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Analytical Method Development and Validation for Simultaneous Estimation of Cinitapride Hydrogen Tartrate and Pantoprazole Sodium in Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

The aim of the research is method development and validation of Reversed-Phase High-Performance Liquid chromatography (RP-HPLC) method for simultaneous determination of Cinitapride hydrogen tartrate and Pantoprazole sodium in its pharmaceutical dosage form. The method is simple, precise, economic, less time consuming and suitable for routine quality control analysis of both the drugs in formulation. The chromatographic separation was achieved on Thermoscientific BDS Hypersil C18 (250 × 4.6 mm, 5µl) column using a mixture of methanol and 0.1% v/v triethylamine (pH 6) in the ratio 85: 15 % v/v at a flow rate of 1.0 ml/min and UV detection at 264 nm. The retention times of Cinitapride and Pantoprazole were found to be 4.73 and 2.86 min respectively. The method shows linearity in the concentration range of 0.5-1.3 µg/ml for both the drugs with r^2=0.9922 for Cinitapride and r^2 =0.9974 for Pantoprazole. The LOD of Cinitapride and Pantoprazole were found to be 0.00164 µg/ml and 0.00042 µg/ml respectively. The LOQ of Cinitapride and Pantoprazole were found to be 0.00496 µg/ml and 0.00126 µg/ml respectively. The percentage recovery was found to be within the limits. The method for the determination of assay was below 2.0% RSD. Hence the developed HPLC method was applied for the estimation of Cinitapride and Pantoprazole in its pure form as well as in tablet dosage form and results was found to be in good agreement with the labeled claim. The developed method was found to be simple, accurate, precise, and specific and is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: RP-HPLC, Cinitapride hydrogen tartrate, Pantoprazole sodium, Simultaneous estimation, Validation.

INTRODUCTION

Cinitapride hydrogen tartrate (Figure 1) is chemically 4-Amino-N-[1-(cyclohex-3-en-1yl methyl) piperidin-4-yl]-2-ethoxy-5-nitrobenzamide. It is gastroprokinetic agent and anti-ulcer agent of Benzamide class [1]. It acts as an agonist of $5HT_1$ and $5HT_4$ receptors and as an antagonist of $5HT_2$ receptors. Cinitapride is indicated for gastrointestinal disorders associated with motility disturbances such as gastrooesophageal reflux disease, non-ulcer dyspepsia and delayed gastric emptying.

Pantoprazole (Figure 2) is chemically 6-(diflouromethoxy)-2- {[(3,4-dimethoxypyridine-2yl)methane]sulfinyl}-1H-1,3-benzodiazole. It is a proton-pump inhibitor that inhibits gastric acid by blocking H^+/K^+ adenosine triphosphate enzyme system (proton pump) of gastric parietal cells. It is substituted benzimidazole indicated for stomach ulcers, intestinal ulcers, gastrooesophageal disease (GERD) by reducing amount of acid production in stomach. It is used to treat stomach ulcers caused due to medication with NSAIDs and by bacteria called *H. pylori*.

Cinitapride and Pantoprazole are available in combined dosage form as hard gelatin capsule (CINTODAC). Each capsule contains 3 mg of Cinitapride hydrogen tartrate equivalent to Cinitapride (as extended release pellets) and 40 mg of Pantoprazole sodium equivalent to Pantoprazole (as enteric coated tablet) [2-6].

The combination of Cinitapride and Pantoprazole is used to treat gastro intestinal disorders in particular hyperacidity associated with gastro-intestinal dismotility [7]. Extensive literature survey reveals that several analytical methods have been reported for the estimation of Cinitapride and Pantoprazole in pharmaceutical dosage form.

The aim of the current research is to develop simple and accurate RP-HPLC method for simultaneous determination of Cinitapride and Pantoprazole and extend it for their determination in formulation and validate as per the ICH guidelines [8-10].

