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AN ISOCRATIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LEVOSULPIRIDE AND PANTOPRAZOLE IN BULK SAMPLES AS WELL AS IN TABLET DOSAGE FORMS BY USING RP-HPLC

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ABSTRACT
A simple, precise, accurate, rapid and economical reverse phase high-pressure liquid chromatographic method has been developed as per ICH norms for the simultaneous estimation of levosulpiride and pantoprazole sodium from pharmaceutical formulation. The method was carried out on a Phenomenex column C_{18} (250 mm X 4.6 mm; 5 μ) with a mobile phase consists of buffer (adjusted to pH 2.8 with 1 % OPA): methanol (50:50 v/v) and filtered through a 0.45 μ cellulose nitrate filters. The flow rate was maintained at 1.0 mL/min. The detection was carried out at 232 nm. The run time was 7 min. The retention time of levosulpiride and pantoprazole was 1.533 and 5.098 min respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Keywords: RP-HPLC, Levosulpiride, Pantoprazole, Validation, ICH Guidelines

INTRODUCTION
Pantoprazole sodium (The Merck Index, 2006; Drugs.com) (PNT) is a (RS) 5-(difluoromethoxy)-2-[[(3, 4-dimethoxy]-2-pyridinyl) methyl] sulfinyl]-1Hbenzimidazole. It is a proton pump inhibitor drug that inhibits gastric acid secretion used for short-term treatment of erosion and ulceration of the esophagus caused by gastro esophageal reflux disease. Levosulpiride (The Merck Index, 2006) is an N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxy-5-sulf amoyl benzamide. It is a new orally effective antipsychotic and a prokinetic agent reported to be a selective antagonist of dopamine D2 receptor activity on both central and peripheral levels. Pantoprazole (Protonix) is in a group of drugs called proton pump inhibitors. It decreases the amount of acid produced in the stomach. Pantoprazole is used to treat erosive esophagitis (damage to the esophagus from stomach acid), and other conditions involving excess stomach acid such as Zollinger-Ellison syndrome. Pantoprazole is not for immediate relief of heartburn symptoms [4]. The chemical structure of pantoprazole is presented in figure 1.
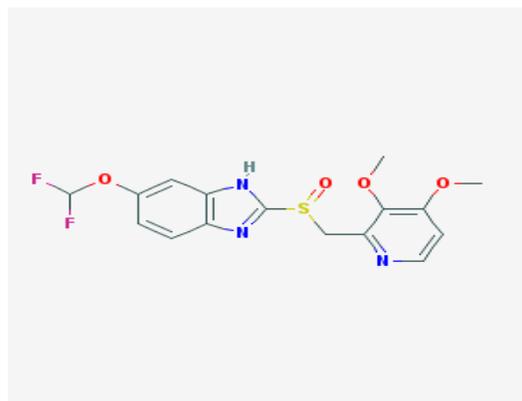
Figure 1: The chemical structure of Pantoprazole

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Levosulpiride (The Merck Index, 2006; 4; Jain *et al.,* 2012) is also claimed to have mood elevating property and used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome, and premature ejaculation. Levosulpiride and pantoprazole sodium of combined tablet dosage form in the ratio of 75 mg and 40 mg. Literature survey reveals that Levosulpiride can be estimated by spectrophotometric methods (Jain *et al.,* 2012; Shobha *et al.,* 2011; Dhwani *et al.,* 2012; Yogesh *et al.,* 2012), HPLC (Sravan *et al.,* 2013; Rakesh *et al.,* 2013; Atulita *et al.,* 2013) and by HPTLC (Shreenidhi *et al.,* 2013) individually or with other drugs in bulk. In pantoprazole sodium can estimate by RP- HPLC (Sivakumar *et al.,* 2007; Prasanna and Kiran, 2009; Tated *et al.,* 2011; Prasanna *et al.,* 2010; Kampati *et al.,* 2013; Mohideen *et al.,* 2011; Suryadevara *et al.,* 2013; Saurabh *et al.,* 2013). But combination of pantoprazole sodium and levosulpiride not estimated by HPLC method .So we planned to develop a RP- HPLC method and to validate according to ICH norms (ICH, Q2B (R1), 1996; ICH, Q2 (R1), 2005). The chemical structure of levosulpiride is presented in figure 2.
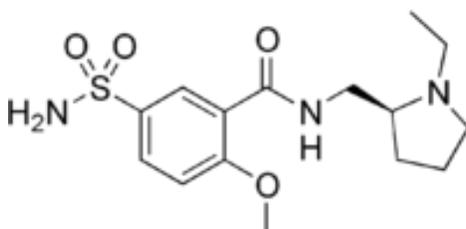

