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## Development and Validation of RP-HPLC Method for Estimation of Chiral Purity of S (-) Pantoprazole Sodium in S (-) Pantoprazole and Mosapride Capsule Dosage Form



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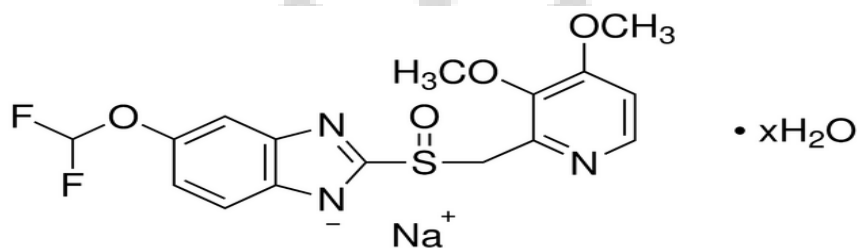
**Keywords:** S (-) Pantoprazole, Capsules, Chiral Purity, Development, Validation

### ABSTRACT

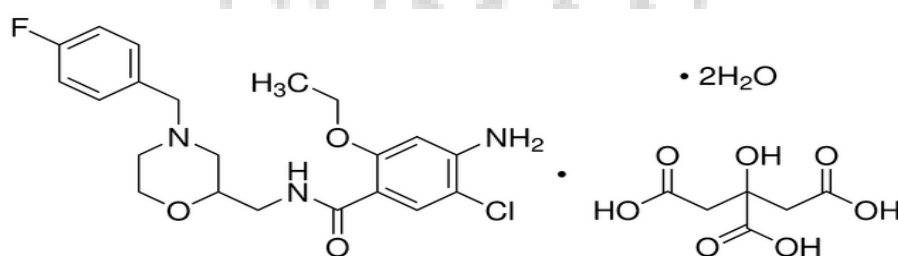
S (-) Pantoprazole and Mosapride Citrate are used in combined dosage form for treating various gastrointestinal disorders, particularly for hyperacidity which is frequently associated with gastrointestinal dysmotility. Present research work was undertaken to develop and validate a novel, rapid, accurate, sensitive, precise High-performance liquid chromatographic method (RP-HPLC) for determination of chiral purity of S(-)Pantoprazole in S(-)Pantoprazole and Mosapride Citrate capsule dosage form. The chromatographic separation was achieved with the use of Chiralcel OJ-RH (4.6 mm X 150 mm, 5 $\mu$ ) analytical column at 25° C column temperature. The mobile phase consisting of 10mM Sodium perchlorate buffer and acetonitrile in the ratio of 75:25 v/v, used at a flow rate of 0.5mL/min with injection volume of 10 $\mu$ L and the detection was done at 290 nm using UV detector. The retention times of S (-) Pantoprazole and R (+) Pantoprazole were found to be 14.7 min and 17.4 min respectively. The method was validated for System suitability criteria, specificity, accuracy, precision, linearity, filter validation and solution stability. The results obtained are well within limit as per ICH guideline. In addition, the proposed method was effectively applied for the routine analysis for determination of chiral purity of S (-) Pantoprazole in S (-) Pantoprazole and Mosapride Citrate capsule dosage form.

## INTRODUCTION

Chemically, S (-) Pantoprazole sodium is Sodium 5-(difluoromethoxy)-2-[[[3,4-dimethoxy-2-pyridinyl) methyl]sulfinyl]-1H benzimidazole [1] [Fig-1]; it is a proton pump inhibitor drug used for short term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease [2]. Mosapride is chemically 4-Amino-5-chloro-2-ethoxy-N-[[4-[(4-fluorophenyl) methyl]-2-morpholinyl] methyl] benzamide citrate dihydrate [Fig-2]; it is listed in Martindale – the complete drug reference [3]. It is used in reflux esophagitis and to enhance gastric motility [4]. This drug is selective 5HT4 agonist [5]. The complementary pharmacological actions of Mosapride and S (-) Pantoprazole make their use in combined dosage form which is useful for treating various gastrointestinal disorders, like hyperacidity which is frequently associated with gastrointestinal dysmotility. The combination drug products are widely used in the treatment of gastro esophageal reflux disease and non-ulcer dyspepsia.