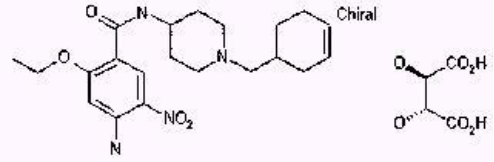

Figure 1: Chemical structure of Cinitapride hydrogen tartrate

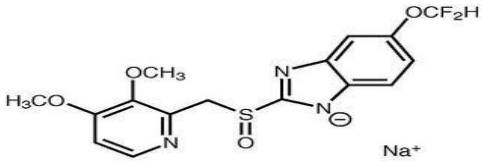

Figure 2: Chemical structure of Pantoprazole sodium

MATERIALS AND INSTRUMENTS

Chemicals and solvents used [11-15]

- Acetonitrile
- Water
- Methanol
- Orthophosphoric acid
- Triethylamine

All reagents and chemicals were used of HPLC grade.

Pure drug samples (Table 1) [16]

Table 1: Pure drug information

Drugs	Supplier	Quantity	Purity
Cinitapride hydrogen tartrate	Comprime Labs	10.0 g	99.98% w/w
Pantoprazole sodium	Comprime Labs	10.0 g	99.98% w/w

The drugs used for the present investigation were donated as gift samples.

Marketed formulation available (Table 2) [17]

Table 2: Marketed formulation information

Brand Name	Mfg By	Content	Quantity
CINTODAC hard gelatin capsule	Zydus Cadila Healthcare Ltd.	Cinitapride hydrogen tartrate	3 mg
		Pantoprazole sodium	40 mg

The marketed formulation was purchased from local market.

Instrumentation (Table 3) [18]

Table 3: Required Instruments information

Name of Equipment	Make	Model
HPLC	Shimadzu	996 PDA Detector
		LC Solutions Software
pH Meter	Lab India®	SAB 5000
Digital Electronic Balance	Shimadzu	BL 220H
Column	Thermoscientific (5 µm)	BDS HYPERSIL C18 [4.6 x 250 mm(id)]

Experimental

HPLC method [19]

From the various trials (Figures 3a-3e) (Table 4), chromatograms using mobile phase containing different ratios of methanol and 0.1% triethylamine such as 90: 10, 85: 15, 80: 20, 75: 25 and 70: 30 %v/v were recorded at 264 nm at a flow rate of 1.0 ml/min. The ratio of methanol and 0.1% triethylamine in 85: 15 %v/v (Figure 1b) gave good resolution with symmetric peaks.

Effect of pH of mobile phase

The mobile phase consisting methanol and 0.1 % triethylamine in ratio 85:15 %v/v was adjusted to different pH such as 3, 4, 5, and 6 using 1% Orthophosphoric acid.

From the trials (Figures 4a-4d and Table 5), at the pH of 6 (Figure 4d), both the drugs showed symmetric peaks and hence selected for the study. Chromatographic condition also determined (Table 6).

Preparation of standard solutions

Accurately weighed 10 mg of Cinitapride and 10 mg of Pantoprazole were transferred into a separate clean, dry 100 ml volumetric flasks and dissolved with sufficient volume of mobile phase. The volume was made up to 100 ml with mobile phase to get 100 µg/ml concentration of each drug.

From the stock solution, dilutions were made in the concentration range of 0.5-1.3 µg/ml for both the drugs with mobile phase and chromatograms were recorded at 264 nm. The peak areas were plotted against concentration and calibration graphs were constructed for both the drugs. Concentration range of 0.5-1.3 µg/ml was found to be linear and obeys Beer's law.

Analysis of marketed formulation [20-23]

20 capsules of Cintodac (Label claim: 40 mg Pantoprazole and 3 mg Cinitapride) were weighed, emptied in a glass mortar and powdered. Average weight of capsule was calculated and amount of powder equivalent to 20 mg of Pantoprazole and 18.5 mg of Cinitapride was accurately weighed and added to 50 ml volumetric flask so that sample contains 20 mg equivalent of each drug. It is dissolved in mobile phase, sonicated and made up to the mark and filtered through 0.45 µm membrane filter. Appropriate aliquot of this standard stock solution of formulation (400 µg/ml) was taken into a 10 ml volumetric flask and volume was made up to mark with mobile phase to obtain desired concentration. A 20 µl of sample was injected into injector of liquid chromatographic system and chromatogram was recorded (Figure 5 and Table 7).