Figure 2: Chemical structure of Levosulpiride

MATERIALS AND METHODS
***Chemicals and Reagents Used*:** The following chemicals were used for the process: water [HPLC Grade], pantoprazole sodium and levosulpiride [working standards], methanol [HPLC Grade], and orthophosphoric acid. All the chemicals were procured from Standard Solutions, Hyderabad, Andhra Pradesh, India.

***Apparatus and Chromatographic Conditions*:** The equipment used was High Performance Liquid Chromatography Equipped with Auto Sampler and DAD or UV Detector. The Phenomenex C_{18} column (250 mm X 4.6 mm; 5 μ) was selected. The flow rate was monitored at 1.0 mL/min. The detection was carried out at 232 nm. The injection volume selected 20 μL, the temperature of the column oven was maintained at 25 °C, the detector used was Photo diode array and the run time was 7.0 min.

***Preparation of buffer solution* [23]:** The buffer solution was prepared by 1 X 10^{-5} M of ortho phosphoric acid in a 1000 mL beaker with water [HPLC grade]. Then the pH was adjusted to 2.5 with 1.0 % ortho phosphoric acid.

***Preparation of mobile phase*:** The mobile phase was prepared by mixing a mixture of above buffer solution 500 mL (50 %) and 500 mL of methanol HPLC (55 %) and degas in ultrasonic water bath for 5 minutes. Then, the resultant solution was filtered through a 0.45 μ filter under vacuum.

***Preparation of standard solution of Levosulpiride and Pantoprazole*:** About 100 mg levosulpiride was weighed accurately and transferred into a 100 mL clean and dry volumetric flask. Initially, the drug was mixed with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up to the mark with the same solvent. Similarly, about 100 mg pantoprazole was weighed accurately and transferred into a 100 mL clean and dry volumetric flask. Initially, the drug was mixed with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up to the mark with the same solvent to get a concentration of 1000 μg/mL.

From the above prepared stock solutions 25 mL of levosulpiride and 10 mL of pantoprazole were pipetted out into a 50 mL clean and dry volumetric flask and it was diluted up to the mark with diluent. This mixed stock solution contains 500.0 μg/mL of levosulpiride and 200.0 μg/mL of pantoprazole.

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***Preparation of sample solution of Levosulpiride and Pantoprazole*:** Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg of levosulpiride and 20 mg of pantoprazole were weighed and dissolved in the 70 mL mobile phase with the aid of ultra sonication for 20 min. The content was diluted with 100 mL mobile phase to furnish the preparation of stock solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 25.0 mL and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to get the desired concentration of 500.0 µg/mL of levosulpiride and 200.0 µg/mL of pantoprazole.

System Suitability: The tailing factor for the peaks due to levosulpiride and pantoprazole in standard solution should not be more than 2.0. The Theoretical plates for the levosulpiride and pantoprazole peaks in standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the levosulpiride and pantoprazole. The parameters of system suitability were checked.

Validation Development (ICH, Q2B (R1), 1996; ICH, Q2 (R1), 2005)

1. System Suitability: A Standard solution was prepared by using levosulpiride and pantoprazole working standards as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections levosulpiride and pantoprazole, retention times and peak areas. The data are represented in table 1. The chromatogram is presented in figure 3.

