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Research Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND CINITAPRIDE IN TABLETS

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Abstract: The present work describes a simple reverse phase HPLC method for the determination of omeprazole and cinitapride from tablet formulations. The determination was carried out on a Thermo Hypersil, BDS, C-18 (250x4.6 mm, 5 micron) column using a mobile phase of 0.1 N phosphate buffer(pH 3.2): Acetonitrile (60:40). The flow rate was 1 ml/min and the runtime was 7 min. The Column temperature was 35°C and the eluent was monitored at 230 nm. The retention times of Omeprazole and Cinitapride are 3.5minutes and 5.3minutes respectively. The method was reproducible, with good resolution between Omeprazole and Cinitapride. The detector response was found to be linear in the concentration range of 50-150 µg/ml for Omeprazole and Cinitapride. The developed method was validated for specificity, system suitability, precision, linearity, accuracy, Limit of Detection, Limit of Quantification, robustness, and Stability. Recovery of Omeprazole and Cinitapride in formulations was found to be in the range of 99%, 100%, and 101% respectively. And the correlation coefficient was 0.999. Hence, it was concluded that the developed method is suitable for routine analysis of these combination due to its less analysis time.

Key**words:** Omeprazole, Cinitapride, Validation, RP-HPLC.

Introduction:

Omeprazole belongs to a group of drugs called proton pump inhibitors. It decreases the amount of acid produced in the stomach. Omeprazole is used to treat symptoms of gastro esophageal reflux disease (GERD) and other conditions caused by excess stomach acid. It is also used to promote healing of erosive esophagitis (damage to your esophagus caused by stomach acid).Omeprazole may also be given together with antibiotics to treat gastric ulcer caused by infection with helicobacter pylori (H. pylori).Omeprazole is not for immediate relief of heartburn symptoms. IUPAC name of Omeprazole is (RS)-5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl) methylsulfinyl)-1H-benzo[d] imidazole. Molecular weight of Omeprazole is $C_{17}H_{19}N_3O_3S$, molecular mass is 345.4 g/mol.

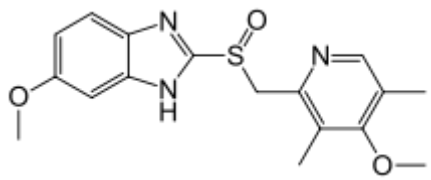

Fig-Structure of Omeprazole

Cinitapride is a new prokinetic agent. It is a substituted benzamide with 5-HT receptor antagonist and 5-HT- receptor agonist activity. It acts as an agonist of the 5-HT1 and 5-HT4 receptors and as an antagonist of the 5-HT2 receptors. IUPAC name of cinitapride is (RS)-4-amino-N-[1-(1-cyclohex-3-enylmethyl)-4-piperidyl]-2-ethoxy-5-nitro-benzamide. Molecular formula of cinitapride is $C_{21}H_{30}N_4O_4$, molecular mass is 402.49 g/mol.

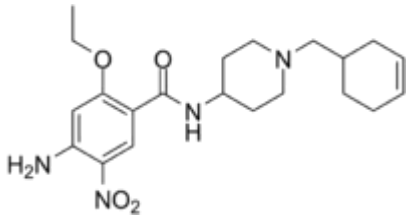

Fig- Structure of cinitapride

Literature survey reveals spectofluorimetric and HPLC methods for determination of cinitapride in pharmaceutical dosage forms as well as in biological fluids. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of omeprazole and cinitapride in their combined dosage forms. Literature survey does not reveal any simple RP-HPLC or other method for simultaneous estimation of omeprazole and cinitapride in combined dosage forms. The present communication describes simple, sensitive, accurate and precise RPHPLC method for simultaneous estimation of both drugs in their combined tablet dosage forms.[1-7]

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 thermo Hypersil BDS column (250mmx4.6mm, particle size 5µm).

Chemicals and Reagents

Omeprazole and cinitapride was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Acetonitrile of

HPLC grade were purchased from E.Merck (India) Ltd., Mumbai. orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd, Potassium dihydrogen ortho phosphate was analytical reagent grade supplied by Fischer Scientific Chemicals and Potassium phosphate dibasic was supplied by Rankem. HPLC grade water was obtained from a Milli-QRO water purification system. Methanol was supplied by Rankem.