Method validation

The method was developed and validated according to ICH guidelines.

Linearity

The calibration curves for Cinitapride and Pantoprazole were constructed by plotting peak area against concentration and regression equations were calculated. Both the drugs showed linearity in the concentration range of 0.5-1.3 µg/ml. Aliquots 20 µl of each solution injected under the operating chromatographic condition (Figures 6a, 6b and Table 8).

Accuracy

Accuracy studies were expressed as recovery (%), which is determined by the standard addition method at 50%, 100%, 120% of the label claim according to ICH guidelines. The %Recovery and %RSD were calculated and reported (Figures 7a-7c and Table 9).

Precision

The intraday and interday precision of the proposed method was evaluated by analyzing samples of two different concentration of Cinitapride (0.8, 0.9 µg/ml) and Pantoprazole (0.8, 0.9 µg/ml) in triplicates on same and different days (Tables 10 and 11).

Repeatability

Repeatability was determined by analyzing standard solutions of 0.8 µg/ml concentration of Cinitapride and Pantoprazole by injecting six times. The precision and % RSD were calculated and reported (Table 12).

Limit of detection and limit of quantitation (LOD and LOQ)

The LOD and LOQ of Cinitapride and Pantoprazole were calculated based on standard deviation of the response and slope values of two drugs according to ICH guidelines.

System suitability studies

System suitability is a pharmacopoeial requirement and used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done. The parameters like theoretical plate count, retention time, tailing factor and resolution of both the drugs were reported (Table 13).

RESULTS AND DISCUSSION

Mobile phase

Effect of ratio of mobile phase

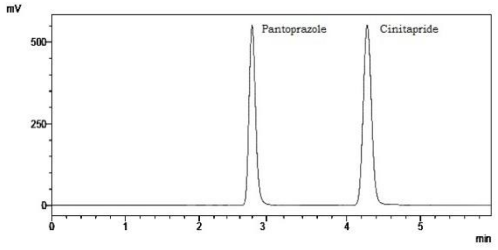

Figure 3a: Methanol: 0.1% triethylamine buffer (90:10 %v/v)

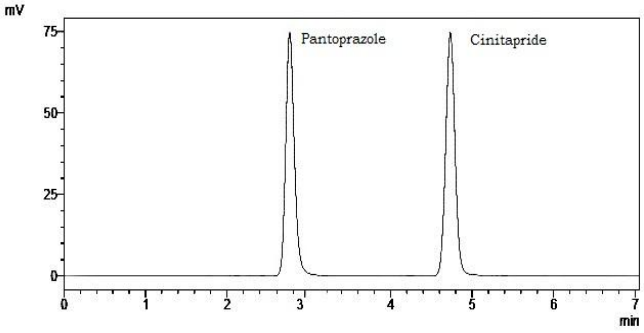

Figure 3b: Methanol: 0.1% triethylamine buffer (85:15 %v/v)

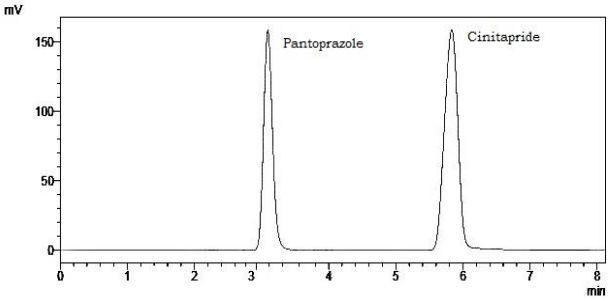

Figure 3c: Methanol: 0.1% triethylamine buffer (80:20 %v/v)

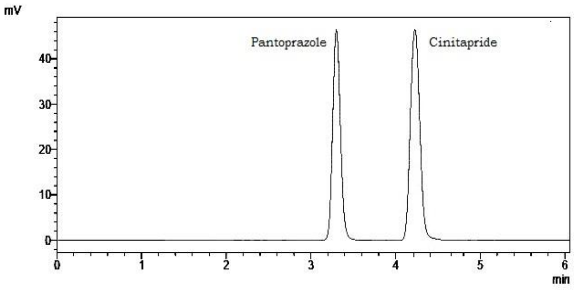

Figure 3d: Methanol: 0.1% triethylamine buffer (75:25 %v/v)