Acceptance Criteria: The % RSD for the retention times of principal peak from 5 replicate injections of each standard solution should be not more than 2.0 %. The number of theoretical plates (N) for the levosulpiride and pantoprazole peaks is NLT 2000. The Tailing factor (T) for the levosulpiride and pantoprazole peaks is NMT 2.0.

Table 1: The system suitability parameters for Levosulpiride and Pantoprazole

System suitability parameters	Levosulpiride	Pantoprazole
% RSD for six replicate injections of standard	1.56	1.69
Tailing factor	1.38	1.14
Theoretical plates	2627	6531
Resolution	-	14.7
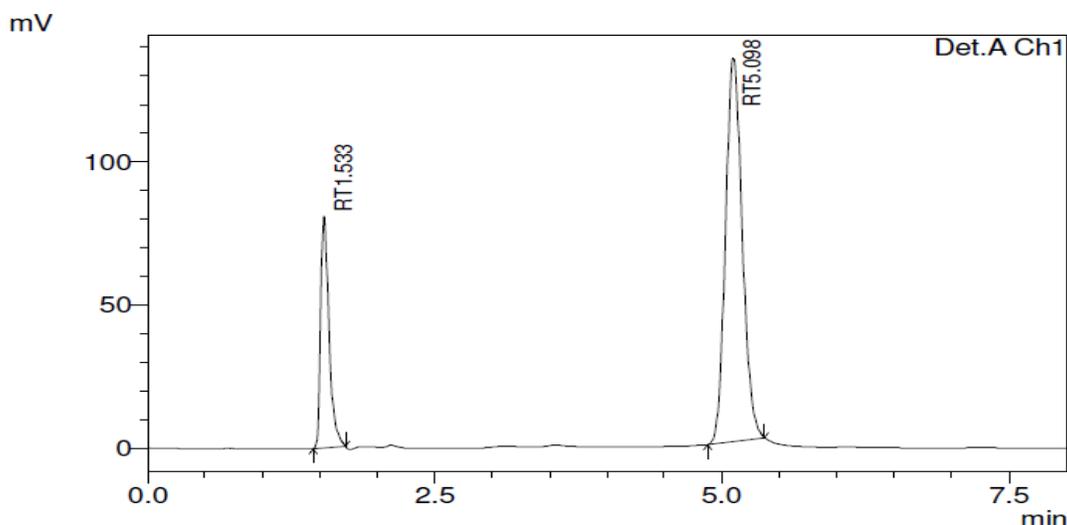
Figure 3: A typical chromatogram for Levosulpiride and Pantoprazole

2. Specificity: Solutions of standard and sample were prepared as per the test method are injected into chromatographic system. The chromatograms of standard and sample should be identical with near retention time. The specificity is represented in figure 4.

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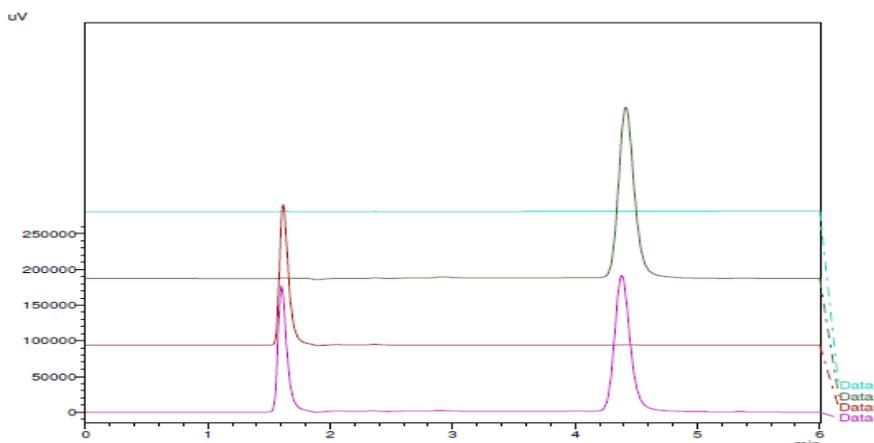
Figure 4: An overlay chromatogram for specificity for Levosulpiride and Pantoprazole

