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Analytical method development and method validation of Cinitapride and pantoprazole in pharmaceutical dosage forms by RP-HPLC

Dr. G SubbaRao , ChekkaSujith Kumar and DoddiSravani

St. Xavier Institute of Pharmacy, Deenapur, Phirangipuram, Guntur

Corresponding author: Dr. G Subba Rao

ABSTRACT

A new rapid, precise and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of cinitapride and pantoprazole simultaneously in combined dosage form. The two components cinitapride and pantoprazole were well resolved on an isocratic C18 column, utilizing a mobile phase composition of acetonitrile: phosphate buffer (50:50, v/v, pH 6.8) at a flow rate of 1.0 mL/min with UV detection at 281 nm. The retention time of cinitapride and pantoprazole were 4.5min and 5.4min respectively. The developed method was validated for specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness as per ICH guidelines. Linearity for cinitapride and pantoprazole were found in the range of 1.5-10.5µg/ml and 20-140µg/ml, respectively. The percentage recoveries for cinitapride and pantoprazole ranged from 97.9-103.44 % and 98.9-103.1%, respectively. The proposed method could be used for routine analysis of cinitapride and pantoprazole in their combined dosage forms.

Keywords: Cinitapride; Pantoprazole; RP-HPLC

INTRODUCTION

Pharmaceutical analysis deals not only with medicaments (drugs and formulations), but also with their precursors i.e. with the raw material whose degree of purity, which in turn decides the quality of medicaments. The quality of a drug is determined, after establishing its authenticity, which is carried by testing its purity and the quality of the pure substance in the drug and its formulations.

High-performance liquid chromatography (HPLC):

High-performance liquid chromatography is a chromatographic technique used to separate the components in a mixture, to identify each component, and to quantify each component .The method involves a liquid sample being passed over a solid adsorbent material packed into a column using a flow of liquid solvent. Each analytic in the sample interacts slightly differently with the adsorbent material, thus retarding the flow of the analyses. If the interaction is weak, and the analysts flow off the column in a short amount of time, and if the interaction is strong, then the elution time is long.

Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases**.** (Sharma B.K.1994)

The HPLC method was considered the choice of estimation, since this method is the most powerful of all chromatographic and other separate methods. The HPLC method has enabled analytical chemist to attain great success in solving his analytical problems. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise, and accurate and the limit of detection is low and also it offers the following advantages.

The schematic representation of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components .A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis, photodiode array (PDA) or Refractive index (RI).

MATERIALS AND METHODS

METHOD VALIDATION

Preparation of Standard Stock Solution

25 mg each of standard Cinitapride and Pantoprazole were weighed accurately and transferred in to two separate 25mL flasks, and dissolved in 10mL of solvent, the volume was made up to the mark with solvent to obtain a solution of concentration of 1000 µg/mL of each Cinitapride and Pantoprazole (standard stock solutions A1 and A2 respectively). From the above stock solution A1 and A2 respectively 1.5mL and 20 mL aliquots were pipetted in to a 50mL volumetric flasks and dissolved in 25mL of the solvent and made up to the mark with the solvent to obtain a final concentration of 400 and 30µg/mL of Pantoprazole and Cinitapride respectively (working stock solution). The stock solutions were filtered through a 0.45µm membrane filter and sonicated for 15min in an ultrasonic bath sonicate.

Preparation of Standard Solution

Transfer 5mL aliquot of the working standard stock solution A was diluted to 20 mL to obtain a concentration of 7.5 and 100µg/mL of Cinitapride and Pantoprazole respectively.

Preparation of Sample Stock Solution

The contents of twenty marketed Cintodac capsules were weighed accurately and their average weight was determined. A mass equivalent to 40 mg Pantoprazole and 3mg Cinitapride from the contents of the capsule were taken in a 100mL volumetric flask and dissolved in 50mL of the solvent. The solution was kept for sonication 15min. The solution was made up to the mark with the solvent and filtered through a 0.45 µ membrane filter paper sample stock solution ‘A’.

Preparation of Sample Solution

Transfer 5.0 mL aliquot of the working sample stock solution A was diluted to 20 mL to obtain a concentration of 7.5 and 100µg/mL of Cinitapride and Pantoprazole respectively.

SYSTEM SUITABILITY

The chromatographic system was equilibrated using the initial mobile phase composition, followed by 6 injections of the standard solution of concentration 7.5 µg/mL Cinitapride and100 µg/mL Pantoprazole. The system suitability parameters including Theoretical plates, resolution, tailing factor and asymmetric factor were determined. The results were statistically analysed for the determination of standard deviation and %RSD.

Table 9: Data of system suitability chromatograms

S.No	Retention time		Peak area	
	CIN	PAN	CIN	PAN
1	4.547	5.397	7968111	92478206
2	4.537	5.397	8085965	93227923
3	4.540	5.400	7901974	92562832
4	4.537	5.403	7938349	91486225
5	4.530	5.407	7871160	91603127
6	4.527	5.400	8062795	93050876
Mean	4.535	5.400	7971392	92401532
SD	0.005	0.006	86544.1	722731.5
% RSD	0.12	0.12	1.09	0.78
No. of Theoretical Plates			13127	14273
Resolution			2.79	
Tailing factor			1.1	1.3

Table: 10 System suitability parameters

Parameters	CIN	PAN
Retention Time (min)	4.535	5.400
Tailing	1.1	1.3
Resolution	-	2.79
Theoretical Plates	13127	14273
%RSD	1.09	0.78

Observation

From the system suitability studies it was observed that all the parameters are within limit, hence it is concluded that the Instrument, Reagents and Column are suitable to perform Assay.

Acceptance criteria

The % RSD of Cinitaprideand Pantoprazole peak areas should be NMT 2%.The number of theoretical plates (N) for the Cinitaprideand Pantoprazole peaks is NLT 2000.The Tailing factor (T) for the Cinitaprideand Pantoprazole peaks is NMT 2.0.

LINEARITY

Aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 mL of working stock solution was serially diluted to 10mL in separate volumetric flasks to obtain a solution of concentrations in the range 1.5-10.5µg/mL of Cinitapride and 20-140µg/mL of Pantoprazole.

The prepared solutions with increasing concentrations of Cinitapride and Pantoprazole reference standards were analysed chromatographically. For every concentration in μg/mL was measured the peak response value at 281nm. The experimental results were subjected to linear regression analysis.