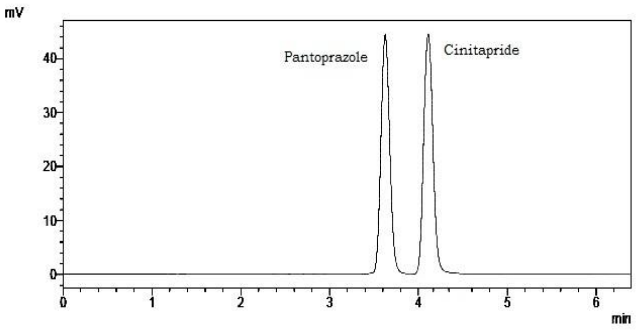

Figure 3e: Methanol: 0.1% triethylamine buffer (70:30 %v/v)

From the above five trials the observations are follows:

Table 4: Observations for trial of effect of ratio of mobile phase

S. No.	Methanol: 0.1%triethyl amine(% v/v)	Retention time	
		CIN	PAN
1	90:10:00	4.29	2.88
2	85:15:00	4.73	2.86
3	80:20:00	5.82	3.1
4	75:25:00	4.27	3.29
5	70:30:00	4.12	3.62

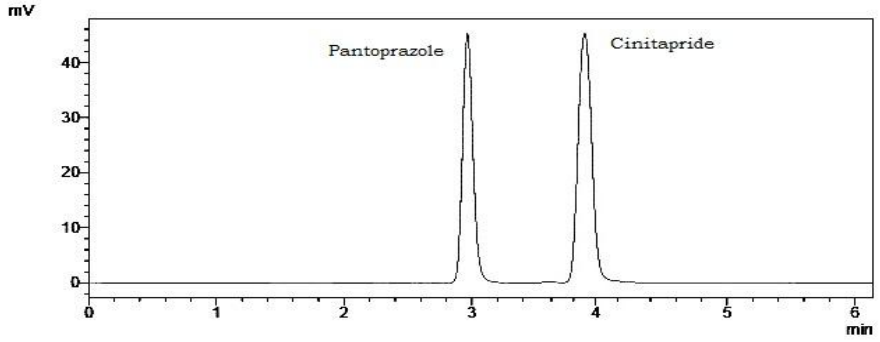

Figure 4a: Methanol: 0.1% triethylamine buffer (85:15 %v/v) (pH 3)

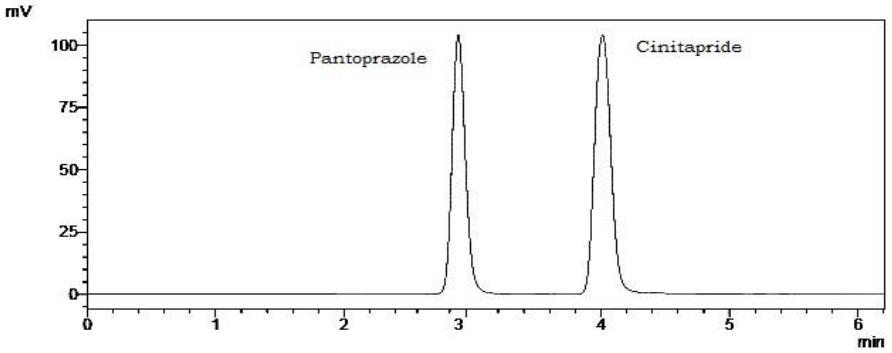

Figure 4b: methanol: 0.1% triethylamine buffer (85:15 %v/v) (pH 4)