3. Accuracy and Precision
*a. Preparation of LLOQ sample***:** From the stock solutions 0.2 mL of pantoprazole and 0.5 mL of levosulpiride were taken and diluted up to 10 mL with diluent to get a concentrations of 10 μg/mL pantoprazole and levosulpiride.
*b. Preparation of LQC sample***:** From stock solutions 0.5 mL of pantoprazole and 1.25 mL of levosulpiride were taken into a 10 mL volumetric flask and made up the volume with mobile phase to get a concentrations of 25 μg/mL pantoprazole and levosulpiride.
*c. Preparation of MQC sample***:** From stock solutions 1.0 mL of pantoprazole and 2.5 mL of levosulpiride were taken into a 10 mL volumetric flask and made up the volume with mobile phase to get a concentrations of 50 μg/mL pantoprazole and levosulpiride.
*d. Preparation of HQC sample***:** From stock solutions 1.5 mL of pantoprazole and 3.75 mL of levosulpiride were taken into a 10 mL volumetric flask and made up the volume with mobile phase to get a concentrations of 75 μg/mL pantoprazole and levosulpiride.
e. Accuracy of the assay method was determined for both intra-day and inter-day variations using the triplicate analysis of 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 a laboratory over a short period of time that was evaluated by assaying the QC samples during same day. Intermediate precision was assessed by comparing assays on different days (3days). The data are represented in table 2 and 3.

Table 2: Accuracy and precision results for levosulpiride

DAY 1	LLOQ	LQC	MQC	HQC
Nominal Conc.'s (μg/ml)	10.00	25.00	50.00	75.00
Mean Conc.'s (μg/ml)	10	24.73	51.98	75.24
SD	0.15	0.19	0.15	0.75
% RSD	0.15	0.19	0.14	0.75
Recovery (%)	100.43	98.92	103.97	100.32
DAY 2	LLOQ	LQC	MQC	HQC
Mean Conc.'s (μg/ml)	10.01	24.36	51.99	75.24
SD	0.16	0.47	0.15	0.76
% RSD	0.16	0.48	0.14	0.76
Recovery (%)	100.1	97.43	103.97	100.32
DAY 3	LLOQ	LQC	MQC	HQC
Mean Conc.'s (μg/ml)	10.32	24.73	51.98	75.21
SD	0.18	0.19	0.15	0.79
% RSD	0.17	0.19	0.14	0.79
Recovery (%)	103.2	98.92	103.97	100.28

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Table 3: It shows accuracy and precision results for pantoprazole

DAY 1		LLOQ	LQC	MQC	HQC
Nominal Conc.'s (µg/ml)		10.00	25.16	50.31	**75.47**
Mean Conc.'s (µg/ml)		10.06	25.50	46.26	82.58
		0.16	0.32	1.0	1.18
SD		0.16	0.32	1.09	1.08
% RSD		100.50	101.33	91.96	109.42
Recovery (%)					
DAY 2		LLOQ	LQC	MQC	**HQC**
Mean Conc.'s (µg/ml)		10.25	25.68	46.26	82.58
		0.13	0.09	1.01	1.19
SD		0.13	0.09	1.10	1.09
% RSD		102.5	102.06	91.96	109.41
Recovery (%)					
DAY 3		LLOQ	LQC	MQC	**HQC**
Mean Conc.'s (µg/ml)		10.14	25.52	45.48	81.79
		0.11	0.33	0.73	1.39
SD		0.11	0.3	0.81	1.28
% RSD		101.4	101.43	90.39	108.37
Recovery (%)					

4. Ruggedness: The ruggedness was determined by using the data obtained by the analysis performed by two different analysts. Each analyst prepared 5 MQC samples of the same batch and the results obtained were compared. The data are represented in table no.4.

