noise ratio of 3). The LOD for pantoprazole and levosulpride was found to be 0.00321µg/ml and 0.000549µg/ml respectively (Table 2).

Precision:

Intraday and interday precision studies for pantoprazole and levosulpride are given respective chromatograms and in Table 3 respectively. The RSD values for intraday precision and interday precision studies were < 2.0 % for pantoprazole and levosulpride confirming that the developed method was precise.

Table 3: Method Precision (Inter and Intraday) Studies for Pantoprazole and Levosulpride by the Proposed Method

Method precision by proposed method		
For pantoprazole		**For levosulpride**
Method precision (Inter & Intraday)		**Method precision (Inter & Intraday)**
Set-1	3001254	4680424
Set-2	3006331	4689046
Set-3	3001511	4687279
Set-4	3008369	4680589
Set-5	3001990	4685276
Set-6	3004348	4682396

Over All Avg.	3003967	4684168
Over All Std Dev.	2912.459	3596.321
Over All %RSD	0.0969	0.07677

Accuracy:

Recovery studies of pantoprazole and levosulpride were determined at three different concentration levels. The mean recovery for pantoprazole and levosulpride was 100 %respectively indicating that the proposed RP-HPLC method is accurate.

Robustness:

The robustness study of the developed assay method for pantoprazole and levosulpride were established in all variance conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed is robust (**Table 4**).

Table 4: Robustness Studies of the Proposed RP-HPLC Method

Robust conditions		**Pantoprazole**		**Levosulpride**	
		Rt	**Peak area**	**Rt**	**Peak area**
Flow rate	0.8 ml/min	2.187	1500539	3.856	2354729
	1.2 ml/min	2.157	1519345	3.675	2445729
Temp	30^{o}C	2.159	1545780	3.746	2348729
	40^{o}C	2.240	1698039	3.879	2441729

Assay in Marketed Formulations:

The proposed method was applied to the determination of pantoprazole and levosulpride in their combined capsule dosage forms. The results of the assays (n = 6) undertaken yielded 99.87% and 99.98% of label claim for pantoprazole and levosulpride, respectively. The results of this assay (**Table 5**) indicated that the developed method is selective for the analysis of both pantoprazole and levosulpride without interference from the excipients.

Table 5:Analysis of Marketed Tablets by the Proposed Method

Drug	**Label claim**	**Quantity found***	**%Assay**
Pantoprazole	40	39.95	99.87
Levosulpride	**75**	74.99	99.98

*Average of six determinations

CONCLUSIONS

A novel simple, precise, accurate, and sensitive isocratic RP- HPLC method have been developed by the author for the simultaneous estimation of pantoprazole and levosulpride in pure and marketed formulations. The method gave good resolution for both the drugs with a short analysis time below 5 minutes. The good % recovery in tablet forms suggested that the excipients present in the dosage forms have no interference in the determination. The percentage

assay for Pantoprazole and Levosulpiride was found to be 99.87% and 99.98% respectively. The present method was validated as per the ICH guideline so it can be adopted for its routine analysis of pantoprazole and levosulpridein combined dosage forms.

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