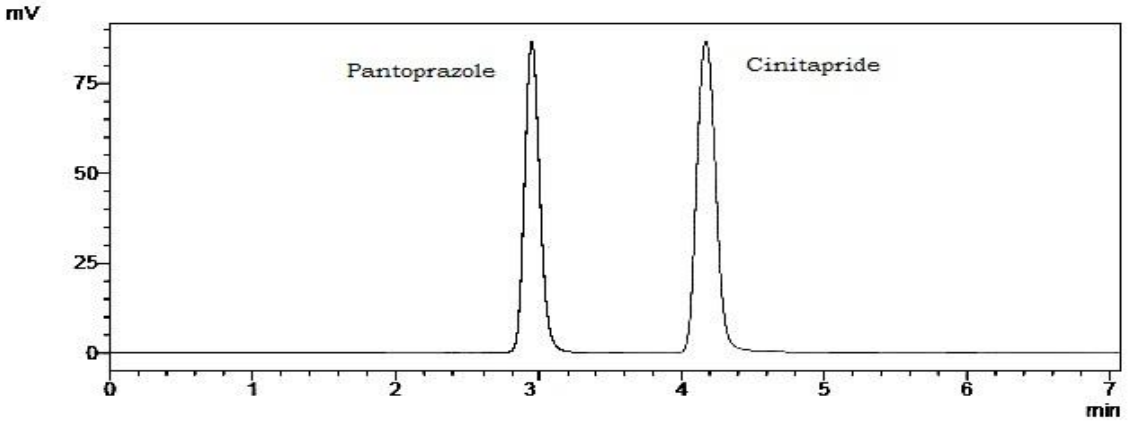

Figure 4c: Methanol: 0.1% triethylamine buffer (85:15 %v/v) (pH 5)

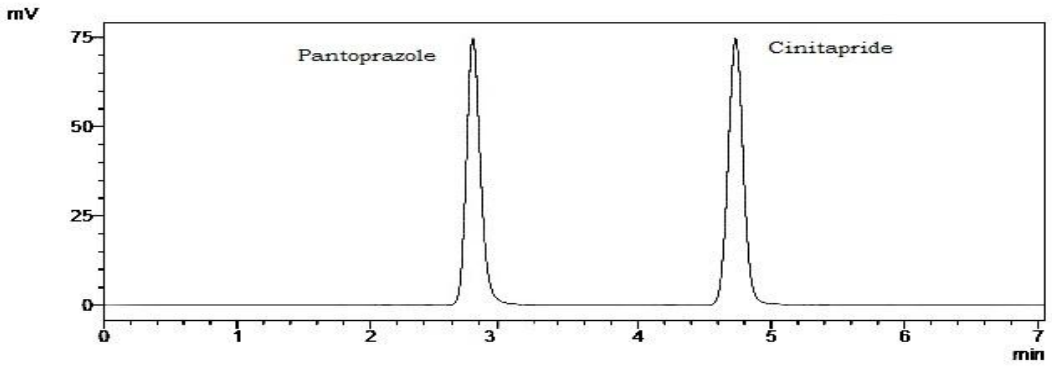

Figure 4d: Methanol: 0.1% triethylamine buffer (85:15 %v/v) (pH 6)

Table 5: Observations for trial of effect of pH of mobile phase

S. No.	pH	Observation
1	pH 3	Asymmetric peaks
2	pH 4	Asymmetric peaks
3	pH 5	Slight tailing
4	pH 6	Symmetric peaks

Chromatographic conditions

Table 6: Chromatographic condition

Column	Thermoscientific (250 x 4.6 mm, 5 µ)
Particle size packing	10 µm
Stationaray phase	BDS HYPERSIL C18 (5 µm)
Mobile phase	Methanol: 0.1% triethylamine(pH 6)
Detection wavelength	264 nm
Flow rate	1 ml/min
Run time	07 min
Temperature	Ambient
Sample size	20 µl
Diluent	Methanol

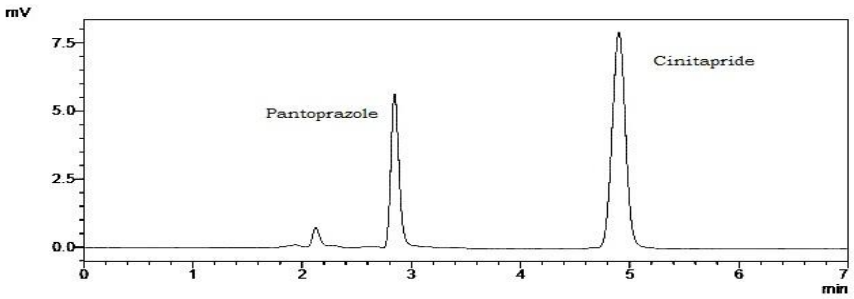

Figure 5: Chromatogram of marketed formulation

Table 7: Analysis of marketed formulation

Drugs	Labeled amount, mg tablet^{-1}	Amount found, mg tablet^{-1}	% Label claim
Cinitapride hydrogen tartrate	3	3.06	102
Pantoprazole sodium	40	41.05	102.87