Table 4: Ruggedness results for levosulpiride and pantoprazole

	Drug name	Rt	Tailing factor	Theoretical plates	USP Resolution
Analyst 1	Levosulpiride	1.57	1.39	2519	--------
Analyst 2	Pantoprazole	4.36	1.14	6260	14.42
	Levosulpiride	1.57	1.39	2530	--------
	Pantoprazole	4.37	1.14	6224	14.43

5. Robustness: The MQC sample solution prepared was analysed under different chromatographic conditions stated below. The data are represented in table 5.

Table 5 (b): Robustness results for levosulpiride and pantoprazole

|	Parameters	Variation	Rt	Tailing factor	Plate count
		1.1 mL/min	1.39	1.62	1745
	Flow rate	0.9 mL/min	1.80	1.55	2182
Levosulpiride					
		45 % organic phase	1.63	1.60	2140
	Mobile phase	55 % organic phase	1.33	1.25	1991
Pantoprazole		1.1 mL/min	3.85	1.22	4654
	Flow rate	0.9 mL/min	5.21	1.22	5863
		45 % organic phase	6.21	1.27	6016
	Mobile phase	55 % organic phase	2.34	1.21	5498

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6. Limit of detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
1. Based on Signal-to-Noise
2. Based on the Standard Deviation of the Response and the Slope.
7. Limit of Quantification: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The data for LOD and LOQ are represented in table 6. The chromatograms are represented in figure 5 and 6.

Table 6: It shows LOD and LOQ results for levosulpiride and pantoprazole

Drug name	Parameter	Peak area	Tailing factor	Theoretical plates
Levosulpiride	LOD	12883	1.31	2745
	LOQ	23667	1.38	2200
Pantoprazole	LOD	25512	1.14	6159
	LOQ	44066	1.16	5865
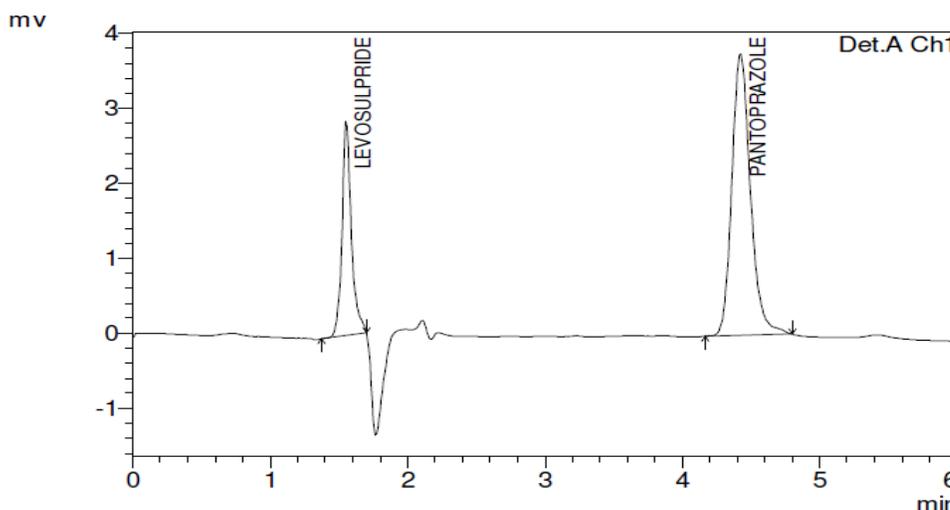
**Figure 5: An overlay chromatogram for LOD for Levosulpiride and Pantoprazole**
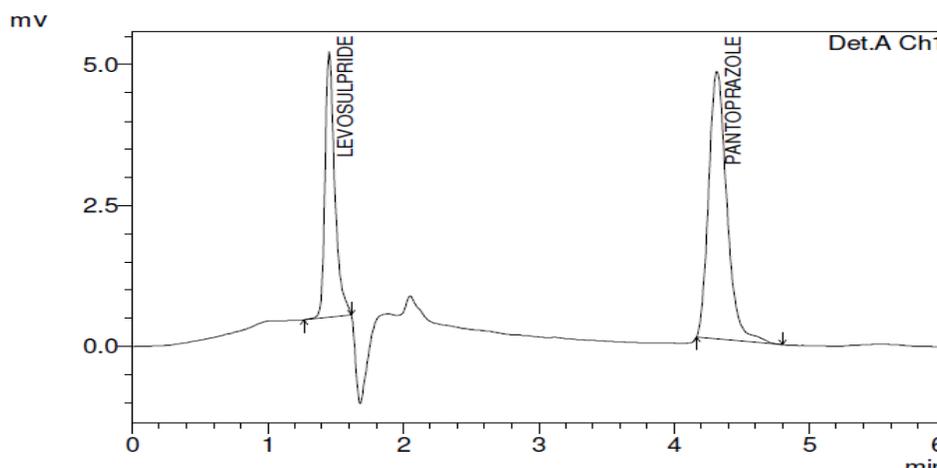
Figure 6: An overlay chromatogram for LOQ for Levosulpiride and Pantoprazole