The drugs Cinitapride and Pantoprazole follow the beer's lamberts law in the concentration range of 1.5-10.5 μg/mL and 20-140 μg/mL respectively. Linearity determinations were carried in triplicate (n=3). Regression equation was established and the correlation coefficient was determine

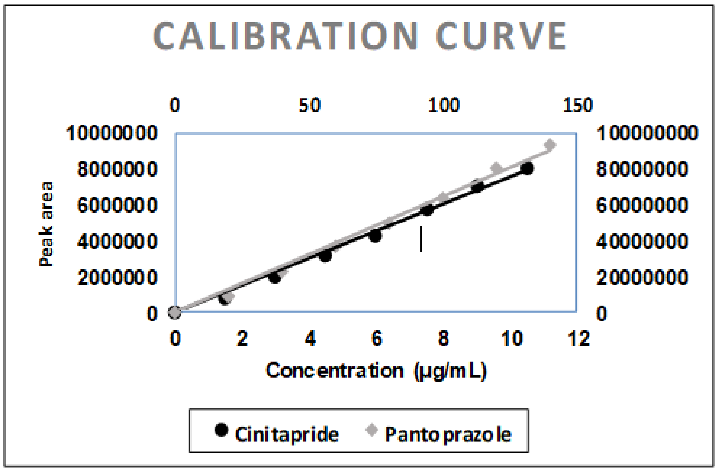

Fig.: 1 Calibration Curve

Table 1: HPLC Linearity Data of Cinitapride and Pantoprazole

Cinitapride			
Concentration μg/mL	**Peak area n=1**	**Peak area n=2**	**Peak area n=3**
Level 1-1.5	1271341	1294718	1095714
Level 2-3.0	2302253	2376086	2176603
Level 3-4.5	3518631	3650508	3155130
Level 4-6.0	4729404	4848928	4248871
Level 5-7.5	5923659	5814888	5328019
Level 6-9.0	7265947	7157380	6251377
Level 7- 10.5	8155958	8168715	7368677
Correlation coefficient	0.9995	0.9996	0.9999
Slope	786823.2	777850.3	697674.94
y- intercept	15077.17	80188.67	40255.41
Pantoprazole			
Level 1-20	10005542	10703227	11703227
Level 2-40	25379145	23830602	24832691
Level 3-60	38938675	36537859	37765210
Level 4-80	52442612	49024228	49417502
Level 5-100	65873861	63834941	64910451
Level 6-120	81936017	77674361	78676174
Level 7-140	93249699	91640930	93641899
Correlation coefficient	0.9993	0.9993	0.9992
Slope	682969.3	660024.74	668002.1
y intercept	1829654.167	-2045963.41	1641755.75

Table 2: Statistical Linearity Validation Data of Cinitapride and Pantoprazole

Parameter	Cinitapride	Pantoprazole
Linearity (µg/mL)	1.5-10.5	20-140
Correlation coefficient	0.9997	0.9993
Slope	754116.2	670332
y intercept	45173.75	-1839124
Limit of detection (µg/mL)	0.144	0.996
Limit of quantitation (µg/mL)	0.431	2.987

ACCURACY:

Procedure for the Determination of Accuracy

Recovery studies were performed by applying standard addition method. To a known amount of the pre-analysed drug sample an 80%, 100%, and 120% of standard drug substance was added and suitably diluted. The peak areas of the resultant solutions were measured at 281nm. The amount recovered at each recovery level was determined by substituting the peak response values in the regression equation.

In 80% recovery level concentration the amount of standard added was 2.4mg Cinitapride and 32mg Pantoprazole (80% addition). In 100% recovery level concentration the amount of standard added was 3mg Cinitapride and 40mg Pantoprazole (100% addition). In 120% recovery level concentration the amount of standard added is 3.6mg Cinitapride and 48mg Pantoprazole (120% addition). To each of the above three recovery levels a sample concentration equivalent to 0.6mg of Cinitapride and 8mg of Pantoprazole of the capsule dosage form was added.

The contents corresponding to the three recovery levels were transferred in to three separate 100mL volumetric flasks and dissolved in a small quantity of methanol and sonicated in an ultrasonic bath sonicator for 10min. finally the volume was made up to the volume with methanol. This solution was filtered through What man filter paper No. 41.

The solution corresponding to the three recovery levels were prepared in triplicates. A 20µL volume of these solutions were injected to the chromatographic system and the respective chromatograms were recorded at 281nm. The %recovery at each level was calculated by substituting the peak area values in the regression equation and the results were statistically validated.

Table 3: Data of Assay chromatograms

S.No	Retention time		Peak area	
	CIN	PAN	CIN	PAN
1	4.520	5.397	7925458	92781379
2	4.526	5.391	8008349	93005443
3	4.538	5.400	7891102	92620009
4	4.540	5.408	8002877	91753925
5	4.529	5.411	8024688	91980341
6	4.533	5.397	7973557	92706273

Table 4: Assay data of Cintodac Marketed Formulations

S.No.	Conc. present (µg/mL)		Conc. obtained (µg/mL)		% recovery (%w/w)	
	CIN	PAN	CIN	PAN	CIN	PAN
1	7.5	100	7.472	99.77	99.63	99.77
2	7.5	100	7.550	100.01	100.67	100.01
3	7.5	100	7.440	99.60	99.20	99.60

4	7.5	100	7.545	98.67	100.60	98.67
5	7.5	100	7.565	98.91	100.87	98.91
6	7.5	100	7.517	99.69	100.23	99.69

Statistical validation data

Drug	Mean	Standard deviation	%RSD
Cinitapride	7.515	0.05	0.66
Pantoprazole	99.44	0.53	0.53

Table 5: Accuracy Data of CIN and PAN

Recovery level	Amount of standard		Amount of test added		Total amount recovered		% Recovery	
	CIN	PAN	CIN	PAN	CIN	PAN	CIN	PAN
80%	2.4	32	0.6	8	3.04	39.59	101.5	98.9
	2.4	32	0.6	8	2.99	39.41	99.8	98.5
	2.4	32	0.6	8	3.01	40.09	100.5	100.2
100%	3.0	40	0.6	8	3.61	48.21	100.5	100.4
	3.0	40	0.6	8	3.56	48.11	99.1	100.2
	3.0	40	0.6	8	3.64	48.68	101.1	101.4
120%	3.6	48	0.6	8	4.28	56.11	101.9	100.2
	3.6	48	0.6	8	4.20	56.76	100.2	101.4
	3.6	48	0.6	8	4.24	56.77	101.2	101.4

