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Research Article

A VALIDATED RP HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND CINITAPRIDE IN COMBINED DOSAGE FORMS

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ABSTRACT

An accurate, Precise, Simple and Economical High Performance Liquid Chromatographic method for the estimation of Omeprazole and Cinitapride was developed and validated. The determination was performed by the using of two phases one is stationary phase it's a Thermo BDS Hypersil C18 column having 250 x 4.6mm 5µ, and another one is mobile phase containing 0.1N phosphate buffer and Acetonitrile at the ratio 50:50%v/v. The flow rate was 1ml/min and effluents were monitored at 282nm. The retention time of Omeprazole and Cinitapride was 3.5 and 5.4 min respectively. The developed method was validated for specificity, system suitability, precision, linearity, accuracy, Limit of Detection, Limit of Quantification, robustness, and ruggedness. 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.

Keywords: Cinitapride, Omeprazole, RP HPLC, method development and validation.

INTRODUCTION

Omeprazole (OME) is 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulphinyl]-1*H*-benzimidazole (Figure.1), a substituted benzimidazole compound and prototype anti-secretary agent, it is a proton pump inhibitor, used for the prophylaxis and treatment of gastro duodenal ulcers and for the treatment of symptomatic gastro-esophageal reflux disease[1] Cinitapride (CNT), 4-amino-N-[1-(cyclohex-3-en-1-ylmethyl)piperidin-4-yl]-2-ethoxy-5-Nitrobenzamide (Figure. 2), it is a substituted benzamide gastro enteric prokinetic agent, it shows synergistic effects on serotonergic 5-HT2 (inhibition) and5-HT4 (stimulation) receptor and inhibits the dopaminergicD2 receptors in the neuronal synapses of the myenteric plexus, astimulating gastrointestinal moiety agent and commercially successful anti-ulcerativedrugsubstance. Combination of these two drugs are essential constituent of Gastro esophageal reflux disease (GERD). Omeprazole is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United State Pharmacopoeia (USP). The IP, BP and USP describe HPLC method for estimation of Omeprazole. Literature survey reveals that there is no of spectro photometric[4], derivative UV Spectroscopy[5], spectrofluorimetric[6], voltammetric[7] LC-MS and HPLC methods for determination of individual Omeprazole in pharmaceutical dosage forms as

well as in biological fluids and determination of Cinitapride hydrogen tartarate individually in pharmaceutical dosage forms. Cinitapride is not official in any pharmacopeia. The combination of these two drugs is not official in any pharmacopoeia so no official method is available for the simultaneous estimation of CNT and OME in their combined dosage forms. Literature survey reveals that there is no simple RP-HPLC method available for estimation of OME and CNT in combined dosage form. So, there is a need for the development of RP-HPLC method, which will be used for simultaneous determination of OME and CNT in combined dosage form[2,3].

MATERIALS AND METHODS

Apparatus

Waters e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software.

Materials

Acetonitrile was HPLC grade and collected from E. Merck, Darmstadt, Germany. Potassium di hydrogen ortho phosphate was analytical reagent grade supplied by Fischer Scientific Chemicals and Potassium phosphate di basic was supplied by Rankem. HPLC grade Water was obtained from a Milli-QRO water purification system. Methonal was supplied by Rankem.

Commercial Formulation

OME and CNT in combined capsules are available in the market as BURPEX. Those are Fixed-dose combinations of 20mg and 3mg. The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Buffer Preparation

Weighed 0.2625g of Potassium phosphate dibasic and 1.3625g of Potassium di hydrogen orthophosphate and dissolved in 500 ml of water and filtered through 0.45 µ filter and degassed.

Preparation of mobile phase

Buffer solution and Acetonitrile were mixed in the ratio of 50:50% and degassed.

Preparation of Omeprazole standard solution

Accurately weighed quantity of 20mg Omeprazole was transferred to a100ml volumetric flask, dissolved in 25ml of methonal and sonicated for 20 mins and the solution was made up the volume with methanol. From the above stock solution take 5ml was transferred to 50ml volumetric flask and make up the volume with methanol. The solution was filtered with 0.45µ filter and sonicated for 15min.

Preparation of Cinitapride standard solution

Accurately weighed quantity of 12mg Cinitapride was transferred to a 200ml volumetric flask, dissolved in 25ml of methonal and sonicated for 20 mins and the solution was made up the volume with methanol. From the above stock solution take 2.5ml was transferred to 50ml volumetric flask and make up the volume with methanol. The solution was filtered with 0.45µ filter and sonicated for 15min.

Preparation of sample solution

The capsule dosage form contains Cinitapride as tablet and Omeprazole as granules.

