

Validated RP-HPLC Method for Simultaneous Quantification of Cinitapride and Omeprazole in Combined Tablet Dosage Form

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Abstract

A rapid and precise reverse-phase high-performance liquid chromatographic method has been developed and validated for the simultaneous estimation of Cinitapride and Omeprazole, in pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6 × 150 mm, 5 µm) column using a mixture of acetonitrile:water:methanol (60:20:20 v/v) as the mobile phase at a flow rate of 1.0 mL/min, with detection at 230 nm. The retention times of Cinitapride and Omeprazole were 2.8 and 3.8 ± 0.02 min, respectively. The method produced linear responses in the concentration range of 10–50 µg/mL for Cinitapride and 66.6–330 µg/mL for Omeprazole. The method precision for the determination of assay was below 2.0% RSD. The method is suitable for the quality control of bulk and pharmaceutical formulations.

Keywords: Cinitapride; Omeprazole; RP-HPLC; method validation; ICH guidelines; tablet dosage form

1. Introduction

High-performance liquid chromatography is at present one of the most sophisticated tools of pharmaceutical analysis, and reverse-phase separations on chemically bonded silica columns remain the workhorse of modern quality control laboratories. The estimation of Cinitapride and Omeprazole in a fixed-dose combination product requires a single, stability-indicating and cost-effective chromatographic procedure capable of resolving two analytes of markedly different polarity and absorptivity within a short run time.

Cinitapride is a prokinetic benzamide-type agent that acts on gastrointestinal motility, while Omeprazole is a substituted benzimidazole proton pump inhibitor that suppresses gastric acid secretion through inhibition of the gastric proton pump. Because the two actives differ appreciably in their therapeutic dose and in their chromophoric response, a simultaneous assay demands careful optimisation of mobile-phase composition, column chemistry and detection wavelength. The present work reports the development of such a method, its optimisation across several mobile-phase and column trials, and its full validation in accordance with ICH and USP requirements with respect to system suitability, precision, accuracy, linearity, limit of detection, limit of quantitation and robustness.

2. Materials and Methods

2.1 Instruments used

Table 1. Instruments used

SL.No	Instrument	Model
1	HPLC	WATERS, software: Empower 2, 2695 separation module. 996 PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV
3	pH meter	Lab India
4	Weighing machine	Sartorius
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Enertech

2.2 Chemicals used

Table 2. Chemicals used

S.No	Chemical	Brand names
1	Cinitapride	Sura labs
2	Omeprazole	Sura labs
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	Merck
6.	Anhydrous di hydrogen phosphate	Finar chemicals
7	Citric acid	Finar chemicals

2.3 HPLC method development

2.3.1 Mobile-phase optimisation

Initially the mobile phase tried was methanol:orthophosphoric acid and phosphoric acid (pH 3): acetonitrile, and methanol: ACN and methanol: ammonium acetate buffer, with varying proportions. Finally, the mobile phase was optimised to acetonitrile: water in the proportion 70:30 v/v, respectively.

2.3.2 Optimisation of column

The method was performed with various columns, including a C18 column, an X-bridge column, Xterra and a C8 column. Hypersil C18 (4.6 × 150 mm, 5 µm, Make: Waters) was found to be ideal, as it gave good peak shape and resolution at 1 mL/min flow.

2.4 Chromatographic conditions

Table 3. Chromatographic conditions

Mobile phase	Acetonitrile: Water: Methanol
Flow rate	1 ml/min
Wavelength	230 nm
Column temp	Ambient

Volume	10 μ l
Run time	7 minutes

2.5 Preparation of mobile phase

Accurately measured 700 mL (70%) of acetonitrile and 300 mL of water (30%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through a 0.45 μ m filter under vacuum filtration.

Diluent preparation

The mobile phase was used as the diluent.

2.6 Validation parameters

2.6.1 Method precision

preparation of standard solution

Accurately weigh and transfer 10 mg of Cinitapride and 10 mg of Omeprazole working standard into 10 mL clean dry volumetric flasks individually, add about 7 mL of diluent to each volumetric flask and sonicate to dissolve completely, then make up the volume to the mark with the same solvent (stock solution).

