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Research Article

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

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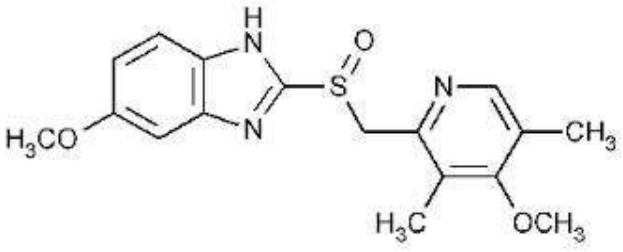
Abstract: The present work describes a simple reverse phase HPLC method for the determination of Omeprazole and Cinitapride from capsules formulations. The determination was carried out on a Thermo Hypersil, BDS, C-18 (250x4.6 mm, 5 micron) column using a mobile phase of 0.1 N phosphate buffer(pH 3.2): Acetonitrile (60:40). The flow rate was 1 ml/min and the runtime was 7min. 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 and robustness. 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, ICH Guidelines

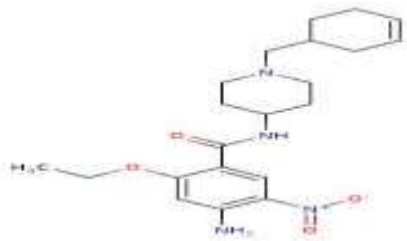
INTRODUCTION

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 C17H19N3O3S , molecular mass is 345.4 g/mol.



Omeprazole structure

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-nitrobenzamide.Molecular formula of cinitapride is C21H30N4O4,molecular mass is 402.49 g/mol.



Cinitapride structure

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 capsuls dosage forms.

MATERIAL AND METHODS

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 was purchased from E. Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

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 5ml into 50ml volumetric flask and dilute up to the mark with water.

Preparation of sample Solution:

Accurately weighed equivalent to 8 capsules and calculated average weight and crushed. Transfer the 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

Table1: System Suitability Parameters

Parameters	Omeprazole	Cinitapride
Correlation Coefficient	0.999	0.999
Regression Equation	y = 6479x – 5029	y = 6797x – 4989
LOD	0.571	1.904
LOQ	0.8396	2.7986
Theoretical plates	16612	16544
Tailing	1.128	1.039

SPECIFICITY:

The specificity was established by preparing a Omeprazole and Cinitapride standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test

Fig. 1: Standard chromatogram for omeprazole and cinitapride

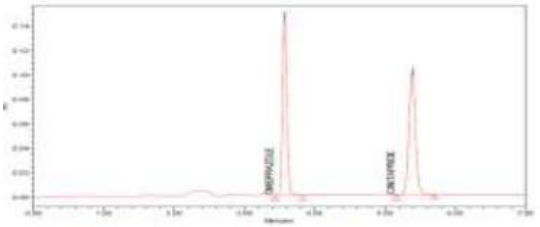


Fig1: standard chromatogram:

Fig. 2: Formulation chromatogram for Omeprazole and Cinitapride

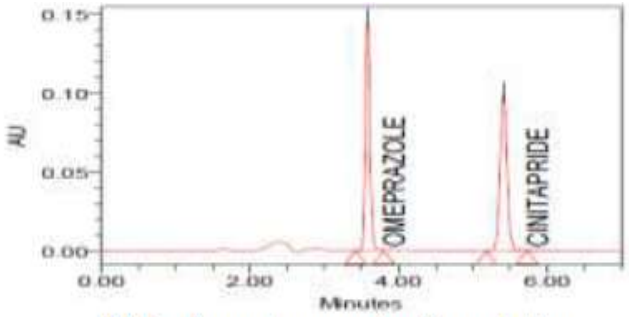


Fig2: chromatogram for formulation:

The method was validated as per ICH guidelines:

SYSTEM SUITABILITY STUDIES:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table1). 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.

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.

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).