Table 6: Statistical Validation Data of Accuracy

Level of recovery	Mean		Standard deviation		% RSD	
	CIN	PAN	CIN	PAN	CIN	PAN
80 %	3.018	39.698	0.0258	0.3504	0.856	0.882
100 %	3.608	48.339	0.0372	0.3070	1.032	0.635
120%	4.245	56.551	0.0368	0.3787	0.867	0.669

PRESICION

Procedure for the Determination of Precision

The precision of the analytical method was determined a minimum of 6 determinations at the 100% test concentrations. An amount equivalent to 3mg Cinitapride and 40mg of Pantoprazole was weighed accurately and transferred to a 100mL volumetric flasks and dissolved in a small quantity of solvent and the content was kept in a sonicator for 10min. finally the volume was made up to the mark with the solvent. The solution was filtered through 0.45µ Nylon filter. The above sample solution was suitably diluted with the solvent to obtain a solution of concentration 7.5µg/mL Cinitapride and 100µg/mL Pantoprazole.

Intra-day Precision

In intraday precision six replicate sample matrices containing 7.5µg/mL Cinitapride and 100µg/mL Pantoprazole were chromatographically analysed at different time intervals on the same day. The variation of the results within the same day was analysed and statistically validated.

Table 7: Intraday Precision Areas of CIN and PAN

S.No	Retention time		Peak area	
	CIN	PAN	CIN	PAN
1	4.523	5.387	8002877	92081040
2	4.529	5.411	7978349	92005111
3	4.514	5.401	7991125	91127487
4	4.522	5.384	8022087	91237418

5	4.529	5.408	7924688	91304394
6	4.505	5.391	7903049	93004714

Table 8: Intraday Precision Data of CIN and PAN

S. No	Conc. present (µg/mL)		Result in mg/mL		Result in %	
	CIN	PAN	CIN	PAN	CIN	PAN
1	7.5	100	3.005	40.03	100.59	99.71
2	7.5	100	2.996	39.99	99.77	100.32
3	7.5	100	3.001	39.61	101.50	101.27
4	7.5	100	3.012	39.66	101.15	100.97
5	7.5	100	2.976	39.69	99.07	101.72
6	7.5	100	2.968	40.43	100.28	100.16

Table 9: Statistical Validation Data of Intraday Precision

Drug component	Mean	Standard deviation	%RSD
Cinitapride	99.77 %	0.58	0.58
Pantoprazole	99.76 %	0.78	0.78

Inter-day Precision

In inter-day precision six replicate sample matrices containing 7.5µg/mL Cinitapride and 100µg/mL Pantoprazole were chromatographically analysed on three consecutive (1st, 2nd and 3rd) days. The variation of the results within the same day was analysed and statistically validated

..Table: 10 Data of Interlay precision chromatograms

S.No	Retention time		Peak area	
	CIN	PAN	CIN	PAN
1	4.520	5.397	7925458	92781379
2	4.526	5.391	8008349	93005443
3	4.538	5.400	7891102	92620009
4	4.540	5.408	8002877	91753925
5	4.529	5.411	8024688	91980341
6	4.533	5.397	7973557	92706273

Table 11: Inter day Precision Data CIN & PAN

Sl. No	Conc. present (µg/mL)		Result in mg/mL		Result in %	
	CIN	PAN	CIN	PAN	CIN	PAN
1	7.5	100	2.989	39.91	99.63	99.77
2	7.5	100	3.020	40.00	100.67	100.01
3	7.5	100	2.976	39.84	99.20	99.60
4	7.5	100	3.018	39.47	100.60	98.67
5	7.5	100	3.026	39.56	100.87	98.91
6	7.5	100	3.007	39.88	100.23	99.69

Table :12 Statistical Validation Data of Inter day Precision

Drug component	Mean	Standard deviation	%RSD
Cinitapride	100.20 %	0.65	0.65
Pantoprazole	99.44 %	0.53	0.53

Acceptance criteria

The % RSD for the sample of Cinitapride and Pantoprazole should be NMT 2.0%.

LIMIT OF DETECTION

Limit of detection was determined based on the standard deviation of y intercepts of the regression line. The standard deviation of y intercepts obtained from the replicate measurements (n=3) was substituted for σ in the equation 3.3σ/S, and S is the mean of slope of the calibration curves.

LIMIT OF QUANTITATION

Limit of quantitation was determined based on the standard deviation of y- intercepts of the regression line. The standard deviation of y intercepts obtained from the replicate measurements (n=3) was substituted for σ in the equation 10σ/S, and s is the mean slopes of the three calibration curves.

TheLODandLOQvalues weredetermined bythe formulae

LOD=3.3XS/m and LOQ=10 S/m

Where, Sisthe standard deviationoftheresponses
Mismeanoftheslopesof thecalibrationcurves.

The results were given in the table.

SPECIFICITY

Specificity is defined as the degree to which the analyse measured is due only to the analyse of the interest and not to any other substances expected to be present in the sample matrix. For determining the specificity of the method the test solution was injected to the chromatographic system. Interferences due to the presence of excipients were not traced in the chromatogram and the chromatographic parameters were not affected. Thus the selected method is specific for the determination of the marketed formulation.

ROBUSTNESS

As part of evaluation of robustness, deliberate changes were made in the flow rate and Wavelength to evaluate the impact on the method.

Effect of Variation of Flow Rate

Standard solution prepared as per the test method was injected into the chromatographic system maintaining flow rates, less flow (0.8ml/min) and more flow (1.2 ml/min).

Table: 13 Robustness Data of CIN and PAN (0.8mL/min)

S. No	**CIN**		**PAN**	
	R_t(min)	**Peak area**	**R_t(min)**	**Peak area**
1	5.040	8592577	5.890	96167944
2	5.057	8533424	5.893	97467968
3	5.037	8516499	5.911	97162006
4	5.041	8448044	5.880	95721698
5	5.022	8575998	5.890	96478365
6	5.048	8442165	5.896	96747880
mean	8518118		96624310	
SD	62945		641938	
%RSD	0.74		0.66	

Table: 14 Robustness Data of CIN and PAN (1.2mL/min)

S. No	CIN		PAN	
	R_t(min)	Peak area	R_t(min)	Peak area
1	4.220	7822305	5.090	89688853
2	4.201	7738652	5.000	90397831
3	4.198	7806791	5.054	90650360
4	4.207	7744041	5.094	90106974
5	4.205	7861332	5.039	91325064
6	4.221	7876529	5.067	91038385
mean	7808275		90534578	
SD	57700		601480	
%RSD	0.74		0.66	

Effect of Variation of Wavelength

Standard solution prepared as per the test method was injected into the chromatographic system maintaining flow rates, less wavelength 280nm, more wavelength 282nm and actual wavelength 281nm. (Fig.: 35-37).