Method validation

Linearity

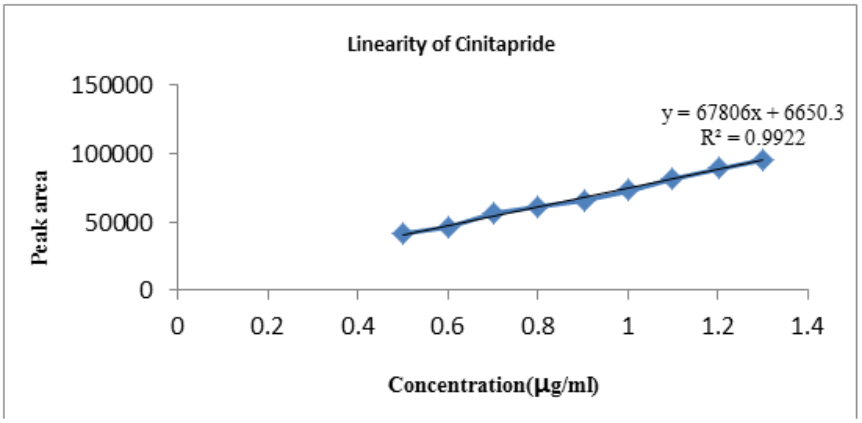

Figure 6a: Calibration curve of Cinitapride

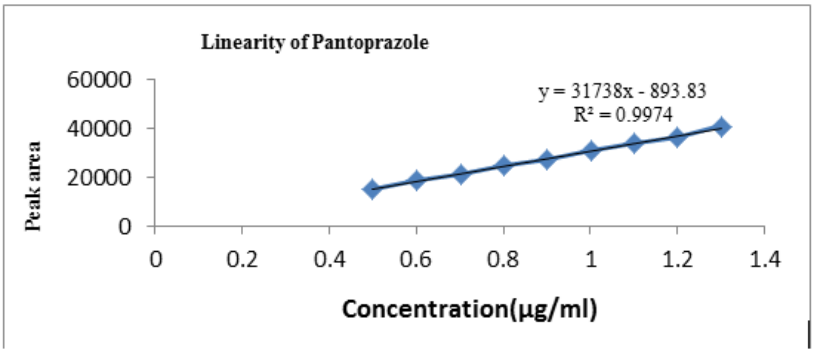

Figure 6b: Calibration curve of Pantoprazole

Table 8: Linearity observation

Parameters	Cinitapride	Pantoprazole
λ_{max}, nm	264	264
Beer's Law limit (µg/ml)	0.5-1.3	0.5-1.3
Regression equation (Y*)	Y = 67806x+6650	Y = 31738x-893.8
Correlation coefficient (r^2)	0.992	0.997
Slope (b)	67806	31738
Intercept (a)	6650	893.8