Cinitapride

20 tablets from formulation were weighed and powdered, 12 mg of sample was transferred in to 25ml volumetric flask and methanol was added to dissolve the sample. To make up the volume with methanol & filtered with 0.45µ filter paper. From the above stock solution take 10ml was transferred to 50 ml volumetric flask and make up the volume with methanol. The solution was filtered with 0.45µ filter and sonicated for 15min.

Omeprazole

OME granules in twenty capsules were weighed accurately and crushed to fine powder and powder equivalent to 50mg of OME was accurately weighed and transferred in to 50ml volumetric flask and mobile phase was added to dissolve the sample. To make up the volume with mobile phase & filtered with 0.45µ filter paper. From the above stock solution take 5ml was transferred to 100 ml volumetric flask and make up the volume with mobilephase. The solution was filtered with 0.45µ filter and sonicated for 15min.

Chromatographic Conditions

The mobile phase pumped at a flow rate of 1 ml/min through the column (C18; 4.6 X 250 mm, 5µ, Thermo BDS column) at 30ºC, and injection volume is 10 µl, wavelength used was 282nm, and runtime was 7 mins.

Recommended procedure

The HPLC system was stabilized for 30 minutes by following the chromatographic conditions as

described. One blank followed by 5 Injections of standard solution was injected. The retention time and the average peak areas for each standard were recorded. Two Injections of working sample solution was injected and the results were recorded. The amount of drug present in sample solution was calculated. Chromatograms for the standards are shown in figure 3 and figure 4.

Validation of HPLC method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of OME and CNT in capsule dosage form. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, LOD, LOQ, and robustness.

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of OME and CNT. The developed method was validated according to ICH Guidelines. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters. The result was shown in the table 1.

Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard samples of OME and CNT were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another. Chromatograms shown in figure 4 and explain that retention time for standard and sample are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of OME and CNT of different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients were 0.999 for the drugs which prove that the method is linear. The graphs were shown in the figure 5 and figure 6.The linearity data for OME and CNT was shown in table 2.

Recovery

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 100% and 6 replicate injections at 50% and 150%. Known amounts of standard OME and CNT were added to pre-analyzed samples and were subjected to the proposed HPLC method. The measured value was obtained by recovery test. Spiked amount of both the drugs were compared against the recovery amount. % Recovery was 100%. The results are shown in table 3.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. The values are shown in table 4.

Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºc change in temperature. The values are shown in table 5.

Ruggedness

System to system variability

System to system variability on two HPLC systems was carried out to get the ruggedness of assay method. The result was shown in Table 6.

HPLC column to column variability

Column to column variability on two HPLC systems was carried out to get the ruggedness of assay method. The result was shown in Table 7.

RESULTS AND DISCUSSION

System suitability parameters, selectivity, linearity, precession, accuracy, robustness, and

ruggedness, these all values are within the limit so method development and validation are validated. It will be use full for further analysis in quality control and other departments.

