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Research Article

DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND CINITAPRIDE IN BULK AND CAPSULE DOSAGE FORM

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Omeprazole and Cinitapride in bulk and Capsule dosage form. Chromatographic analysis was performed on a Symmetry C8 column (150x 4.5 mm, 5µm) column ambient temperature with a mixture of mixed phosphate buffer and Acetonitrile in the ratio 50:50 (mixed phosphate buffer preparation; 1.625 gm of potassium Dihydrogen phosphate and 0.3 gm of Di potassium hydrogen phosphate in 550 mL HPLC grade water, pH= 6.0 adjust with phosphoric acid) as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 287 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Omeprazole and Cinitapride were 2.49 and 3.650 min, respectively. Calibration plots were linear over the concentration ranges

5–30 µg mL^{-1} and 0.75-4.5 µg mL^{-1} for Omeprazole and Cinitapride, respectively. The Limit of detection was 1.43570 and 0.086 µg mL^{-1} and the quantification limit was 4.35 µg mL^{-1} and 0.26 µg mL^{-1} for Omeprazole and Cinitapride, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98.62% to 100.37%. Commercial capsule formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Omeprazole and Cinitapride in bulk and capsule dosage form.

Keywords: Cinitapride, Omeprazole, RP-HPLC, Validation.

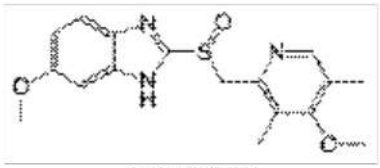
INTRODUCTION

Omeprazole ($C_{17}H_{19}N_3O_3S$)

Omeprazole is chemically 6-methoxy -2-{[(4-methoxy-3, 5-dimethylpyridin -2-yl)methane] sulfinyl -1H-1,3-benzodiazole}, it is white crystalline powder. It is soluble in methanol, di chloromethane, chloroform and slightly soluble in water and melting point is 156 ˚C, it is belongs to a new class of anti secretory compound, the substituted benzimidazole[1]. A highly effective inhibitor of gastric acid secretion used in the therapy of stomach ulcers and zollinger-ellison syndrome. The drug inhibits the H()-K()-ATPase (H()-K()-exchanging ATPase) in the proton pump of gastric parietal cells. For the treatment of acid-reflux disorders (GERD), peptic ulcer disease, H. pylori eradication, and prevention of gastroinetestinal bleeds with NSAID use.

Omeprazole is a compound that inhibits gastric acid secretion and is indicated in the treatment of gastroesophageal reflux disease (GERD), the healing of erosive esophagitis, and H. pylori eradication to reduce the risk of duodenal ulcer recurrence. Omeprazole belongs to a new class of antisecretory compounds, the substituted benzimidazoles, that do not exhibit anticholinergic or H_2 histamine antagonistic properties, but that suppress gastric acid secretion by specific inhibition of the H+/K+ ATPase at the secretory surface of the gastric parietal cell. As a result, it inhibits acid secretion into the gastric lumen. This effect is dose-related and leads to inhibition of both basal and stimulated acid secretion irrespective of the stimulus [1].

Omeprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H+/K+-ATPase in the gastric parietal cell. By acting specifically on the proton pump, omeprazole blocks the final step in acid production, thus reducing gastric acidity. Symptoms of overdose include confusion, drowsiness, blurred vision, tachycardia, nausea, diaphoresis, flushing, headache, and dry mouth. There are few analytical methods reported for the estimation of omeprazole which is include HPLC methods [5,6,8,11&12], UV-Spectrometric methods [13&19] and HPTLC methad[18].



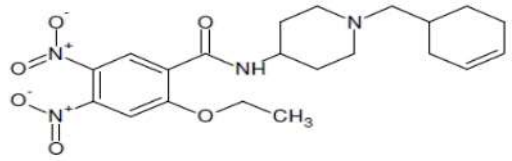
mol.wt:345.416

Figure 1. Structure of Omeprazole.