Further pipette 0.3 mL and 1.98 mL of the above Cinitapride and Omeprazole stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution

Accurately weigh 10 combination tablets, crush in a mortar and pestle, and transfer an amount equivalent to 10 mg of Cinitapride and Omeprazole (marketed formulation — dose of Cinitapride is 25 mg and dose of Omeprazole is 50 mg in the combination tablet) into a 10 mL clean dry volumetric flask; add about 7 mL of diluent and sonicate to dissolve completely, then make up the volume to the mark with the same solvent (stock solution).

Further pipette 1.98 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

The standard and sample solutions of 30 μ g/mL of Cinitapride and 198 μ g/mL of Omeprazole were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

2.6.2 Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on a different day using a different make column of the same dimensions.

Preparation of stock solution. Accurately weigh 10 combination tablets, crush in a mortar and pestle, and transfer an amount equivalent to 10 mg of Cinitapride and Omeprazole (marketed formulation — dose of Cinitapride is 3 mg and dose of Omeprazole is 20 mg in the combination tablet) into a 10 mL clean dry volumetric flask; add about 7 mL of diluent and sonicate to dissolve completely, then make up the volume to the mark with the same solvent (stock solution).

Further pipette 1.98 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

The standard and sample solutions contained concentrations of 30 μ g/mL of Cinitapride and 198 μ g/mL of Omeprazole.

Procedure. The standard solution was injected five times and the area was measured for all five injections

in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.6.3 Accuracy

Preparation of standard stock solution. Accurately weigh and transfer 10 mg of Cinitapride and 10 mg of Omeprazole working standard into 10 mL and 10 mL clean dry volumetric flasks, add about 7 mL and 7 mL of diluent and sonicate to dissolve completely, then make up the volume to the mark with the same solvent (stock solution).

Further pipette 0.3 mL and 1.98 mL of the above Cinitapride and Omeprazole stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent.

Preparation of sample solutions.

For preparation of 50% solution (with respect to target assay concentration):

Accurately weigh 10 combination tablets, crush in a mortar and pestle, and transfer an amount equivalent to 10 mg of Cinitapride and Omeprazole (marketed formulation — dose of Cinitapride is 3 mg and dose of Omeprazole is 20 mg in the combination tablet) into a 10 mL clean dry volumetric flask; add about 7 mL of diluent and sonicate to dissolve completely, then make up the volume to the mark with the same solvent (stock solution). Further pipette 0.99 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. The standard and sample solutions contained concentrations corresponding to 50%.

For preparation of 100% solution (with respect to target assay concentration): the same sample preparation was followed, and further pipette 1.98 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. The standard and sample solutions contained concentrations corresponding to 100%.

For preparation of 150% solution (with respect to target assay concentration): the same sample preparation was followed, and further pipette 2.97 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. The standard and sample solutions contained concentrations corresponding to 150%.

Procedure. Inject the standard solution, accuracy-50%, accuracy-100% and accuracy-150% solutions. Calculate the amount found and amount added for Cinitapride and Omeprazole and calculate the individual recovery and mean recovery values. These solutions were filtered through a 0.45 μ m membrane and then, for each concentration, three replicate injections were made under the optimised conditions. The chromatograms were recorded and the peak responses measured.

2.6.4 Linearity

Preparation of stock solution. Accurately weigh 10 combination tablets, crush in a mortar and pestle, and transfer an amount equivalent to 10 mg of Cinitapride and Omeprazole (marketed formulation — dose of Cinitapride is 3 mg and dose of Omeprazole is 20 mg in the combination tablet) into a 10 mL clean dry volumetric flask; add about 7 mL of diluent and sonicate to dissolve completely, then make up the volume to the mark with the same solvent (stock solution).

- **Level – I (10 ppm Cinitapride and 66.6 ppm Omeprazole):** 0.66 mL of stock solution taken in a 10 mL volumetric flask and diluted up to the mark with diluent.
- **Level – II (20 ppm Cinitapride and 132 ppm Omeprazole):** 1.32 mL of stock solution taken in a 10 mL volumetric flask and diluted up to the mark with diluent.
- **Level – III (30 ppm Cinitapride and 198 ppm Omeprazole):** 1.98 mL of stock solution taken in a 10 mL volumetric flask and diluted up to the mark with diluent.
- **Level – IV (40 ppm Cinitapride and 264 ppm Omeprazole):** 2.64 mL of stock solution taken in a

10 mL volumetric flask and diluted up to the mark with diluent.

- **Level – V (50 ppm Cinitapride and 330 ppm Omeprazole):** 3.3 mL of stock solution taken in a 10 mL volumetric flask and diluted up to the mark with diluent.