**Fig. 1. Chemical structure of S (-) Pantoprazole Sodium**



**Fig. 2. Chemical structure of Mosapride citrate dihydrate**

It is estimated that sales of unichiral drugs could reach \$200 billion in 2008 [6]. A number of factors have contributed to the introduction and popularity of unichiral products since 1980 and more so 1992 onwards. These are: introduction of enantioselective analytical methods, new synthetic methods for unichiral molecules, chromatographic methods for separation and more

importantly US FDA statement in 1992 stating that development of racemates would require justification for inclusion of both the isomers [7-8]. From the literature review, revealed that HPLC methods have been reported for determination and separation of the enantiomers and impurities in S (-) Pantoprazole drug substance [9-12]. Several capillary electrophoresis methods have been reported for the enantioseparation of Pantoprazole and related benzimidazole [13-15].

A comprehensive literature research revealed that no RP-HPLC method has been reported for estimation of Chiral purity of S (-) Pantoprazole in S (-) Pantoprazole and Mosapride in fixed dose pharmaceutical dosage form. Therefore, the present study aimed to develop and subsequently validate a high-performance liquid chromatography (HPLC) method for the determination of chiral purity of S (-) Pantoprazole in S (-) Pantoprazole and Mosapride in fixed dose combination dosage form. So, the purpose of present study was to develop and validate RP-HPLC analytical method for the estimation of Chiral purity of S (-) Pantoprazole in S (-) Pantoprazole and Mosapride citrate in capsule dosage form. Moreover, the current method has been validated for accuracy, precision, specificity, linearity, ruggedness, robustness and solution stability as per the recommendations of ICH guidelines [16-18].

## **MATERIALS AND METHODS**

### **Chemicals and Reagents:**

S (-) Pantoprazole Sodium, S (+) Pantoprazole Sodium, Mosapride Citrate and drug product (Each capsule contains 10 mg of S (-) Pantoprazole and 5 mg of Mosapride Citrate) were provided by Emcure Pharmaceuticals Ltd. Pune, India. HPLC Water was generated in-house by using Merck Millipore, Milli-Q water purification system. Sodium perchlorate monohydrate and Acetonitrile were procured from Merck, India Ltd.

### **Instrumentation:**

Waters HPLC system with Empower-2 software (Waters Corporation) was. The analytical balance and pH meter used were manufactured by Mettler Toledo.

### **Chromatographic Conditions:**

The separation was achieved using Chiralcel OJ-RH (4.6 mm X 150 mm, 5 $\mu$ ) analytical column. The mobile phase consists of 10mM Sodium perchlorate buffer was prepared by dissolving 7.02 gm of Sodium perchlorate monohydrate in 1000 mL of water, mixed well and filter through 0.45  $\mu$ m nylon membrane filter. Then prepare a homogeneous mixture of buffer and acetonitrile in the ratio of 75:25 v/v and degas. The flow rate and column temperature was maintained at 0.5 mL/min and 25 °C respectively throughout the analysis. The sample cooler temperature was maintained at 6 °C. The injection volume was kept at 10  $\mu$ L and wavelength was optimized at 290 nm which was found suitable for detection and quantification of both Pantoprazole isomers. The Selectivity study was performed using Photo Diode Array detector in the wavelength range of 200-400 nm.

### **Preparation of diluent:**

Mobile phase used as a diluent.

### **Preparation of System suitability solution:**

Weighed and transferred about 5 mg each of S (-) Pantoprazole sodium working standard and R (+) Pantoprazole sodium working standard into a 100 mL volumetric flask, added about 50 mL diluent and sonicate to dissolve and diluted to volume with diluent.