8. Linearity: It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line.

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Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in figure 7 and 8.
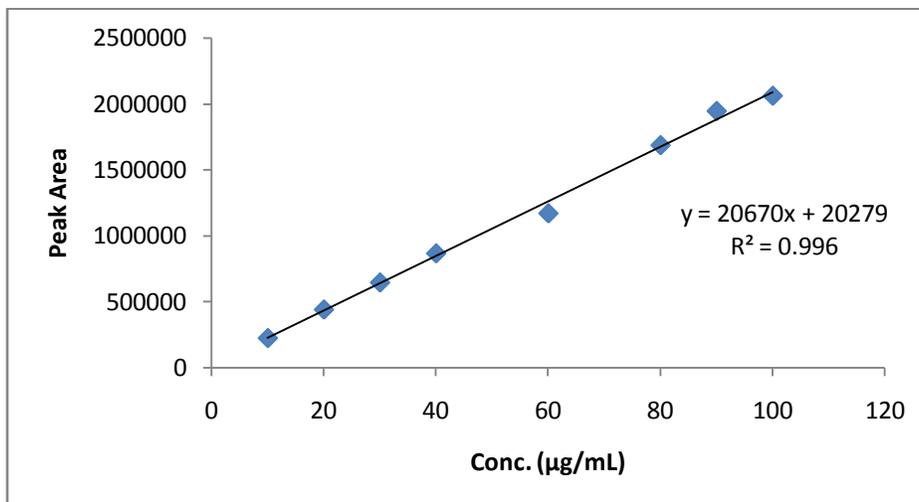
Figure 7: Linearity graph of levosulpiride
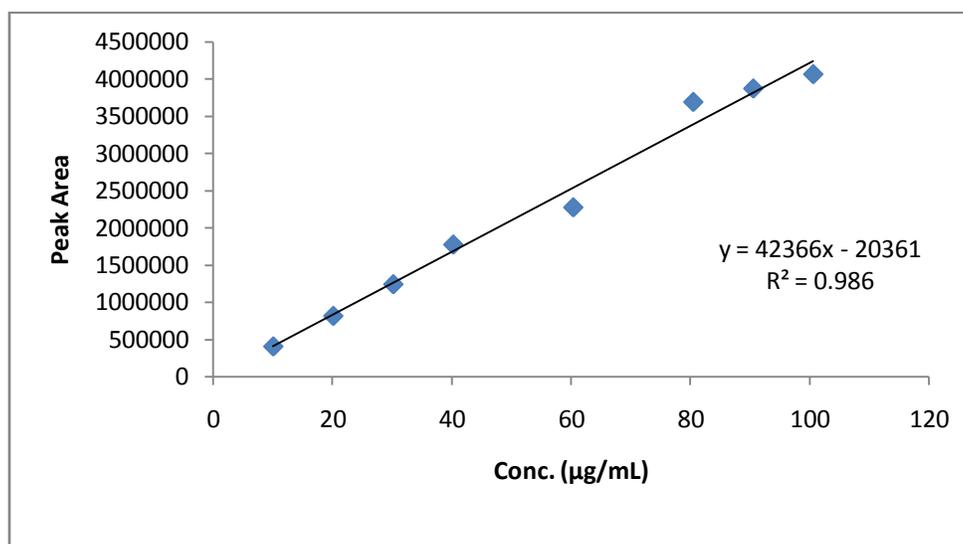
Figure 8: Linearity graph of pantoprazole