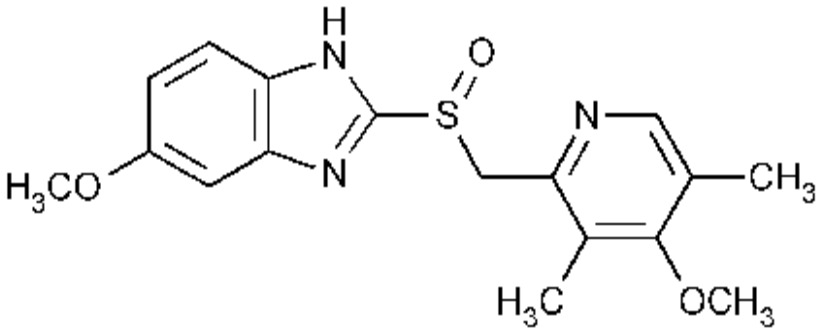

Fig. 1: Structure of Omeprazole

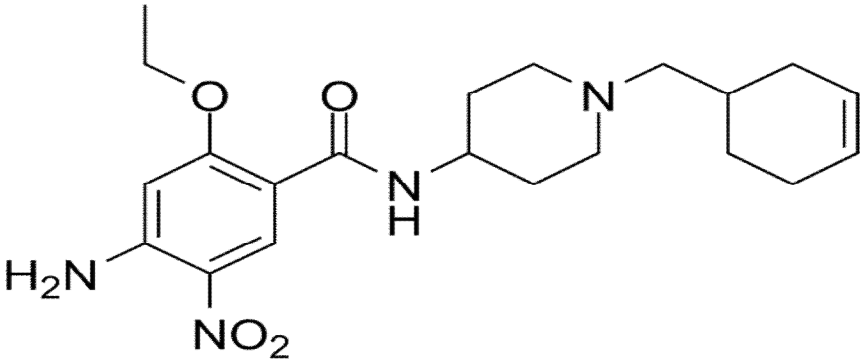

Fig. 2: Structure of Cinitapride

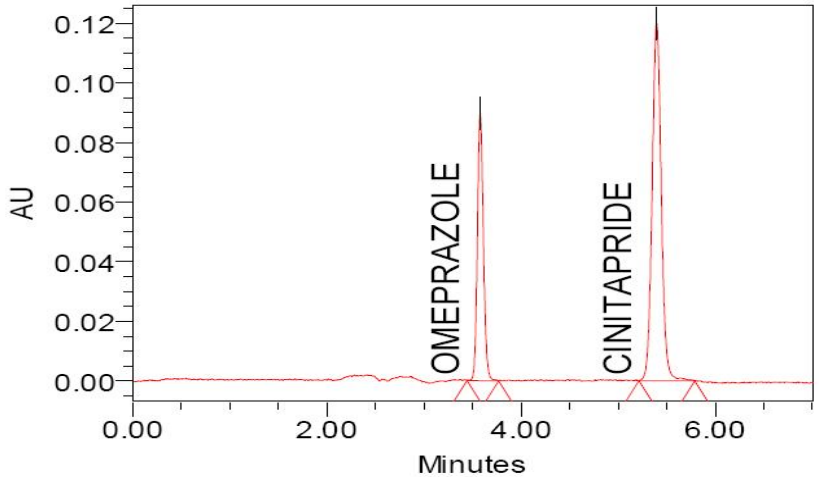

Fig. 3: Chromatogram for standard

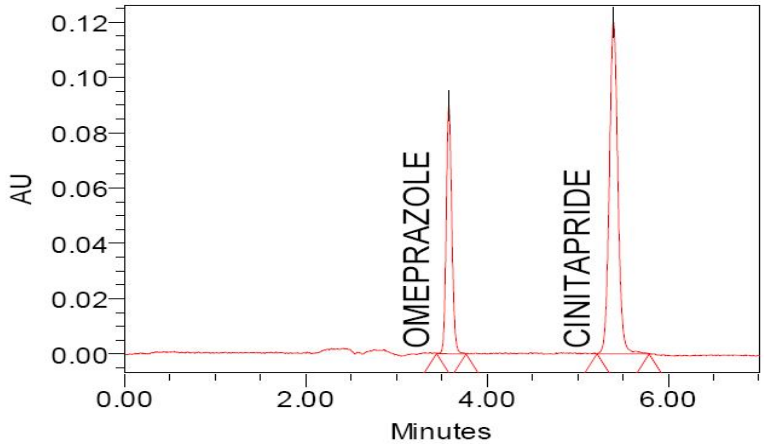

Fig. 4: Chromatogram for sample

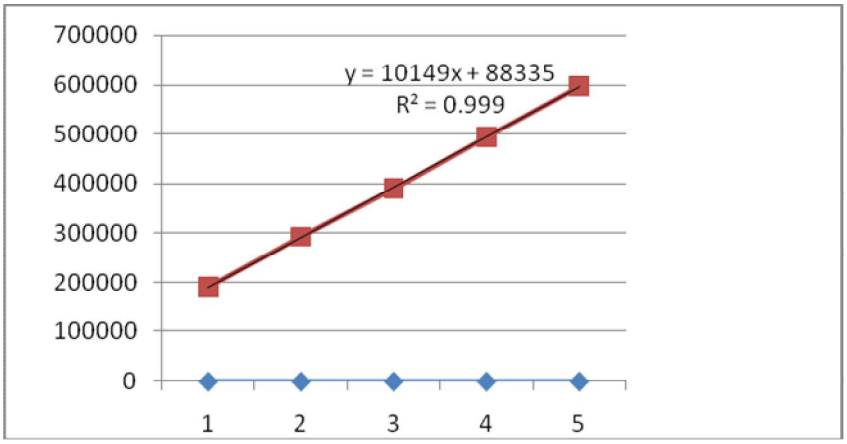

Fig. 5: Linearity of Omeprazole

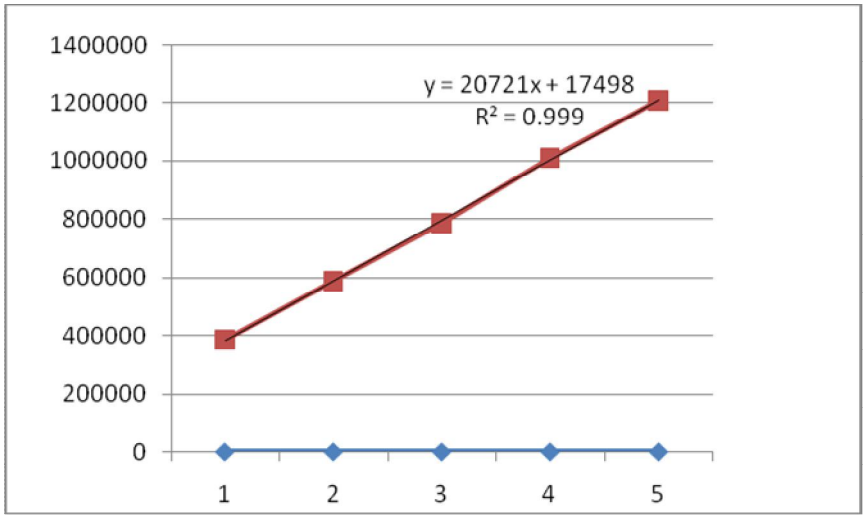

Fig. 6: Linearity of Cinitapride

Table 1: Result of System Suitability Tests of Omeprazole and Cinitapride

PARAMETERS	OME	CNT
Linearity range	50 to 150%	50 to 150%
Correlation coefficient	0.999	0.999
Retention time	3.5	5.4
Resolution Factor	000	12.6
USP plate count	16643	16217
Tailing factor*	1.132	1.06

Table 2: Linearity of Omeprazole and Cinitapride

S. No.	Conc.	Area of OME	Area of CNT
1	50	190346	385528
2	75	292774	588932
3	100	389755	786947
4	125	494030	1011304
5	150	597193	1210404

Table 3: Accuracy of Omeprazole and Cinitapride

S. No.	Spike Level	% Recovery of OME	Mean % Recovery	% Recovery of CNT	Mean % Recovery
1	50%	99	99	99	99
2	50%	98		98	
3	50%	98		98	
4	50%	99		99	
5	50%	99		99	
6	50%	100		99	
1	100%	100	100	99	99
2	100%	100		100	
3	100%	100		99	
1	150%	100	100.6	100	100.6
2	150%	101		100	
3	150%	101		100	
4	150%	100		101	
5	150%	101		101	
6	150%	101		102	

Table 4: Precision of Omeprazole and Cinitapride

S. No.	Sample Weight	Sample Area-OME	Sample Area-CNT	% Assay	% Assay
1	194	386741	783482	99	99
2	194	384652	788600	98	99
3	194	385214	787979	98	99
4	194	383573	775005	98	98
5	194	386871	798212	99	101