### **Standard preparation:**

Weighed and transferred about 5.3 mg of R (+) Pantoprazole sodium reference standard / working standard into a 50 mL volumetric flask, added about 30 mL of diluent, sonicate to dissolve and diluted to volume with diluent and mixed. Pipetted 10 mL of this solution in to 250 mL volumetric flask and make up to the volume with diluent and mixed.

### **Preparation of Sample Solution:**

Empty the content of 10 capsules and carefully select yellowish orange colored coated tablets of S (-) Pantoprazole. Calculate the average of 10 tablets. Accurately weighed and transferred the intact tablets (5 Tablets) equivalent to about 50 mg of S (-) Pantoprazole into a 250 mL volumetric flask. Added about 150 mL of diluent and sonicate for 30 minutes with intermittent

shaking (1 to 2 minutes after every 5 minutes). Ensured the complete dispersion of tablets and diluted up to the mark with diluent. Filtered the solution through 0.45  $\mu$  nylon membrane syringe filter by discarding initial 2 mL of filtrate.

## RESULTS AND DISCUSSION

### Optimization of the chromatographic conditions:

The main objective of this chromatographic method was to separate the peaks due to Pantoprazole enantiomers and its impurities within a short run time. For optimizing the chromatographic conditions, analytical column, mobile phase compositions and flow rate were finalized. Based on the pKa values and pH dependant selectivity study of S (-) Pantoprazole over the range of pH 3.0 to 7.5, it was observed that pH 5.0 to 7.5 is the most suitable range for the chromatographic separation. Various trials were taken by using different columns and organic modifiers composition (Acetonitrile and methanol). Effect of column temperature on chromatographic separation was studied over the range of 25 °C to 40°C. It was observed that at 25°C the chromatographic separation was optimum. Thus it was kept as 25°C. S (-) Pantoprazole is highly susceptible to acid hydrolysis thus to increase the solution stability, mobile phase used as diluent.

### Method Validation:

The analytical method validation was carried out as per ICH guidelines. The parameters include System suitability criteria, specificity, accuracy, precision, linearity, filter validation, robustness and solution stability.

### System Suitability Criteria:

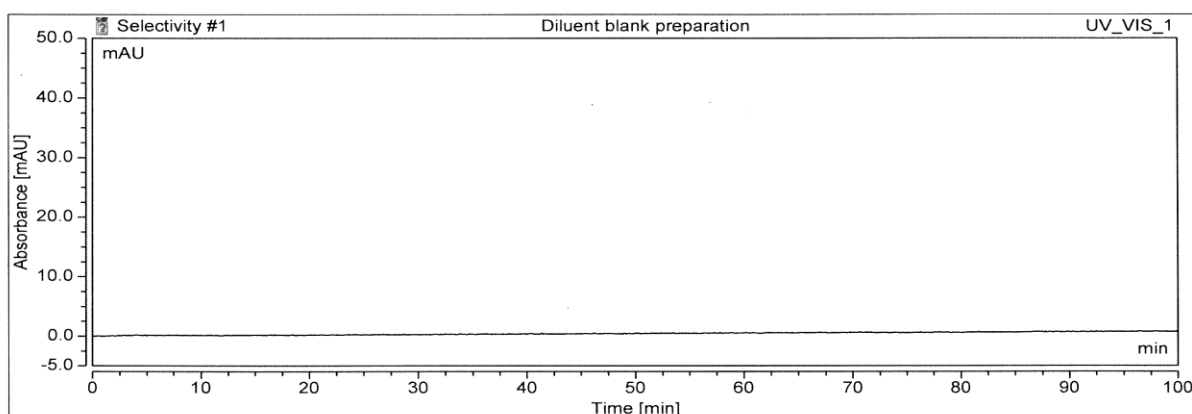
The system suitability test was performed by injecting 6 replicates of standard solution into the chromatography and the chromatograms were recorded, the relative standard deviation of R (+) Pantoprazole from six replicate injections should be less than 5.0%. Resolution between peaks of S-isomer and R-isomer from system suitability solution should be not less than 2.0. Results obtained are summarized in Table No. 1.