Table: 15 Robustness Data of CIN and PAN

S. No	Peak areas			
	280nm		282nm	
	CIN	PAN	CIN	PAN
1	8002877	92620009	7938349	91486225
2	8024688	91753925	7871160	91603127
3	7973557	91980341	7968111	92478206
4	7925458	92706273	8085965	93227923
5	8008349	92781379	7901974	92562832
6	7891102	93005443	7991125	91127487
Mean	7971005	92474562	7959447	92080967
SD	52455	492826	75668	799584
%RSD	0.66	0.53	0.95	0.87

Table 16 Statistical validation Data of Robustness

Operational parameter	Mean	SD	%RSD
Cinitapride			
Flow rate +0.2mL/min	7808275	57700	0.74
Flow rate -0.2mL/min	8518118	62945	0.74
Wavelength +1nm	7959447	75668	0.95
Wavelength -1nm	7971005	52455	0.66

Pantoprazole			
Flow rate +0.2mL/min	90534578	601480	0.66
Flow rate -0.2mL/min	96624310	641938	0.66
Wavelength +1nm	92080967	799584	0.87
Wavelength -1nm	92474562	492826	0.53

RUGGEDNESS

The ruggedness of the developed analytical method was determined by analyst variation (analyst 1 and analyst 2). The results were analysed statistically and the effect of variations were estimated.

The ruggedness of the analytical method was determined by analysis of the solutions prepared by two analyst (analyst 1 and analyst 2). Appropriate aliquots of the working stock solution was transferred to a 20mL volumetric flasks and the volume was made up to the mark with the solvent to obtain a solution of concentration 7.5 µg/mL of Cinitapride and 100 µg/mL of Pantoprazole. Six replicates of this solution was prepared by analyst 1 and analyst 2. A 20 µL of this solutions was analysed injected to the chromatographic system and the chromatograms were recorded. The results were statistically validated

Table: 17 Ruggedness Data of CIN PAN

S. No	Peak areas			
	Analyst-1		Analyst-2	
	CIN	PAN	CIN	PAN
1	8115541	92181733	8083356	91986004
2	8060085	93555226	7971160	92310031
3	7984218	92862802	7992111	92676406
4	7950042	91683225	8005965	93344454
5	8039999	92009411	7951487	91964742
6	7914530	92567000	7931125	92067487
Mean	8010736	92476566	7989201	92391521
SD	74724	671627	53433	538045
%RSD	0.93	0.73	0.67	0.58

Table :18 Statistical Validation Data of Ruggedness

Variation	Mean	SD	%RSD
	Cinitapride		
Analyst 1	8010736	74724	0.93
Analyst 2	7989201	53433	0.67
	Pantoprazole		
Analyst 1	92476566	671627	0.73
Analyst 2	92391521	538045	0.58

Method Validation

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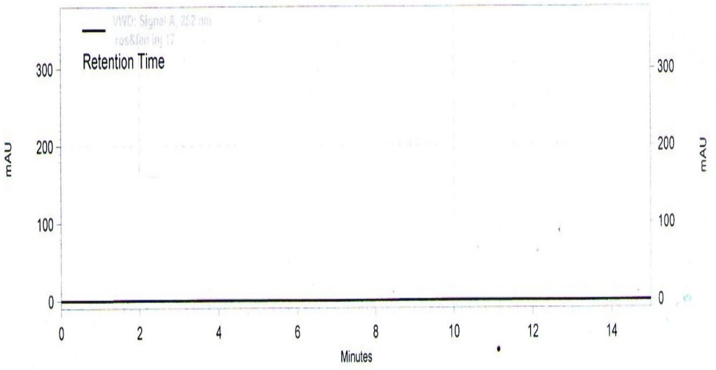

Fig.2

LINEARITY:

Level-1

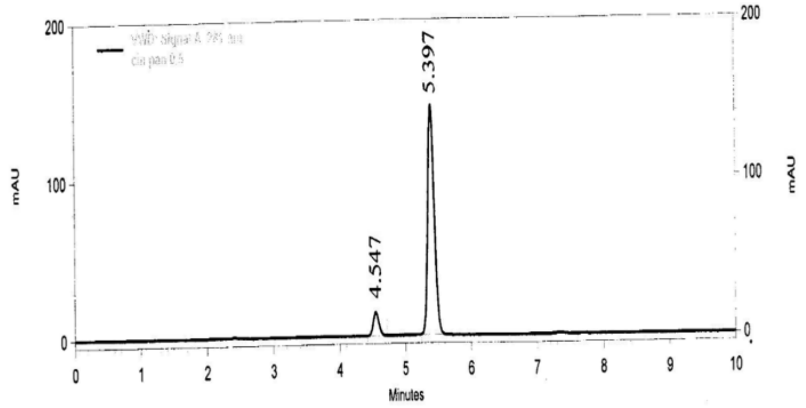

Fig.:3

Level-2

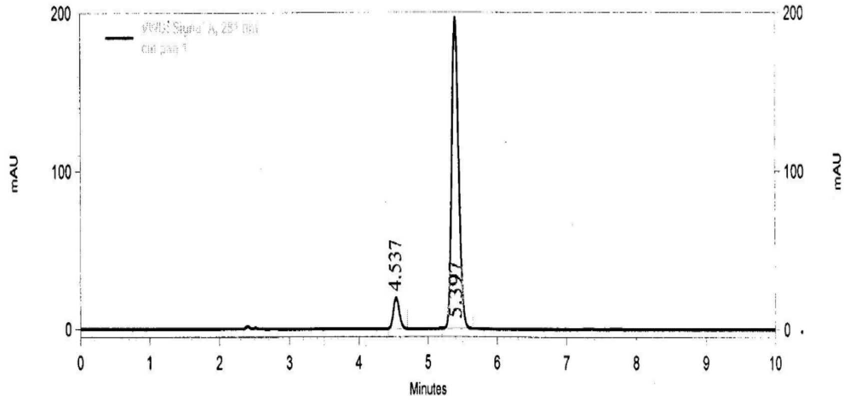

Fig.: 4

Level-3

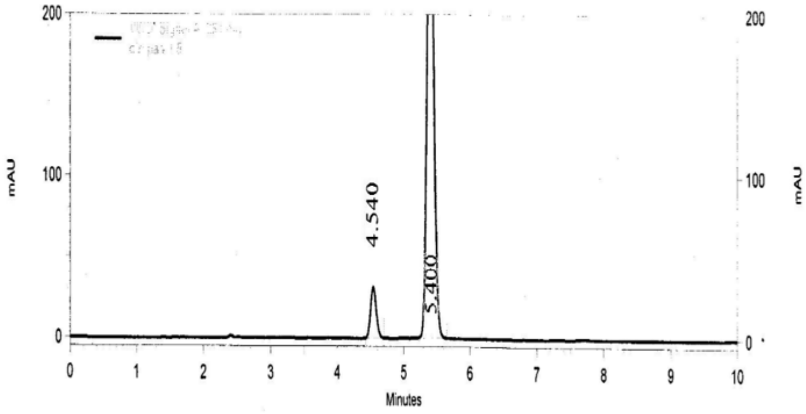

Fig.: 5

Level-4

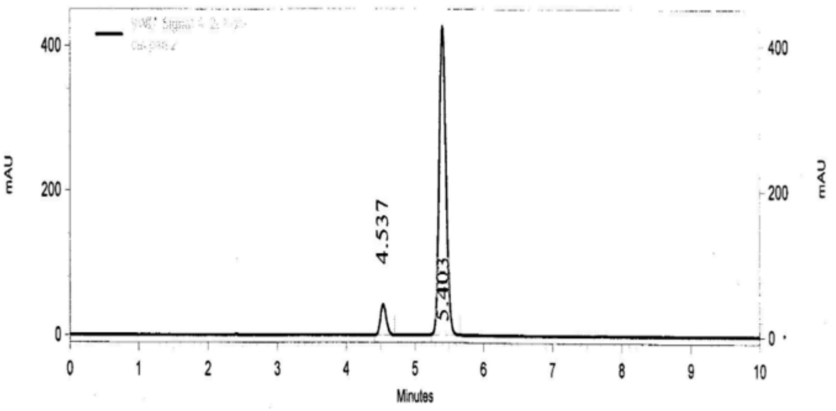

Fig.: 6

Level-5

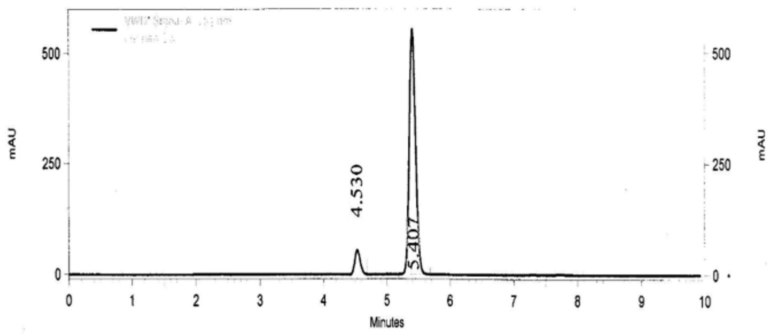

Fig.: 7

Level-6

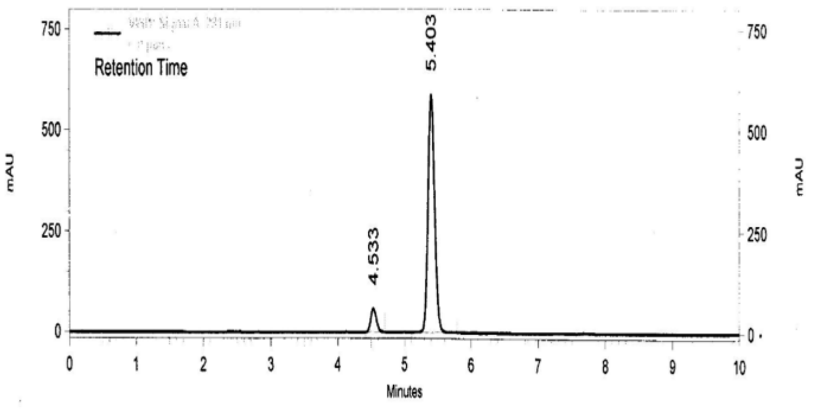

Fig.: 8

Level-7