Procedure. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (concentration on the X-axis and peak area on the Y-axis) and calculate the correlation coefficient.

2.6.5 Robustness

The analysis was performed under different conditions to determine the variability of test results. The following conditions were checked for variation of results.

Preparation of sample solution (75 µg/mL Cinitapride and 90 µg/mL Omeprazole). Accurately weigh and transfer 10 mg of Cinitapride and 10 mg of Omeprazole working standard into 10 mL and 10 mL clean dry volumetric flasks, add about 7 mL and 7 mL of diluent and sonicate to dissolve completely, then make up the volume to the mark with the same solvent (stock solution).

Further pipette 0.3 mL and 1.98 mL of the above Cinitapride and Omeprazole stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent.

- **Effect of variation of flow:** the sample was analysed at 0.8 mL/min and 1.0 mL/min instead of 0.9 mL/min, with the remaining conditions the same. 10 µL of the above sample was injected twice and chromatograms were recorded.
- **Effect of variation of mobile-phase organic composition:** the sample was analysed with variation of the mobile phase, i.e. acetonitrile:water taken in the ratio 60:40 and 80:20 instead of 70:30, with the remaining conditions the same. 10 µL of the above sample was injected twice and chromatograms were recorded.

3. Results and Discussion

3.1 Method development and optimisation

Method development and finally optimising the drugs give following results

Table 4. Results for optimized conditions

S NO.	Peak Name	Rf	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Cinitapride	2.813	399693	47206		1.6	2667.2
2	Omeprazole	3.886	2431064	247830	4.5	1.5	4142.6

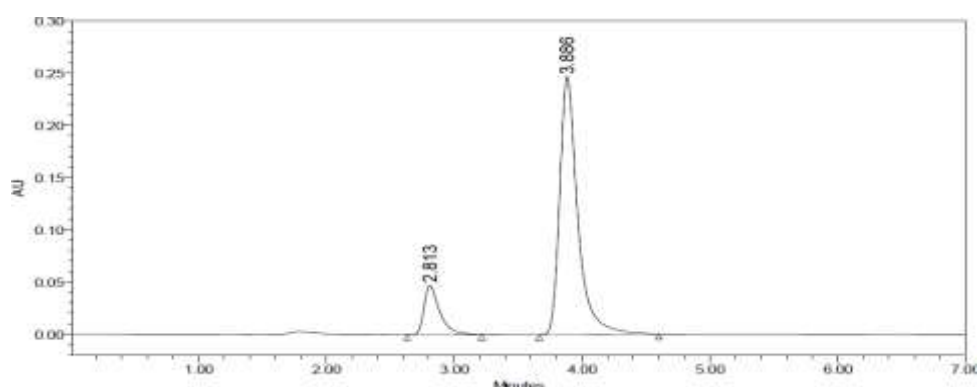


Figure 1: Chromatogram for Cinitapride and Omeprazole

Observation. From the above chromatogram it was observed that the Cinitapride and Omeprazole peaks are well separated and show proper retention time, resolution, peak tailing and plate count. This is therefore the optimised trial. The retention time of Cinitapride was 2.813 min and that of Omeprazole 3.886 min.

3.2 System suitability

Table 5. Results of system suitability parameters for Cinitapride and Omeprazole

S.No	Name	Retention time(min)	Area (μV sec)	Height (μV)	USP resolution	USP tailing	USP plate count
1	Cinitapride	2.816	572745	66043		1.5	2642
2	Omeprazole	3.893	3423737	340922	4.5	1.5	4153

Acceptance criteria

- Resolution between the two drugs must not be less than 2.
- Theoretical plates must not be less than 2000.
- Tailing factor must not be less than 0.9 and not more than 2.

It was found from the above data that all the system suitability parameters for the developed method were within the limits.

3.3 Assay (standard)

Table 6. Assay results

S.No	Name of compound	Label claim	Amount taken(from combination tablet)	%purity
1	Cinitapride	500mg	499.8	99.6 %
2	Omeprazole	600mg	599.9	99.8%

The retention times of Cinitapride and Omeprazole were found to be 2.860 min and 3.886 min, respectively. The % purity of Cinitapride and Omeprazole in the pharmaceutical dosage form was found to be 99.6% and 99.8%, respectively.