**Table 1: System Suitability Parameters**

Peak name	% RSD of 6 injections		Resolution between S & R Isomers	
	Result	Limit	Result	Limits
<b>R (+) Pantoprazole</b>	0.33	< 5.0%	3.39	< 2.0

**Specificity:**

A photodiode array detector was used for analysis of specificity of the method and to evaluate the homogeneity of the analyte peak. The peak purity obtained was found to be acceptable (purity angle < purity threshold) which confirmed that the peaks of the analytes are homogeneous and no additional peaks are co-eluting with the analytes. The results obtained from specificity test proved the ability of method to access unequivocally the analyte of interest in presence potential interference and its degradation products, indicating a high degree of specificity of the proposed method. The diluent blank, excipient blend preparations, Sulphone and Sulphide impurities didn't show any peak at the retention time of R (+) Pantoprazole. Typical chromatograms of blank solution, system suitability solution, standard solution and sample solution were presented in Fig. 3 and 6.



**Fig. 3: Typical chromatogram of blank solution**

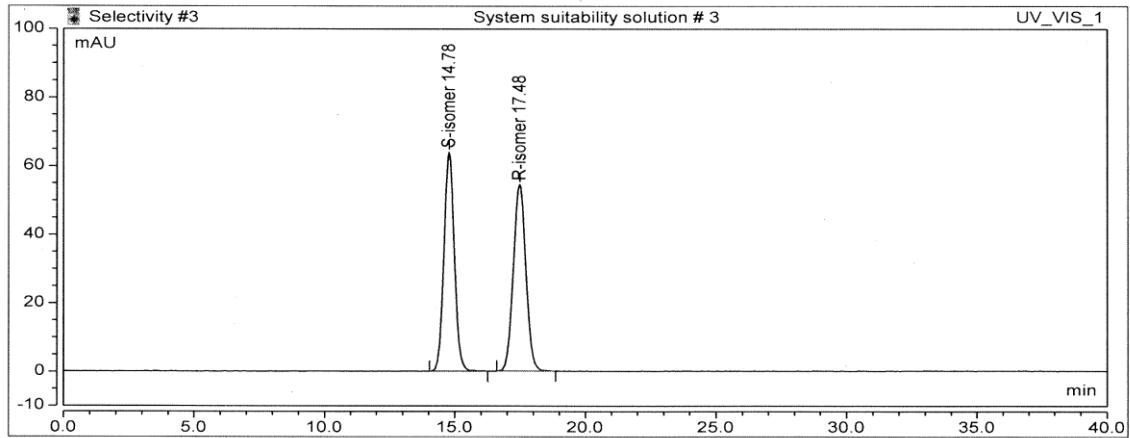


Fig. 4: Typical chromatogram of System suitability solution

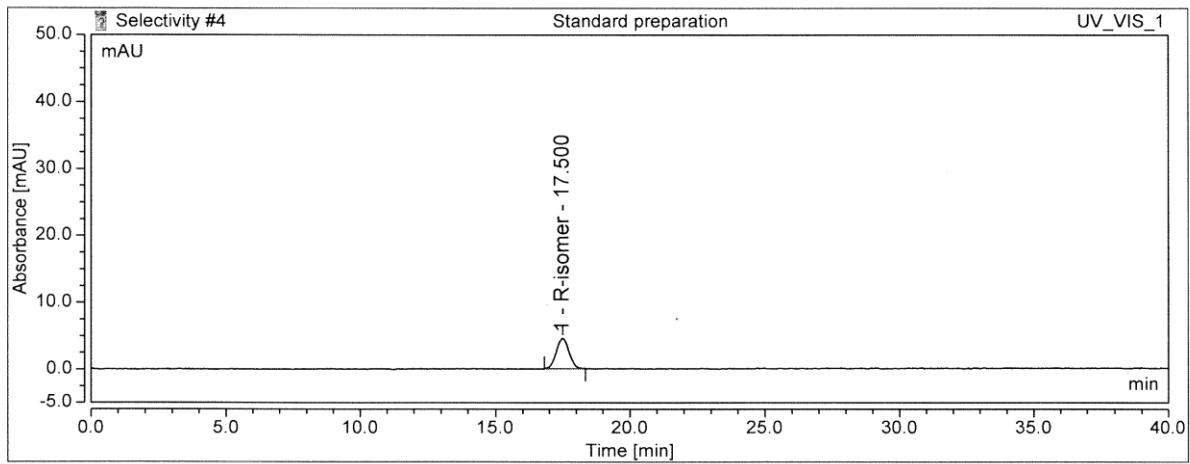


Fig. 5: Typical chromatogram of Standard solution

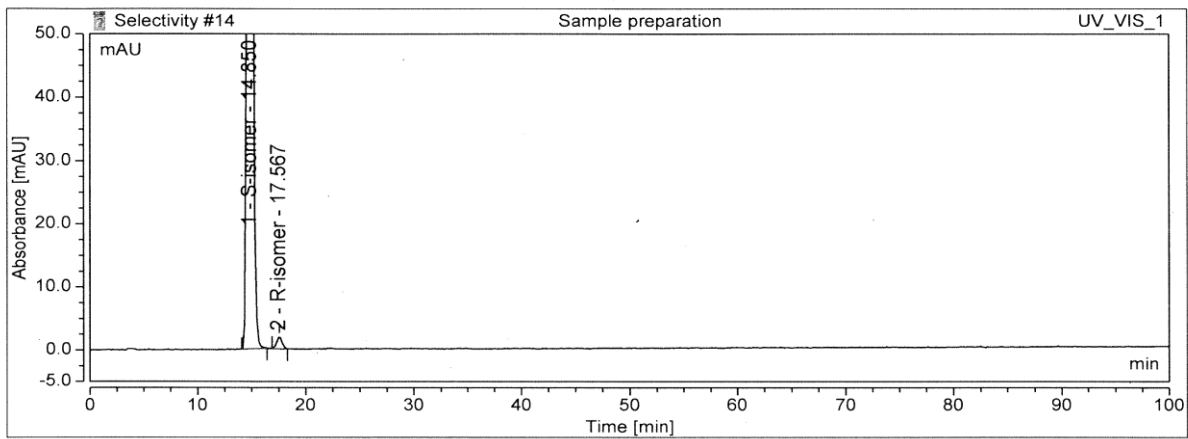


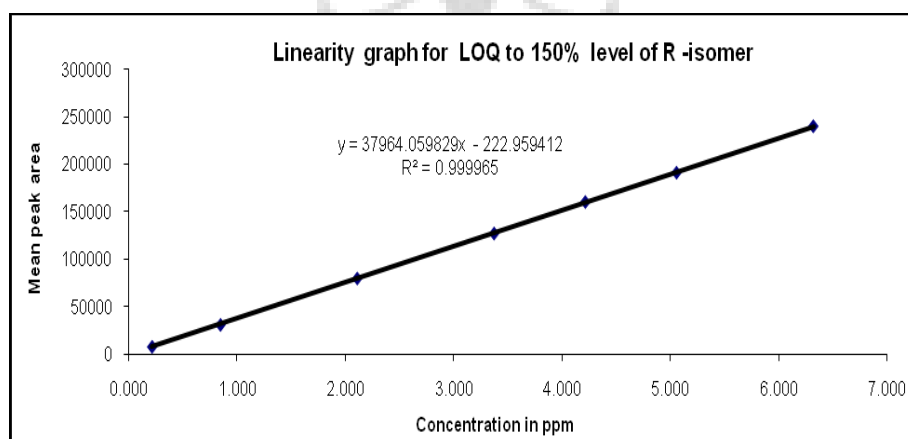
Fig. 6: Typical chromatogram of Sample solution

**Linearity and range:**

Linearity of the method was tested by preparing and injecting in triplicate a series of Standard preparations over a range of 0.4µg/mL to 4.8µg/mL for S (-) Pantoprazole. Average peak areas of each level were plotted against analyte concentration in µg/mL (ppm) and linear regression analysis was performed on the resulting plot. Results obtained are summarized in Table No. 2 and Fig. 7.