Table 7: Linearity data for Levosulpiride and Pantoprazole

Parameters	Levosulpiride	Pantoprazole
Slope	20670	42366
Intercept	20279	20361
Correlation coefficient	0.9960	0.9862

***9. Assay of the marketed product*:** Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg of levosulpiride and 20 mg of pantoprazole were weighed and dissolved in the 70 mL mobile phase with the aid of ultra sonication for 20 min. The content was diluted with 100 mL mobile phase to furnish the preparation of stock solution. The stock solution was filtered through a 0.45 μm Nylon syringe filter and 25.0 mL and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric

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flask to get the desired concentration of 500.0 μg/mL of levosulpiride and 200.0 μg/mL of pantoprazole. The results are presented in table no. 8.

Table 8: Results of levosulpiride and pantoprazole for marketed product

Marketed formulation	Drug	Mean	SD	% RSD
BEULAH	Levosulpiride 70 mg	91.34	1.04	1.14
	Pantoprazole 40 mg	90.61	0.62	0.68

RESULTS AND DISCUSSION
To optimize the mobile phase, various proportions of buffer (adjusted to pH 2.8 with 1 % OPA): methanol were tested. The use of buffer (adjusted to pH 2.8 with 1 % OPA) and methanol [HPLC Grade] in the ratio of 50:50 (v/v) resulted in peak with good shapes and resolution. A flow rate of 1.0 mL /min was found to be optimum in the 0.4-1.5 mL/min range resulting in short retention time, baseline stability and minimum noise. By applying the proposed method, the retention times of levosulpiride and pantoprazole were observed at 1.533 and 5.098 min at 232 nm respectively.

Quantitative linearity was obeyed in the concentration ranges of 10-100 μg/mL for both levosulpiride and pantoprazole. The relevant regression equations were $y = 20670x + 20279$ for levosulpiride ($R^2 = 0.996$) and $y = 42366x - 20361$ for pantoprazole ($R^2 = 0.986$) (where y is the peak area ratio and x is the concentration of levosulpiride and pantoprazole (μg/mL)). The intra-day and inter-day drugs variations by the proposed method showed an RSD less than 2 %, indicating that the method is precise. The corresponding mean recoveries of the drugs were within the limits. This reveals that the method is quite accurate. The tailing factor (1.38 and 1.14 for levosulpiride and pantoprazole), USP plate count (2627 and 6531 for levosulpiride and pantoprazole); obtained were within the acceptance limits, which indicate the sensitivity of the method. The method tolerated minor variations in optimized chromatographic conditions indicating good robustness, which indicate the efficient performance of the column.

No interfering peaks were found in the chromatograms indicating that the excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

Conclusion
The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous determination of levosulpiride and pantoprazole. The method was validated as per ICH guidelines and all the parameters met within the acceptance criteria. Applicability of this method for simultaneous estimation of levosulpiride and pantoprazole from tablet dosage forms was confirmed. Hence, this method is specific and can be successfully used for the simultaneous estimation of levosulpiride and pantoprazole in bulk drug samples, pharmaceutical dosage forms. Hence, this method can be easily and conveniently adopted for routine quality control analysis of the above drugs.

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