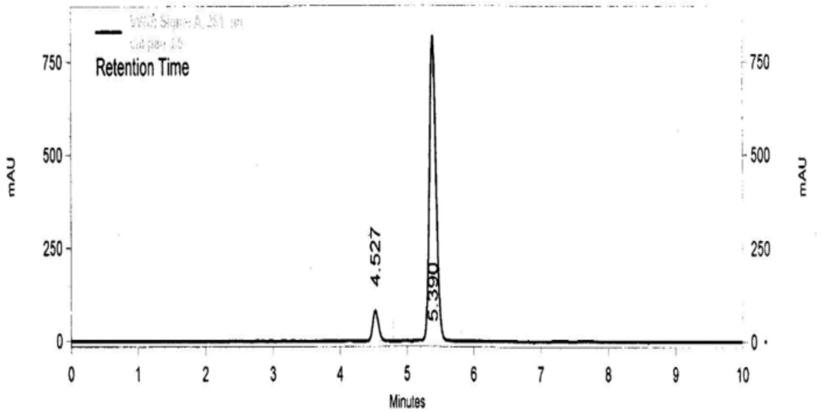

Fig.: 9

ACCURACY

Assay Chromatogram

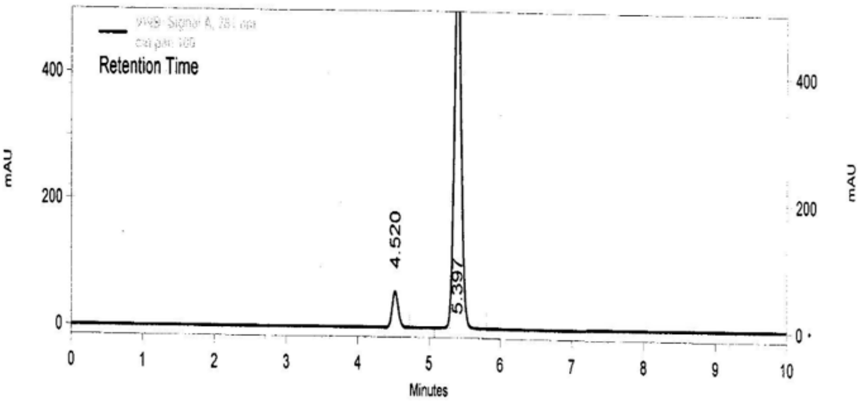

Fig.: 10

Recovery Level-1

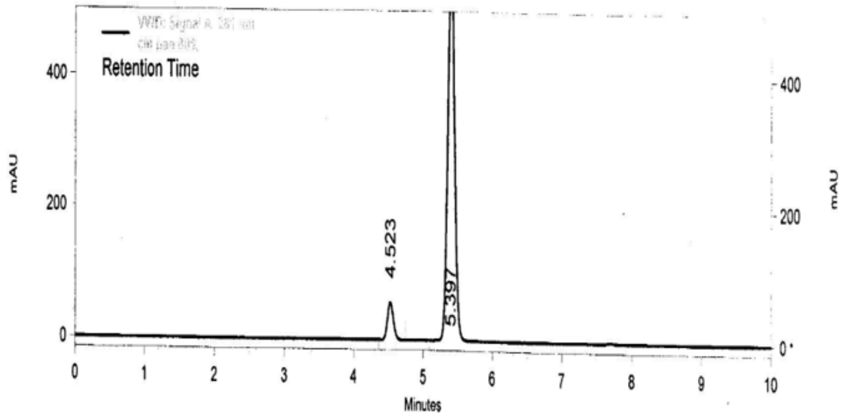

Fig.: 11

Recovery level-2

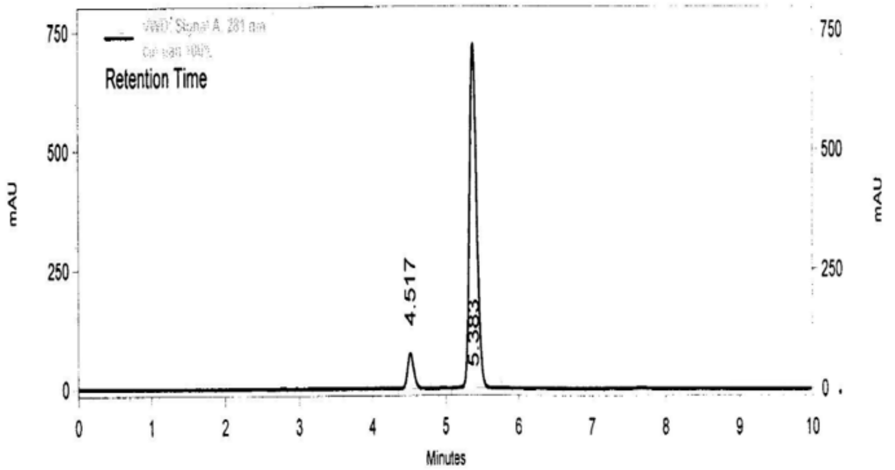

Fig.: 12

Recovery level-3

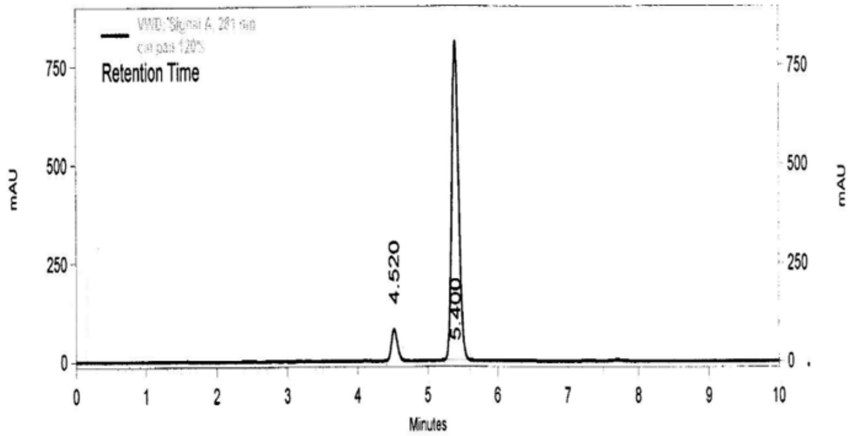

Fig.: 13

PRECISION:

Intraday Precision:

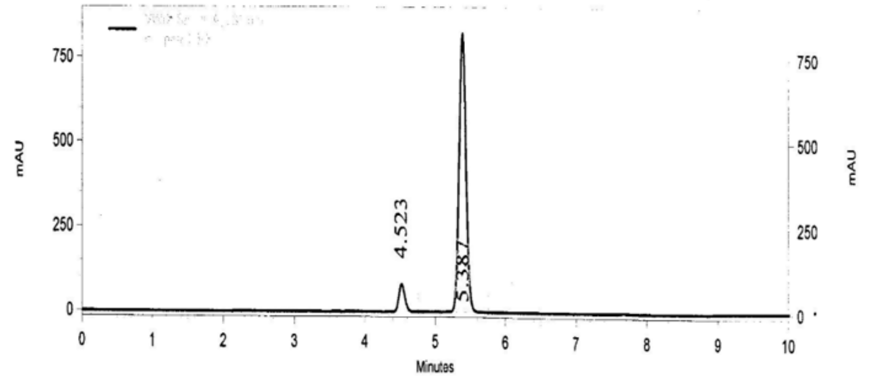

Fig.: 14

Interday Precision

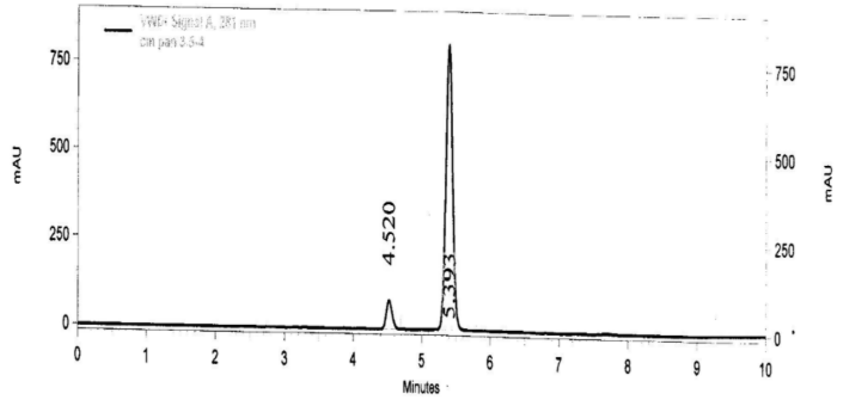

Fig.: 15

RUGGEDNESS

Analyst-1

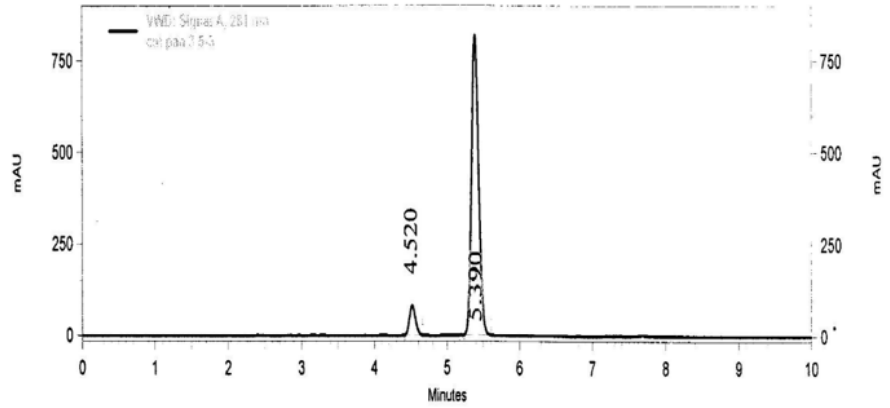

Fig.: 16

Analyst-2

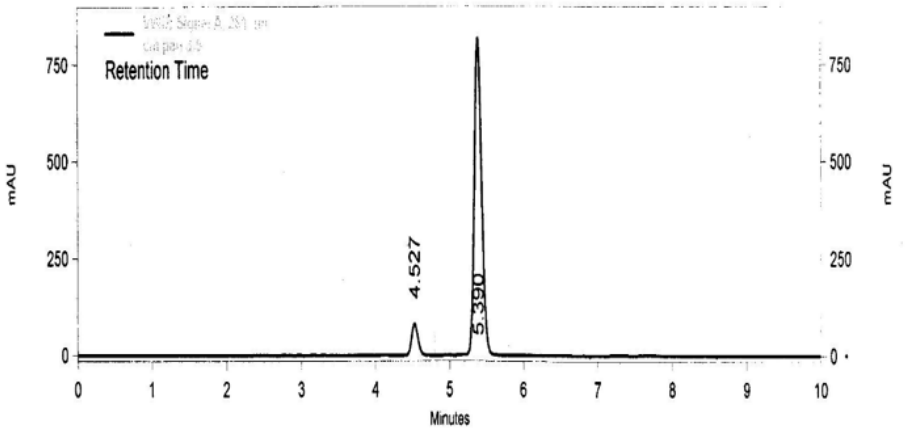

Fig.: 17

ROBUSTNESS

Wavelength at 280 nm:

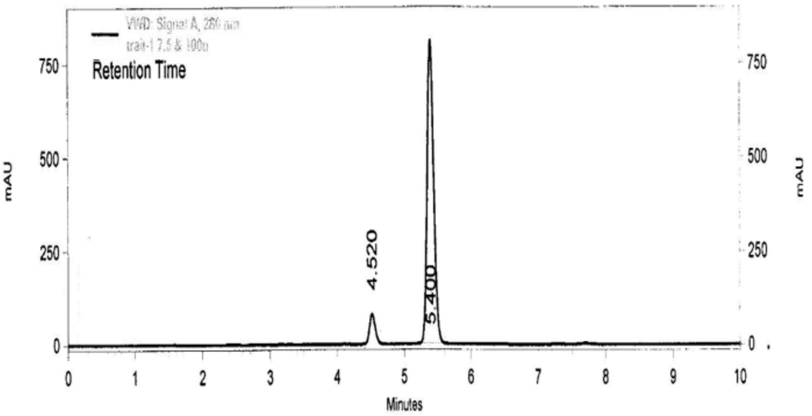

Fig.: 18

Wavelength at 282 nm:

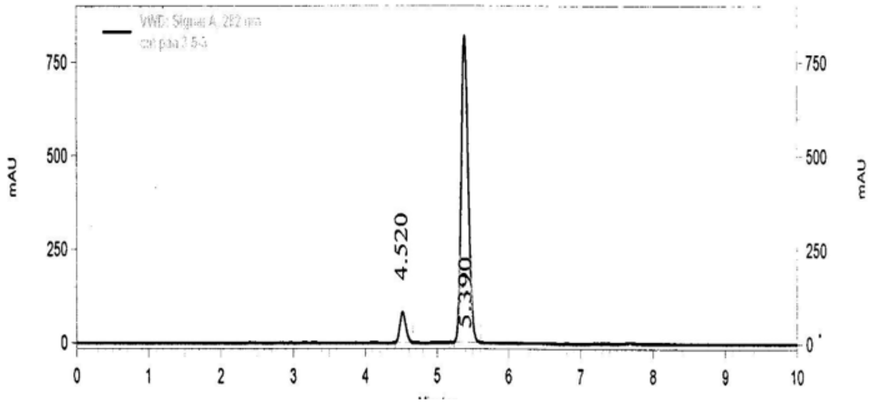

Fig.: 19

Flow rate at 0.8 mL/min:

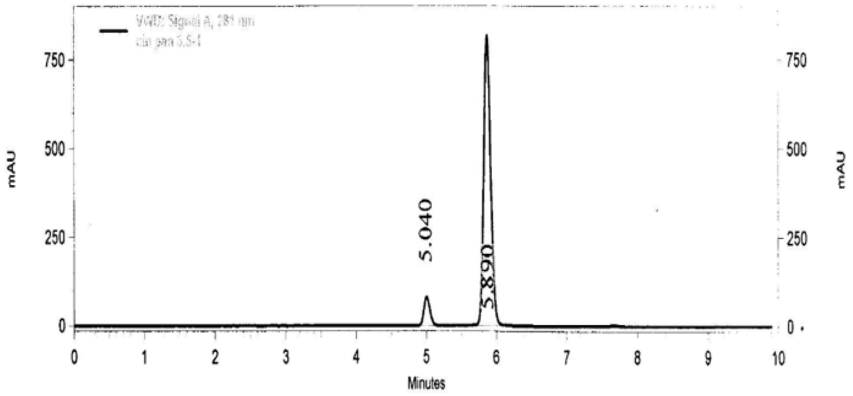

Fig.: 20

Flow rate at 1.2 mL/min:

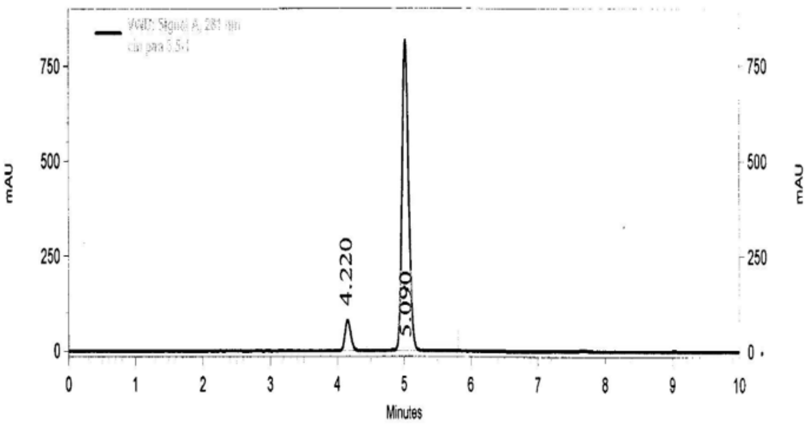

Fig.: 21

RESULTS AND DISCUSSION

The peak areas corresponding to the concentration range of Cinitapride 1.5 -10.5 µg/mL and Pantoprazole 20-140 µg/mL prepared in triplicate were plotted against the respective concentrations. The calibration curves were linear in the range studied for Cinitapride and Pantoprazole, respectively, with mean correlation coefficients (n = 3) of 0.999 and higher, the representative calibration curve was shown in fig. 10. The regression analysis was given in the tables 3 and 4. The chromatogram of linearity was given in figure 11-17.

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product. The recovery of the added standard to the drug product sample was calculated and it was found to be 100.64 %w/w and 100.29%w/w for Cinitapride and Pantoprazole respectively and the % RSD was less than 2 for both the drugs which indicates a good accuracy of the method. Chromatograms depicting the three recovery levels were given in figures 18-21. The results of recovery were given in the table 6-8.

The method was precise with a %RSD of less than 2 for both Cinitapride and Pantoprazole respectively. The results of intraday and inter day precision were given in the table 9, 10, 12 and 13. The statistical validation data is given in the tables 11 and 14. Chromatograms of precision were given in the figure 22, 23. Limit of detection of Cinitapride and Pantoprazole were 0.34 µg/mL and 3.38µg/mL respectively. Limit of quantification of Cinitapride and Pantoprazole were 1.04 µg/mL and 10.15 µg/mL respectively. LOD and LOQ values were given in the table 4.

Specificity of the chromatographic method was tested by injecting sample concentration prepared from marketed formulation. The response was compared with that obtained from the standard drug. The chromatogram confirms the presence of Cinitapride and Pantoprazole at 4.5min and 5.4min respectively without any interference. Thus the developed method was specific to Cinitapride and Pantoprazole. An optimised chromatogram with the retention times of Cinitapride and pantoprazole was given in the figure 18.

Ruggedness was carried out by change in the analyst (1 and 2), and instrument (Agilent). Solution of 100% concentration was prepared and injected in triplicate for each varied operational condition and % R.S.D was found to be less than 2. The Results were given in the table 19 & 20. The chromatograms were shown in the figure 24, 25.

Robustness was carried out by change in the flow rate (± 0.2mL/min), and variation in wavelength (± 1 nm). Solution of 100% concentration was prepared and injected in six for each varied operational condition and % R.S.D was found to be less than 2. The Results were given in the table 16-18. The chromatograms were shown in the figure 26-29.

Assay of marketed formulation; A 20 µL injection volume of test concentration containing 7.5 µg/mL Cinitapride and 100µg/mL Pantoprazole solution was injected in triplicate to the chromatographic system and the peak response was measured. The content of each component in the formulation was estimated by comparing the peak area of the test sample with that of the peak area of the standard. The results of estimation were given in the table 5. The chromatogram was given in figure 18.

Table -19 Analytical method validation report for Cinitapride and Pantoprazole

Parameter	**Results** **Cinitapride**	**Pantoprazole**
wavelength (nm)	281	
Rt (min)	4.5	5.4
Regression equation	y=754116.2x+45173.75	y=670332x-1839124
Correlation coefficient	0.9996	0.9992
Accuracy	100.64 %	100.29 %
LOD (µg/ml)	0.14	0.99
LOQ (µg/ml)	0.43	2.98
Assay	100.2 %	99.4 %
	Precision (%RSD)	
Intraday precision	0.58	0.78
Inter day precision	0.65	0.53
	Robustness (%RSD)	
Flow rate 1.2mL/min	0.74	0.66
Flow rate 0.8mL/min	0.74	0.66
wavelength 280nm	0.66	0.53
wavelength 282nm	0.95	0.87

Ruggedness (%RSD)		
Analyst 1	0.93	0.73
Analyst 2	0.67	0.58

CONCLUSION

A RP-HPLCmethod for Cinitapride and Pantoprazole were developed and validated in capsule dosage form as per ICH guidelines. The results are found to be complying with the acceptance criteria for each of the parameter.

Agilent HPLC (Open Lab software with DAD detector) with Zorbax ODS C_{18} (250X 4.6mm, 5µ) Packed Column, Injection volume of 20µL was injected and eluted with the Mobile phase (Acetonitrile : Phosphate buffer pH 6.8, in the ratio of 50:50%v/v) Which was pumped at a flow rate of 1.0 mL at 281nm. The peak of Cinitapride and Pantoprazole was found well separated at 4.5 min, 5.4 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, linearity, accuracy, precision, specificity, and limit of detection, limit of quantitation, ruggedness, and robustness.

Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity and hence it is suitable for routine analysis as well as for stability analysis.

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