6	194	386061	790204	99	100
Avg Assay				99	99
STD				0.33	0.97
% RSD				0.33	0.98

Table 5: Results for Robustness Test of Omeprazole and Cinitapride

Parameters Count	Changes	RT	USP Tailing	USP Plate
Flow rate(ml/min)	Flow 1(low)	4.46, 6.78	1.05	9331
	Flow 2(high)	2.98, 4.53	1.12	7200
Temperature	Temp1(low)	3.58, 5.43	1.06	8149
	Temp1(high)	3.56, 5.6	1.04	8879

Table 6: System to System Variability for Omeprazole and Cinitapride

S. No.	ASSAY% OF OMEPRAZOLE (SYSTEM 1)	ASSAY% OF CINITAPRIDE (SYSTEM 1)	ASSAY% OF OMEPRAZOLE (SYSTEM 2)	ASSAY% OF CINITAPRIDE (SYSTEM 2)
1	99.2	99	100.4	99
2	99.5	99	99.9	99
3	99.3	99	100.6	100.6
4	99.2	98	100.4	100.4
5	100.2	101	99.5	101
6	99.5	100	100.5	100
Average	99.5	99	100.2	99
% RSD	0.37	0.97	0.44	0.86

Table 7: Column to Column Variability for Omeprazole and Cinitapride

S. No.	ASSAY% OF OMEPRAZOLE (COLUMN 1)	ASSAY% OF CINITAPRIDE (COLUMN 1)	ASSAY% OF OMEPRAZOLE (COLUMN 2)	ASSAY% OF CINITAPRIDE (COLUMN 2)
1	99.2	99	99.6	100.3
2	99.5	99	99.6	99.4
3	99.3	99	98.7	99.7
4	99.2	98	100.0	100.1
5	99.5	101	99.7	99.2
6	100.2	100	99.0	100.5
Average	99.5	99	99.4	99.9
% RSD	0.37	0.97	0.50	0.91

CONCLUSION

The new Reverse phase HPLC method developed and validated for simultaneous determination of Omeprazole and Cinitapride pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid dosage forms. This method was doing in simple manner but founded rapidly accurate values, so method will be use full for quality control department, formulation and other departments.

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