HPLC conditions

The mobile phase consisting of Phosphate buffer pH adjusted with Ortho phosphoric acid (pH 3.2) and Acetonitrile (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60: 40v/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 7min. 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.

Preparation of standard solution:

Omeprazole:

Accurately weighed quantity, 20 mg of omeprazole was transferred into 50ml of volumetric flask and added 30ml of water and sonicate for 5 mins make up the volume with water. Transfer 5 ml into 50ml volumetric flask and dilute up to the mark with water.

Cinitapride:

Accurately weighed quantity, 20mg of cinitapride was transferred into 50ml of volumetric flask and added 30ml of water and sonicate for 5mins make up the volume with water. Transfer 0.8ml into 50ml volumetric flask and dilute up to the mark with water.

Preparation of sample preparation:

Accurately weighed 8 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder weigh about 194mg of sample into 50ml of volumetric flask add 15ml of water and sonicate for 30mins and filter through the 0.45µm filter paper and make up the volume with water. Transfer above solution 5ml into 50ml volumetric flask and make up the volume

The method was validated as per ICH guidelines:

System suitability studies and Specificity:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method**.** The specificity was established by preparing omeprazole and cinitapride standard and test solutions and injected 6 times into HPLC system as per the test procedure.

Table1: System suitability parameters:

Parameters	Omeprazole	Cinitapride
Correlation coefficient	0.999	0.999
Regression equation	y = 6479.x - 5029	y = 6797.x - 4989
LOD	0.0006	0.0019
LOQ	0.0001	0.0004
Theoretical plates	16612	16544
Tailing	1.128	1.039

Precision and Accuracy:

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions (fig-1&2) were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated (Table-2). From the data obtained, the developed HPLC method was found to be precise.

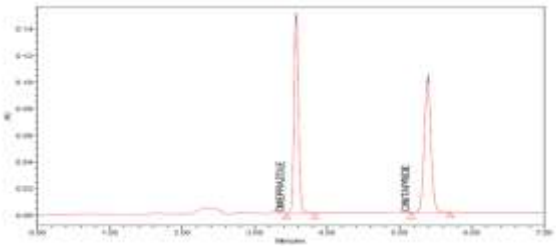

Fig1: standard chromatogram:

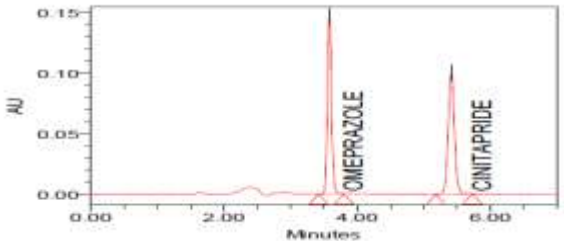

Fig2: chromatogram for formulation:

Table2: Precision for omeprazole and cinitapride:

S NO	Sample weight	Area(ome)	Area(cini)	%Assay(ome)	Assay(cini)
1	198	633201	665444	97	103
2	195	627885	655780	98	103
3	198	627994	658555	97	102
4	199	630534	672508	97	103
5	198	631344	659739	97	102
6	196	628901	656121	98	102

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out

six times and the percentage recovery was calculated .From the data obtained, added recoveries of standard drugs were found to be accurate (Table-3&4).

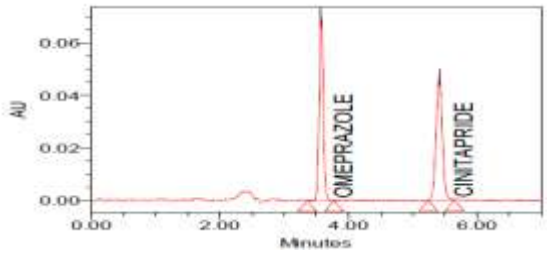

Fig 3: Accuracy chromatograms-50% of omeprazole and cinitapride:

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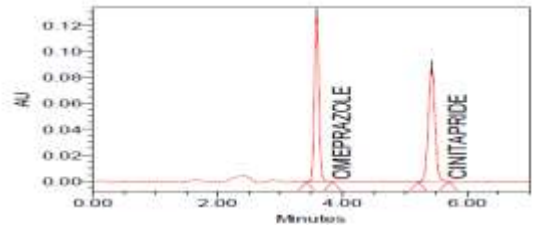


Fig4: Accuracy chromatograms-100% of omeprazole and cinitapride

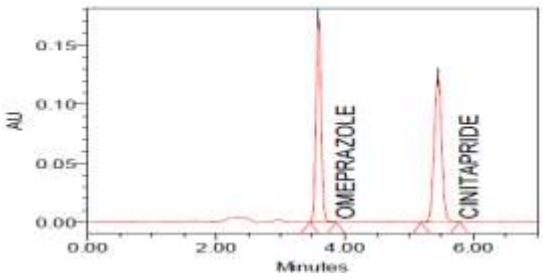


Fig5: Accuracy chromatograms-150% of omeprazole and cinitapride:

Linearity:

The linearity of the method was determined at five concentration levels ranging from 50 to 150 g/ml for omeprazole and 50 to 150µg/ml for cinitapride. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 6479.x - 5029 (R2 = 0.999) for omeprazole and y = 6797.x - 4989 (R2= 0.999) for cinitapride. Then results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above (Fig-6&7).

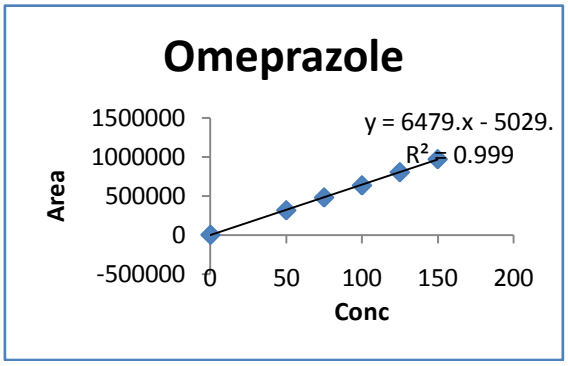


Fig: 6 Linearity curve for omeprazole:

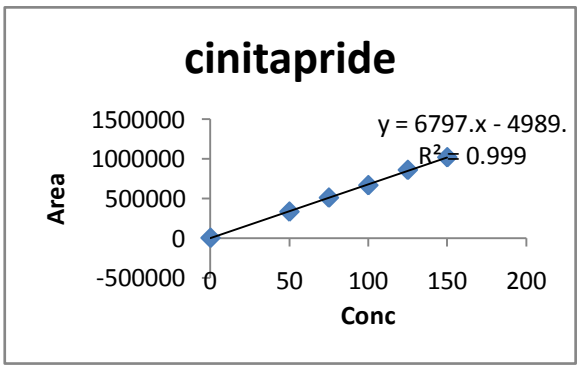


Fig7 Linearity curve for cinitapride:

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 omeprazole and cinitapride was found to be 0.0006µg/ml and 0.0019µg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal-to-noise ratio of 10). The LOQ was 0.0001µg/ml and0.0004µg/ml for omeprazole and cinitapride respectively.

Robustness:

Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed is robust (Table-5&6).

Table3: Accuracy for omeprazole:

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	%recovery	mean
50%	98	311642	19.600	19.56	100	100
50%	99	314729	19.800	19.76	100	
50%	100	312773	20.000	19.64	98	
50%	99	311547	19.800	19.56	99	
50%	99	312768	19.800	19.64	99	
50%	98	316107	19.600	19.85	101	
100%	198	628788	39.600	39.49	100	100
100%	199	631760	39.800	39.67	100	

100%	198	631032	39.600	39.63	100	
150%	302	949475	60.400	59.63	99	99
150%	303	954319	60.600	59.93	99	
150%	304	952741	60.800	59.83	98	
150%	303	955904	60.600	60.03	99	
150%	305	956344	61.000	60.06	98	
150%	305	958777	61.000	60.21	99	

Table4 Accuracy for cinitapride:

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	%recovery	mean
50%	98	324145	3.092	3.13	101	100
50%	99	325795	3.123	3.14	101	
50%	100	326308	3.155	3.15	100	
50%	99	319077	3.123	3.08	99	
50%	99	322200	3.123	3.11	99	
50%	98	322400	3.092	3.11	101	
100%	198	657463	6.246	6.34	101	102
100%	199	658878	6.278	6.35	101	
100%	198	664541	6.246	6.41	103	
150%	302	1014686	9.527	9.78	103	102
150%	303	1011284	9.559	9.75	102	
150%	304	1005969	9.590	9.70	101	
150%	303	1012275	9.559	9.76	102	
150%	305	1001186	9.622	9.65	100	
150%	305	1007018	9.622	9.71	101	

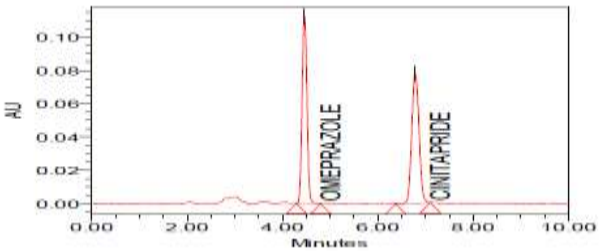


Fig 8: Flow-1

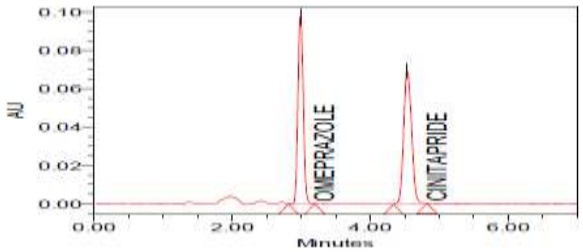


Fig 9: Flow-2

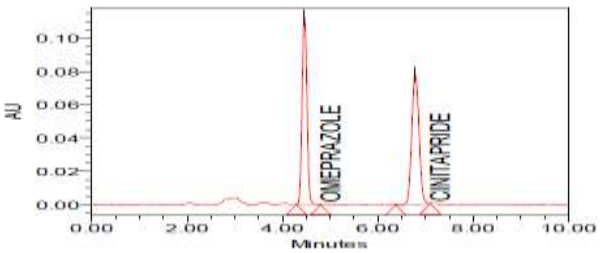


Fig 10:Temparature-1

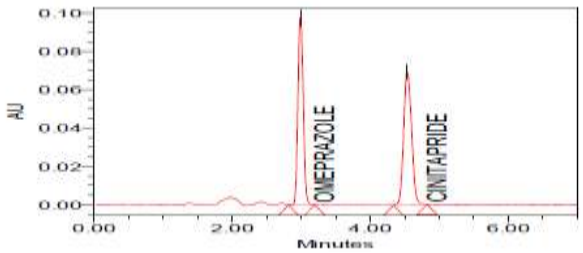


Fig 11:Temparature-2

Table 5: Robustness for omeprazole:

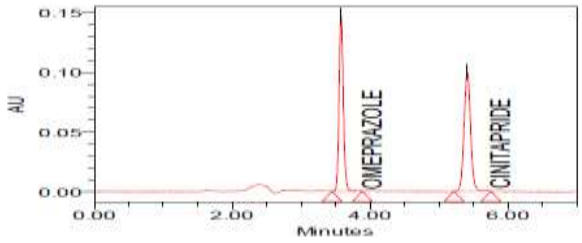
S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Omeprazole	4.464	807731	1.052	9071
2	Flow2	1.2ml/min	Omeprazole	2.987	533311	1.109	7119
3	Temp1	30°C	Omeprazole	4.280	644671	1.071	8098
4	Temp2	40°C	Omeprazole	3.261	648303	1.050	8703

Table 6: Robustness for cinitapride:

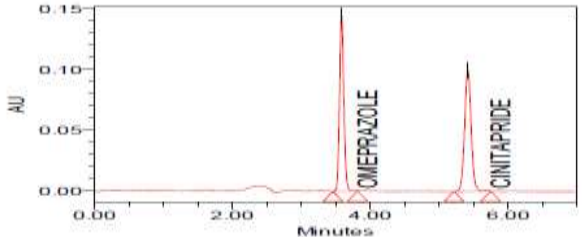
S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Cinitapride	6.307	2118339	1.073	9359
2	Flow2	1.2ml/min	Cinitapride	4.862	1640267	1.110	7084
3	Temp1	30°C	Cinitapride	6.423	1852235	1.081	8369
4	Temp2	40°C	Cinitapride	4.243	1856788	1.071	8408

Forced Degradation studies:

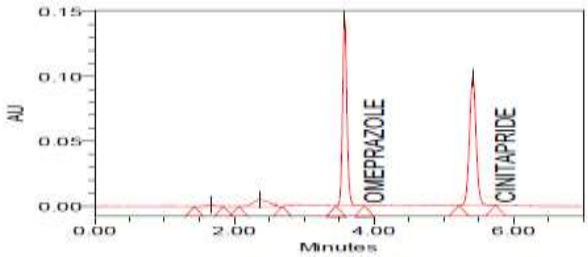
The stability studies were determined by applying the physical stress (acid, base, peroxide, water and light) to the product. It was observed that there were marked degradation in the chromatograms, and the data given in table-7&8).



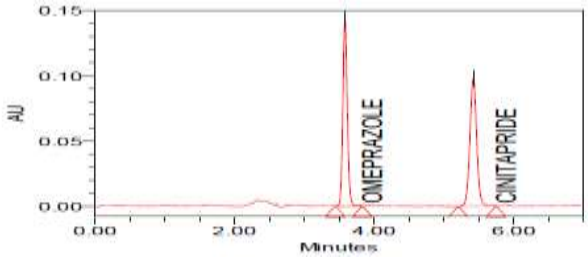
Acid degradation chromatogram:



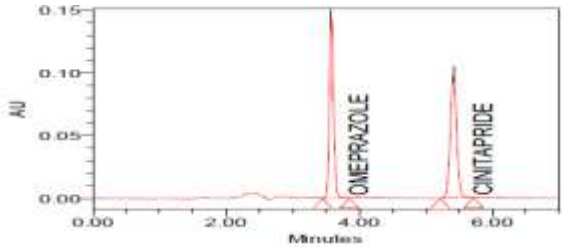
Base degradation chromatogram:



Water degradation chromatogram:



Light degradation chromatogram:



Peroxide degradation chromatogram:

Table 7: Stability studies for Omeprazole:

	Sample weight	Area	%Assay	%deg
Acid	198	606252	93	-4
Base	195	588091	92	-5
Peroxide	198	493068	76	-21
water	199	581344	89	-8
light	199	610349	94	-3

Table 8: Stability studies for Cinitapride:

	Sample weight	Area	%Assay	%deg
Acid	198	568675	90	-12
Base	195	543583	87	-15
Peroxide	198	431614	68	-34
water	199	519765	81	-21
light	199	599044	94	-8

Results and discussion:

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1, so we can say system is suitable for analysis. The method specificity was concluded by fig:1 and fig:2, those figures are omeprazole and cinitapride standard chromatogram and other one is formulation chromatogram There was no Blank, placebo and excipients peaks interference with both analyte peaks. So it was proven that the method is selective. The method accuracy was evaluated by recovery studies. Omeprazole and Cinitapride recovery was founded 98-102% as per ICH 97%- 103% and also percentage RSD was very low. So this method is accurate (table 3&4). Linearity calibration curve was given below fig: 6&7 and plot the graph with three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co efficient. Linear correlation was found to be y = 6479.x - 5029(R^2=0.999) for omeprazole and y = 6797.x - 4989. (R^2=0.999) for cinitapride (fig 3&4). Precision results were shown by table 2. Method robustness results were given by table 5&6. Results for stability studies are shown in table7&8.

Conclusion:

Thus the proposed RP-HPLC method for the simultaneous estimation of omeprazole and cinitapride in combined dosage forms is accurate, precise, linear, robust, simple and rapid. Hence the present RP-HPLC method is

suitable for the quality control of the raw materials, formulations and dissolution studies.

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