Cinitapride ($C_{21}H_{30}N_4O_4$)

Cinitapride is chemically (*RS*) - 4-amino – *N* - [1-(1-cyclohex-3-enylmethyl)-4-piperidyl] -2- ethoxy – 5 –nitro - benzamide. It is yellowish crystalline powder, it is soluble in methanol, chloroform and in soluble in water. Cinitapride is a drug that has against action to the serotoninergic 5-HT2 and D2 dopaminergic receptors that has been indicated in the gastro esophageal reflux and in the functionaldisorders of gastrointestinal motility treatment. The therapeutic effect of cinitapride lies on the capacity of increasing lower esophageal sphinctertone and hasstrong gastro kinetic activity, which generatessignificant increases in the gastric emptiness; besides,through the serotoninergic system it stimulates the intestinal activity. The use of cinitapride is efficient and safe in treatment of patients with disorders in thegastric emptiness related to gastro esophageal refluxand functional dyspepsia as well as in individuals thatpresent irritable bowel syndrome with constipationand abdominal pain [2,3&15].

The symptoms of overdose include drowsiness, confusion and extrapyramidal effects. There are few analytical methods reported for the estimation of Cinitapride, which is include HPLC methods [4,7,9&10], UV-spectroscopic methods[14- 17].



mol.wt: 402.4873
Figure 2. Structure of cinitapride.

MATERIALS AND METHODS

Pure standard of Omeprazole and Cinitapride (Assigned purity 99.98%) was obtained as a gift sample from Shasun Chemicals Pvt Ltd Puducherry India. The gift samples were used as standard without further purification. HPLC grade water, methanol (Qualigens), potassium di hydrogen phosphate, di potassium hydrogen phosphate, phosphoric acid, sodiumperchloricacid and triethylamine (S.D. fine chemicals, Mumbai, India), were used throughout the experiment. Commercial pharmaceutical preparation (BURPEX), ZYDUS (CADILA)) which was claimed to contain 20 mg of Omeprazole and 3 mg of Cinitapride is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies. HPLC grade Acetonitrile from Merck specialties Pvt Ltd, Mumbai. Water HPLC grade was obtained from Rankem laboratories.

Instrumentation and Chromatographic Conditions

High performance liquid chromatography, HPLC (WATERS 2695), UV-VIS detector was used. Isocratic elution of mobile phase comprising of Chromatographic analysis was performed on a Symmetry C8 column (150x 4.5 mm, 5µm) column ambient temperature with a mixture of mixed phosphate buffer and Acetonitrile in the ratio 50:50 (mixed phosphate buffer preparation: 1.625 gm of potassium di hydrogen phosphate and 0.3 gm of di potassium hydrogen phosphate in 550ml HPLC grade water, pH= 6.0 adjust with phosphoric acid) as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 287 nm. The retention times of Omeprazole and Cinitapride were 2.49 and 3.650 min. The column temperature was maintained at ambient and the volume of injection was 20 µL. Prior to injection of analyte, the column was equilibrated for 30 min with mobile phase.

Preparation of mobile phase

The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising of with a mixture mixed phosphate buffer and Acetonitrile in the ratio 50:50 (mixed phosphate buffer preparation: 1.625 gm of potassium di hydrogen phosphate and 0.3 gm of di potassium hydrogen phosphate in 550ml HPLC grade water, pH= 6.0 adjust with phosphoric acid) as mobile phase and filtered before use through a 0.45 µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 mL min^{1}

Standard solution

Standard stock solutions 1 mg mL^{-1} of Omeprazole and Cinitapride were prepared in mobile phase and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain mixture of Omeprazole and Cinitapride in over the linearity range from 5–30 µg mL^{-1} and 0.75-4.5 µg mL^{-1}.

Assay in formulation

Twenty capsules each containing and their average weight was calculated. The capsules were crushed to furnish a homogeneous powder and a quantity equivalent to one capsule were weighed in to a 100 mL volumetric flask, dissolve in mobile phase, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 mL volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 mL of mobile phase, and then the volume was completed to 100 mL with the same solvent. Further add mobile phase to obtain an expected concentration of 20 µg mL^{-1} Omeprazole and 3 µg mL^{-1} Cinitapride. All determinations were conducted in triplicate.

RESULTS AND DISCUSSIONS

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of Omeprazole and Cinitapride were shown in (Fig.3). There was clear resolution between Omeprazole and Cinitapride with retention time of 2.49 and 3.650 minutes, respectively.