Table 2: Precision Studies

S. No.	Sample weight	Area (ome)	Area (cini)	%Assay (ome)	Assay (cini)
1	198	633201	665444	97	98
2	195	627885	655780	98	98
3	194	627994	658555	98	99
4	194	630534	672508	99	101
5	195	631344	659739	98	99
6	196	628901	656121	97	98

Fig. 3: Accuracy Chromatograms-50% of omeprazole and cinitapride

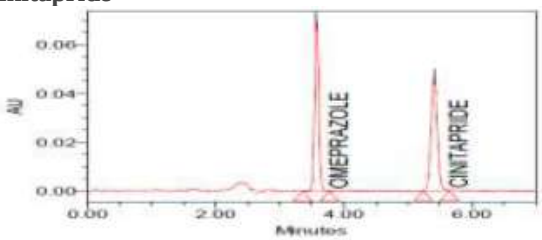


Fig 4: Accuracy Chromatograms-100% of omeprazole and cinitapride

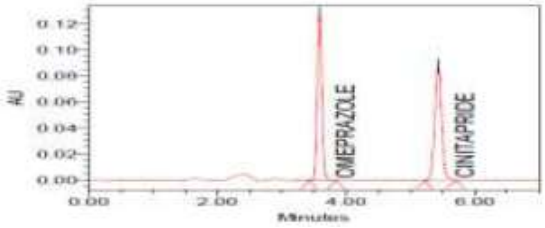


Fig. 5: Accuracy Chromatograms-150% of omeprazole and cinitapride

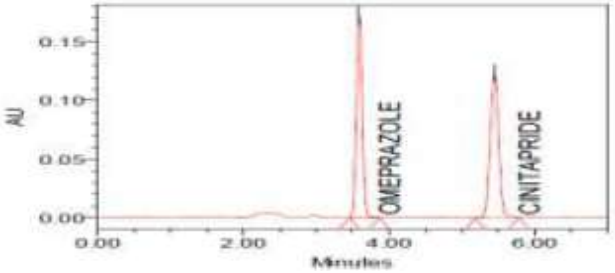


Table 3: Accuracy for Omeprazole

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	98	312768	19.600	19.59	100	100
50%	98	311547	19.600	19.51	100	
50%	97	312773	19.400	19.59	101	
50%	99	314729	19.800	19.71	100	
50%	98	311462	19.600	19.50	100	
50%	98	316107	19.600	19.79	101	
100%	199	631760	39.800	39.56	99	100
100%	198	628788	39.600	39.37	99	
100%	198	631032	39.600	39.51	100	
150%	302	949475	60.400	59.45	98	98
150%	303	954319	60.600	59.76	99	
150%	304	952741	60.800	59.66	98	
150%	303	955904	60.600	59.86	99	
150%	305	956344	61.000	59.88	98	
150%	305	958777	61.000	60.04	98	

Table 4: Accuracy for Cinitapride

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	98	322400	19.802	19.49	98	99
50%	98	322200	20.004	19.48	98	
50%	97	319077	20.206	19.29	98	
50%	99	326308	20.004	19.73	99	
50%	98	325795	19.802	19.70	99	
50%	98	324145	19.802	19.60	99	
100%	199	658878	40.210	39.83	99	100
100%	198	664541	40.008	40.17	100	
100%	198	657463	40.008	39.75	99	
150%	302	1007018	61.023	60.88	100	99
150%	303	1001186	61.225	60.53	99	
150%	304	1012275	61.427	61.20	100	
150%	303	1005969	61.225	60.82	99	
150%	305	1011284	61.629	61.14	99	
150%	305	1014686	61.629	61.34	100	

LINEARITY AND RANGE

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x - 26439 (R^2=0.99) for Omeprazole and y = 19288x - 32595 (R^2=0.99) for Cinitapride. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The results for calibration curves are given in Fig 6&7.

Fig. 6: Linearity Curve for Omeprazole

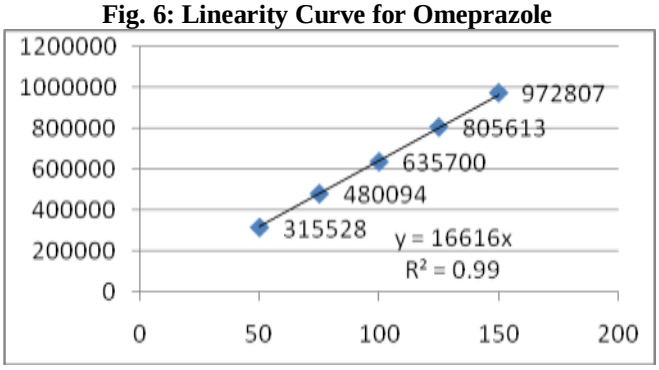
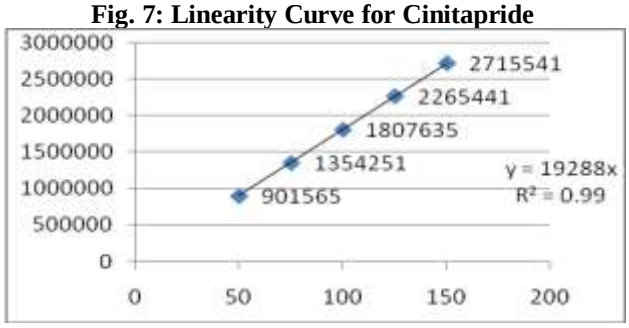