3.4 Precision

Precision of the method was carried out for both sample and standard solutions as described under the experimental work. The %RSD for the sample should be NMT 2. The %RSD for the standard solution is below 1, which is within the limits; hence the method is precise.

Intermediate precision/ruggedness

There was no significant change in assay content and system suitability parameters under the different conditions of ruggedness, such as day-to-day and system-to-system variation.

3.5 Accuracy

Sample solutions at different concentrations (50%, 100% and 150%) were prepared and the % recovery was calculated.

Table 7. Accuracy (recovery) data for Cinitapride

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	569325	5	4.9	98%	

100%	753538	10	10.1	101%	99.8%
150%	955999	15	15.1	100.6%	

Acceptance criteria: the % recovery for each level should be between 98.0 and 102.0%.

Table 8. Accuracy (recovery) data for Omeprazole

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3441832	5	4.9	98%	99.4%
100%	4517559	10	10.1	101%	
150%	5738638	15	14.9	99.3%	

Acceptance criteria: the percentage recovery was found to be within the limit (97–103%).

The results obtained for recovery at 50%, 100% and 150% are within the limits; hence the method is accurate.

3.6 Linearity

The linearity range was found to lie from 10–50 ppm for Cinitapride and 66.6–330 µg/mL for Omeprazole; the chromatograms are shown in the figures.

Table 9. Linearity results for Cinipride

S.No	Linearity Level	Concentration(ppm)	Area
1	I	10	108407
2	II	20	206978
3	III	30	299892
4	IV	40	393459
5	V	50	491862
Correlation Coefficient			0.999

Acceptance criteria: correlation coefficient should not be less than 0.999.

Table 10. Linearity results for Omeprazole

S.No	Linearity Level	Concentration(ppm)	Area
1	I	66.6	606125
2	II	132	1208367
3	III	198	1804843
4	IV	264	2371642
5	V	330	2885708
Correlation Coefficient			0.999

Acceptance criteria: correlation coefficient should not be less than 0.99.

Table 11. Analytical performance parameters of Cinitapride and Omeprazole

Parameters	Cinitapride	Omeprazole
Slope (m)	9735	8804
Intercept (c)	6715	25903

Correlation coefficient (R^2)	0.999	0.999
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Acceptance criteria: correlation coefficient (R^2) should not be less than 0.999.

3.7 Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The result was calculated as $LOD = 3.3 \times \sigma / s$, where σ = standard deviation of the response and s = slope of the calibration curve.

- **Cinitapride:** $= 3.3 \times 1921.9 / 9735 = 0.6 \mu\text{g/mL}$
- **Omeprazole:** $= 3.3 \times 34259 / 8804 = 12.8 \mu\text{g/mL}$

3.8 Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

The result was calculated as $LOQ = 10 \times \sigma / s$, where σ = standard deviation of the response and s = slope of the calibration curve.

- **Cinitapride:** $= 10 \times 1921.9 / 9735 = 1.9 \mu\text{g/mL}$
- **Omeprazole:** $= 10 \times 34259 / 8804 = 38.9 \mu\text{g/mL}$

3.9 Robustness

The standard and samples of Cinitapride and Omeprazole were injected by changing the conditions of chromatography. There was no significant change in the parameters such as resolution, tailing factor, asymmetric factor and plate count.

4. Conclusions

High-performance liquid chromatography is at present one of the most sophisticated tools of analysis. The estimation of Cinitapride and Omeprazole was done by RP-HPLC. The mobile phase was optimised and consisted of acetonitrile:water, with acetonitrile mixed in the ratio 70:30% v/v. A Hypersil C18 column (4.6×150 mm, $5 \mu\text{m}$, Make: Waters) or equivalent, chemically bonded to porous silica particles, was used as the stationary phase. The solutions were chromatographed at a constant flow rate of 1 mL/min. The linearity ranges of Cinitapride and Omeprazole were found to be 10–50 ppm and 66.6–330 ppm, respectively. The linear regression coefficient was 0.999 for both analytes.

The values of %RSD are less than 2%, indicating the accuracy and precision of the method. The percentage recovery of Cinitapride is 99.6% and of Omeprazole 99.8%. LOD and LOQ were found to be within the limit.

The results obtained on the validation parameters met ICH and USP requirements. The method was inferred to be simple, accurate, precise and linear, and was found to have suitable application in routine laboratory analysis with a high degree of accuracy and precision.

5. References

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