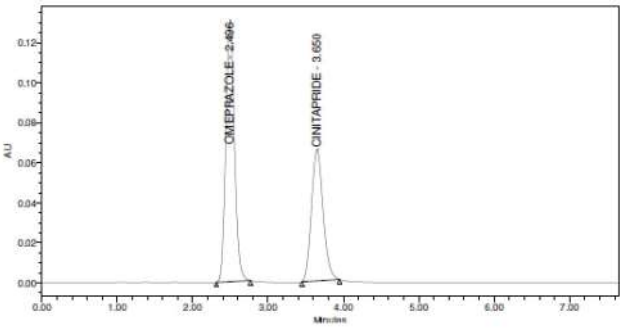


Figure 3. Typical chromatogram of Omeprazole and Cinitapride

Linearity

The response was determined to be linear over the range of 5 µg mL^{-1} to 30 µg mL^{-1} (5, 10, 15, 20, 25, 30) for Omeprazole and 0.75- 4.5 µg mL^{-1} (0.75, 1.5, 2.25, 3, 3.75, 4.5) for Cinitapride. The solutions were injected into HPLC system. Each of the concentration was injected to get reproduciable response. The run time was 15 min and the peak areas were measured (Table 1 & 2). The calibration curve was plotted as concentration of the respective drug versus the response at each level. The purposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study. They were represented by the linear regression equation (Fig 4 and 5 calibration curve).

$Y_{Omeprazole} = 16816x + 776.6$, Coefficient of correlation (r^2) value = 1

$Y_{Cinitapride} = 60256x - 6021$ Coefficient of correlation (r^2) value = 1

Table 1. For Peak Area of Omeprazole

Omeprazole	
Conc µg mL^{-1}	**Area**
0	0
5	825313
10	1689514
15	2536151
20	3374660
25	4202073
30	5034620

Table 2. For Peak area of Cinitapride

Cinitapride	
Conc µg mL^{-1}	**Area**
0	0
0.75	433482
1.5	897758
2.25	1355830
3	1807293
3.75	2250439
4.5	2703493

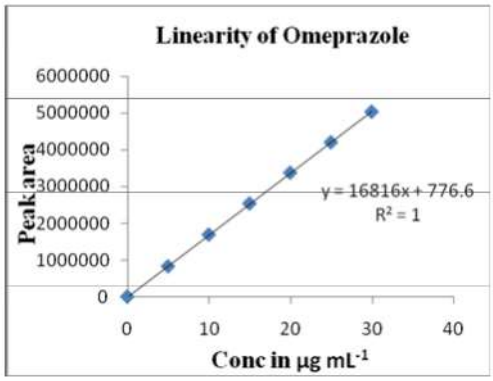
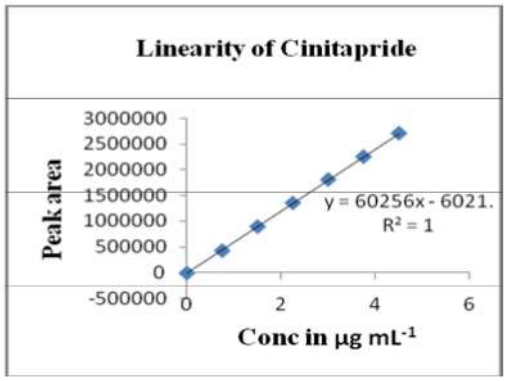


Figure 4. Calibration curve for Omeprazole



Figue 5. Calibration curve for Cinitapride

Table 3. Result of recovery studies

	Omeprazole	**Cinitapride**
Accuracy std	3348808	1793366
	3346729	1792553
	3344522	1791485
Avg	3346686.333	1792468
Accuracy sample	3353076	1795553
50%spike	5017734	2690958
	5015945	2691038
	5016512	2691602
Avg	5016730.333	2691199.333
Amt. recovered	**49.71**	**49.97**
%Recovery	**99.42**	**99.93**
100%spike	6652635	3580528
	6656763	3583244
	6651418	3579640
Avg	6653605.333	3581137.333
Amt. recovered	**98.62**	**99.62**
%Recovery	**98.62**	**99.62**
150%spike	8344114	4488981
	8345064	4490676
	8368344	4503191
Avg	8352507.333	4494282.667
Amt. recovered	**149.38**	**150.56**
%Recovered	**99.59**	**100.37**

Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 50%, 100%, 150% weight of sample drug solution were pipetted into each of three volumetric flasks and prepare the serial dilution to get 23 µg mL^{-1}. To prepare for two each standard drug solution were pipetted into each of six volumetric flasks. To this 10 mL of Omeprazole standard drug solution of 200 µg mL^{-1} was added to each of three volumetric flask respectively. To this 10 mL of Cinitapride standard drug solution of 30 µg mL^{-1} was added to each of three volumetric flask respectively. The volume was made up to 100 mL with mobile phase.20 µL of each solution was injected and chromatograms were recorded. The range was found between 98.62 % to 100.37 % respectively. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients (Table 3).