Accuracy studies

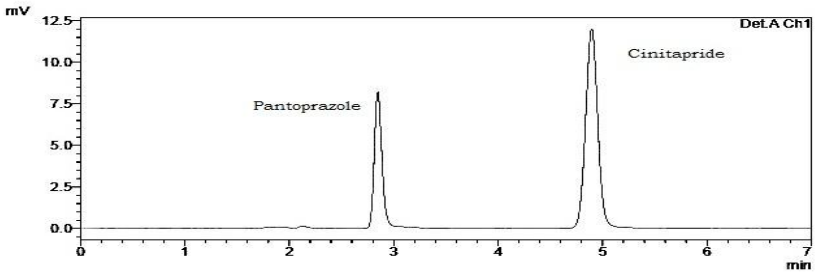

Figure 7a: Chromatogram of 50% recovery studies

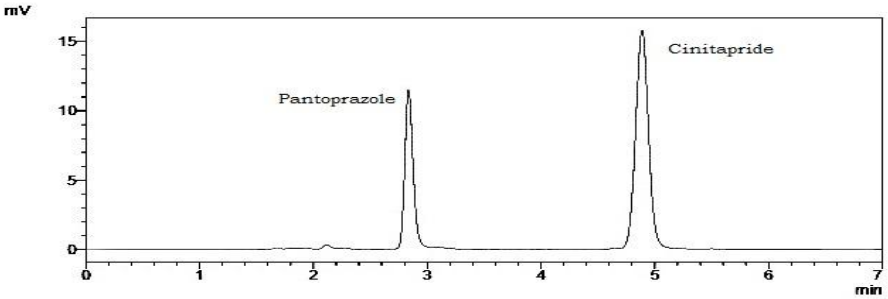

Figure 7b: Chromatogram of 100% recovery studies

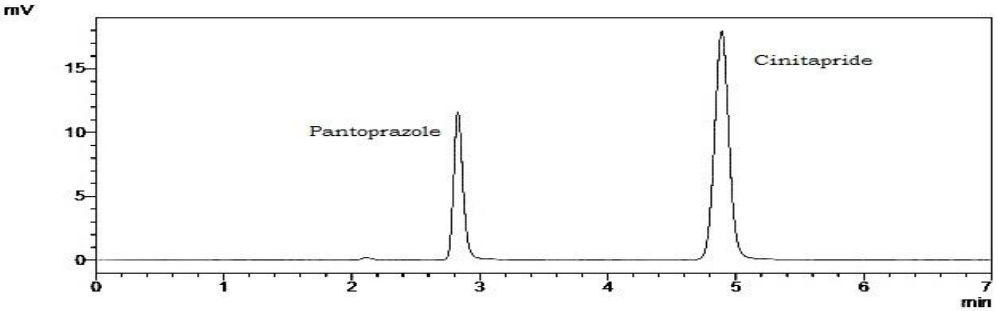

Figure 7c: Chromatogram of 120% recovery studies

Table 9: Accuracy studies

Drugs	% Level	% Recovery	% RSD
Cinitapride	50	103.18	0.72
	100	102.1	1.18
	120	102	1.49
Pantoprazole	50	102.59	0.94
	100	101.09	1.42
	120	102.41	1.09

Precision

Table 10: Intraday precision

Concentration ((µg/ml)		Injection	Peak area		%RSD	
CIN	PAN		CIN	PAN	CIN	PAN
0.8	0.8	1	74340	23988	0.22	0.72
		2	74822	23668		
		3	74549	23992		
		4	74654	23882		
		5	74734	23789		
		6	74594	23567		

Table 11: Interday precision

S. No.	Concentration (µg/ml)		Peak area		%RSD	
	CIN	PAN	CIN	PAN	CIN	PAN
1	0.8	0.8	73450	22452	0.13	1.08
			73650	22942		
			73522	22752		
2	0.9	0.9	80290	26941	0.22	0.38
			80652	26851		
			80428	26734		

Repeatability

Table 12: Observation for Repeatability

Concentration (µg/ml)			Peak area		%RSD	
S. No.	CIN	PAN	CIN	PAN	CIN	PAN
1	0.8	0.8	74340	23988	0.32	
			74822	23668		0.77
			74549	23992		
2	0.9	0.9	81590	27941	0.18	0.12
			81312	27984		
			81341	28009		

System suitability studies

Table 13: Observation for System suitability studies

Drug	Theoretical plate count	Retention time (min)	Tailing factor	Resolution (R_s)
Cinitapride	6806.03	2.86	1.03	17.14
Pantoprazole	5682.03	4.73	1.21	

CONCLUSION

For the simultaneous estimation of Cinitapride hydrogen tartrate and Pantoprazole sodium, RP-HPLC method was developed and validated according to ICH guidelines. The proposed method was found to be simple, precise, economic, less time consuming and proved to be superior to most of the reported methods. The mobile phase was simple to prepare and economical. The sample recovery in the formulation was in good

agreement with their respective label claims and suitable for routine quality control analysis of both the drugs in formulation.

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