**Table 2: Results of Linearity (Correlation Coefficient)**

Analyte	Correlation Coefficient
R (+) Pantoprazole	0.999965



**Fig.7: Linearity of R (+) Pantoprazole**

**Accuracy:**

Accuracy of the method was determined by standard addition method by spiking known concentration of R (+) Pantoprazole in sample solution. The % recovery of R (+) Pantoprazole at different levels was evaluated. The samples for accuracy were prepared at 50%, 100%, and 150% level of sample concentration spiking of API in triplicate. Results obtained for each level of recovery are summarized in Table No. 3.



**Table 3: Recovery of R (+) Pantoprazole**

Level	R (+) Pantoprazole	
	% Mean Recovery	%RSD
LOQ Level	92.33	1.25
Level-1(50%)	97.66	0.84
Level-2(100%)	98.28	0.38
Level-3(150%)	98.21	0.22

**Method Precision:**

In method precision six samples were prepared as per analytical method. The chiral purity result of six samples was calculated and the % RSD for six samples was calculated. Results of method precision are summarized in Table No.4.

**Table 4: Results of Method Precision**

Parameter	% Amount of R (+) Pantoprazole
Method Precision	0.856
	0.848
	0.859
	0.849
	0.852
	0.851
Mean	<b>0.853</b>
%RSD	0.470

**Robustness:**

The robustness of an analytical method is the ability to remain unaffected by small changes in chromatographic parameters. For robustness study one standard solution was prepared and injected in five replicate to determine the system suitability parameters. The parameters altered for robustness study are change in flow rate ( $\pm 0.1$  mL/minute), change in wavelength ( $\pm 2$  nm) and change in column oven temperature ( $\pm 5^\circ\text{C}$ ). Results obtained for each parameter changed for robustness are summarized in Table No. 5.

**Table 5: Results of Robustness**

Parameters	Values	% RSD of Standard Solution	Resolution between R & S Isomers
Control sample	As per method	0.22	3.29
Flow (±0.1 mL/min)	0.4 mL/min	0.16	3.13
	0.6 mL/min	0.28	3.07
Wavelength ± 2 nm)	288 nm	0.41	3.16
	292 nm	0.38	3.16

**Filter validation:**

For filtration study sample solution was filtered using different filters and results compared with centrifuged sample as unfiltered. Results obtained are summarized in Table No. 6.

**Table 6: Results of Filter Validation**

Sample No.	R (+) Pantoprazole	Absolute Difference
Unfiltered Sample	0.828	NA
Filtered Sample -1	0.824	0.004
Filtered Sample -2	0.831	0.003
Filtered Sample -3	0.821	0.007
Filtered Sample -4	0.826	0.002
Filtered Sample -5	0.830	0.002

**Stability of Analytical Solution:**

To determine the stability of solutions, sample solution of S (-) Pantoprazole was prepared as per method of analysis and stored at 2°C to 8°C for a period of 24 hours. Sample and Standard preparations were analyzed initially and after 24 hours from the results obtained solution was found to be stable up to 24 hours at 2°C to 8°C.

## CONCLUSION

In pharmaceutical formulations, the impurities and degradation products can change the pharmacological and toxicological potency of the active pharmaceutical ingredient which has adverse effect on quality, safety and efficacy of the drug. In this study, a rapid, precise, specific and stability indicating RP- HPLC method was developed for accurate quantification of R (+) Pantoprazole. The analytical method was validated and the data reflects satisfactory results for the validation parameters according to ICH guidelines. It could be applied for the routine analysis of Chiral purity test of S (-) Pantoprazole in S (-) Pantoprazole and Mosapride Citrate in capsule dosage form in quality control laboratories, research institutions, industries, approved testing laboratories.

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