Limit of Detection and Quantification

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S)

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response, S = Slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

The LOD was found to be 1.43570 µg mL-1 and 0.08600 µg mL^{-1} and LOQ was found to be 4.35080 µg mL^{-1} and 0.26070 µg mL^{-1} for Omeprazole and Cinitapride respectively which represents that sensitivity of the method is high.

Precision

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 20 µg mL^{-1} for Omeprazole and 3 µg mL^{-1} for Cinitapride showed a RSD of 0.22 % for Omeprazole and 0.09 % for Cinitapride. It was concluded that the analytical technique showed good repeatability (Table 4).

Table 4. Results of repeatability analysis (Method precision and System precision)

Method precision

Omeprazole

S.No.	RT	Area
1	2.633	3360201
2	2.632	3382022
3	2.632	3361575
4	2.632	3353294
5	2.632	3358171
6	2.687	3343379
Avg	2.641333	3359774
SD	0.022376	12741.71
%RSD	**0.85**	**0.38**

Cinitapride

S.No.	RT	Area
1	3.917	1796860
2	3.943	1809912
3	3.94	1799498
4	3.939	1795543
5	3.939	1799331
6	3.994	1797866
Avg	3.945333	1799835
SD	0.025633	5158.728
%RSD	**0.65**	**0.29**

System precision

Omeprazole

S.No.	RT	Area
1	2.633	3354162
2	2.633	3353373
3	2.635	3355997
4	2.634	3356414
5	2.634	3363668
6	2.633	3372308
Avg	2.633667	3359320
SD	0.000816	7335.56
%RSD	**0.03**	**0.22**

Cinitapride

S.No.	RT	Area
1	3.956	1795001
2	3.952	1795147
3	3.964	1798388
4	3.954	1797250
5	3.949	1798146
6	3.945	1798272
Avg	3.953333	1797034
SD	0.006501	1571.179
%RSD	**0.16**	**0.09**

Reproducibility and Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates (Table 5).

Table 5. Results of reproducibility

	Omeprazole	Cinitapride
Average Percentage Recovery	99.21%	99.97 %
SD between set of analysis on same date	12741.71	5158.728
SD between set of analysis on different date	7335.56	1571.179
RSD between set of analysis on same date	0.38%,	0.29%
RSD between set of analysis on different date	0.22%,	0.09%

Robustness

The robustness of the method was determined by deliberate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust.

Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there are no peaks of diluents and placebo at main peak's. Hence, the chromatographic system used for the estimation of Omeprazole and Cinitapride is very selective and specific .Specificity studies indicating that the excipients did not interfere with the analysis. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Fig.6)

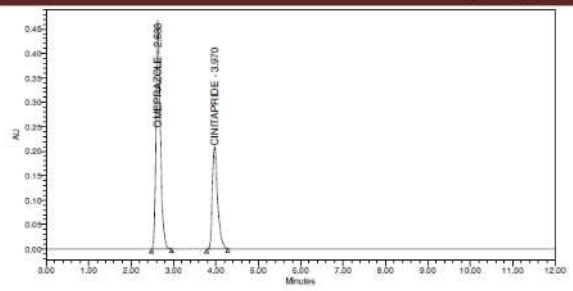


Figure 6. Specificity Chromatograms of Omeprazole and Cinitapride

System Suitability

A binary solution of 20 µg mL^{-1} of Omeprazole and 3 µg mL^{-1} of Cinitapride (in triplicate) was prepared and same was injected, then the system suitability parameters like resolution factor (*Rs*), tailing factor (*Tf*) and theortical plates (*N*) were calculated and recorded in Table 6. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application.

Table 6. Results of system suitability parameters

Parameters	Omeprazole values	Cinitapride values
Theoritical Plates (N)	3178.00	5165.000
Resolution (*Rs*)		6.69
Tailing factor (*Tf*)	1.33	1.4

CONCLUSION

The proposed RP-HPLC method is found to be simple, accurate, precise, linear, and specific for quantitative estimation of Omeprazole and Cinitapride in bulk and its formulation. The proposed RP-HPLC method is cost effective and less time consuming. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application. Hence the present HPLC method is suitable for routine assay of Omeprazole and Cinitapride in raw materials and in pharmaceutical formulations in the quality control laboratories.

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