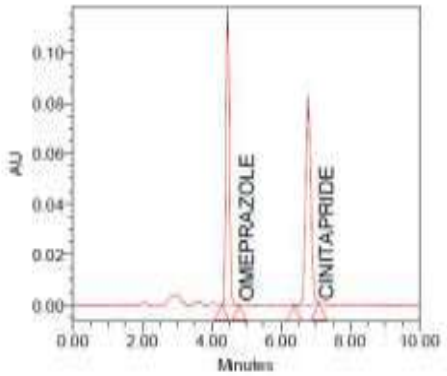
Fig. 7: Linearity Curve for Cinitapride



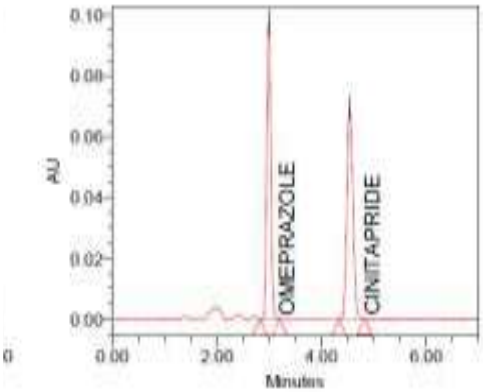
ROBUSTNESS

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust (Table-5&6).

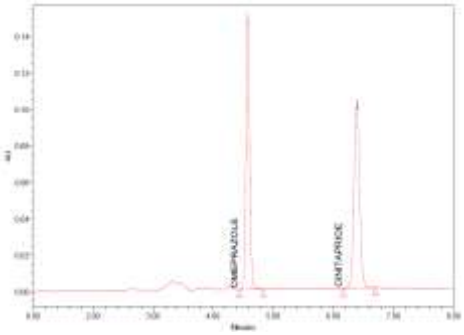
Flow-1(Low)



Flow-2(High)



Tem-1(Low)



Tem-2(High)

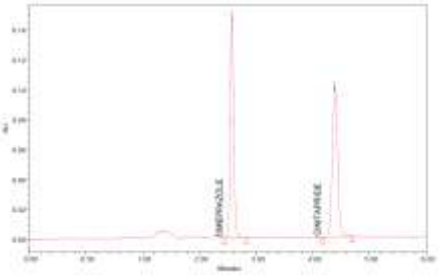


Table 5: Robustness for Omeprazole

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Omeprazole	4.464	807731	1.05	9071
2	Flow2	1.2ml/min	Omeprazole	2.987	533311	1.10	7119
3	Temp1	30°C	Omeprazole	4.624	641284	1.11	17342
4	Temp2	40°C	Omeprazole	2.512	620594	1.02	14122

Table 6: Robustness for Cinitapride

S No	Sample name	change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Cinitapride	6.307	834576	1.073	9359
2	Flow2	1.2ml/min	Cinitapride	4.862	558146	1.110	7084
3	Temp1	30°C	Cinitapride	6.412	662305	1.023	18231
4	Temp2	40°C	Cinitapride	4.312	631284	1.031	16125

RESUTS AND DISCUSSONS:

System suitability results given in table1 and the system suitability parameters are within the limits, so we can say system is suitable for analysis. The method specificity was concluded by fig:1 and fig:2, Omeprazole and Cinitapride standard and formulation chromatograms. There was no interference observed with Blank, placebo and excipients peaks at the RTs of both analyte peaks. So it was proved 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 ploted 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 = 16616x - 26439 (R2=0.999) for Omeprazole and y = 19288x - 32595 (R2=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 table 7&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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