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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF OMEPRAZOLE AND CINITAPRIDE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

The development and validation of a Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the determination of omeprazole and cinitapride in bulk and pharmaceutical formulations are detailed in this study. The RP-HPLC analysis was performed using a Waters HPLC system equipped with an auto sampler and a PDA Detector 996 model. Chromatographic separation was achieved with a Hypersil C18 column (4.6 × 250 mm, 5 µm particle size) at a column temperature of 35°C. The mobile phase consisted of methanol and water in a 80:20 (v/v) ratio. The flow rate was set at 0.8 mL/min, and detection was carried out at a wavelength of 250 nm. An injection volume of 10 µL was used, and the total run time for each analysis was 6 minutes.

The method was rigorously validated in accordance with ICH guidelines, assessing key parameters such as specificity, linearity, precision, accuracy, and robustness. The RP-HPLC method demonstrated excellent resolution and peak symmetry for both omeprazole and cinitapride, with high accuracy and precision. The validated method provides a reliable and efficient analytical tool for the simultaneous quantification of omeprazole and cinitapride in bulk drug substances and pharmaceutical formulations, ensuring compliance with quality control standards and supporting the consistent quality of pharmaceutical products.

Keywords: RP-HPLC, omeprazole and Cinitapride.

1. INTRODUCTION

Omeprazole (OME) is 5-methoxy-2-[[(4- methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulphinyl]-1H-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 disease1 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 photometric4 , derivative UV Spectroscopy5 , spectrofluorimetric 6 , voltammetric7 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. METHODOLOGY

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Omeprazole and Cinitapride working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of the Omeprazole and 0.3ml of the Cinitapride stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

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2.1. Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

2.2. Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 80:20 v/v respectively.

2.3. Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry ODS C18 (4.6mm×150mm, 5µ Particle Size) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

2.4. METHOD VALIDATION PARAMETERS

2.4.1. SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Omeprazole and 10mg of Cinitapride working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Omeprazole and 0.3ml of the Cinitapride stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.4.2. SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Omeprazole and 10mg of Cinitapride working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Omeprazole and 0.3ml of the Cinitapride stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Omeprazole and Cinitapride sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of the Sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

2.4.3. PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Omeprazole and 10mg of Cinitapride working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Omeprazole & 5ppm of Cinitapride):

Pipette out 0.05ml of Omeprazole and 0.1ml of Cinitapride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (20ppm of Omeprazole & 10ppm of Cinitapride):

Pipette out 0.1ml of Omeprazole and 0.2ml of Cinitapride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (30ppm of Omeprazole & 15ppm of Cinitapride):

Pipette out 0.15ml of Omeprazole and 0.3ml of Cinitapride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

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Preparation of Level – IV (40ppm of Omeprazole & 20ppm of Cinitapride):

Pipette out 0.2ml of Omeprazole and 0.4ml of Cinitapride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (50ppm of Omeprazole & 25ppm of Cinitapride):

Pipette out 0.25ml of Omeprazole and 0.5ml of Cinitapride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

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

2.4.4. PRECISION REPEATABILITY

Accurately weigh and transfer 10 mg of Omeprazole and 10mg of Cinitapride working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Omeprazole and 0.3ml of the Cinitapride stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.4.5. INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

DAY 2:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Omeprazole and 10mg of Cinitapride working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.075ml of the Omeprazole and 0.15ml of the Cinitapride stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

2.4.6. ROBUSTNESS:

Accurately weigh and transfer 10 mg of Omeprazole and 10mg of Cinitapride working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Omeprazole and 0.45ml of the Cinitapride stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9ml/min and 1.0ml/min instead of 0.8ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol and Water was taken in the ratio and 40:60, 50:50 instead (80:20), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

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3. RESULTS AND DISCUSSION

3.1. System suitability for Omeprazole

Table 1. system suitability for Omeprazole

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Omeprazole	2.117	658658	67854	6895	1.06
2	Omeprazole	2.118	657893	67582	6847	1.07
3	Omeprazole	2.116	658985	67895	6875	1.06
4	Omeprazole	2.109	65986	67852	6845	1.06
5	Omeprazole	2.102	65874	67456	6865	1.07
Mean			658836.6			
Std. Dev			707.1067			
% RSD			0.106342			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table 2. system suitability for Cinitapride

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Cinitapride	3.547	8658485	845250	8542	1.18	4.65
2	Cinitapride	3.539	8695847	847584	8574	1.19	4.66
3	Cinitapride	3.547	8657474	847612	8569	1.18	4.65
4	Cinitapride	3.565	8625698	846985	8532	1.18	4.65
5	Cinitapride	3.537	8675842	847526	8541	1.19	4.66
Mean			8662669				
Std. Dev			25911.66				
% RSD			0.299119				

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

3.2. SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Omeprazole and Cinitapride in drug product.

Table 3. Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Omeprazole	2.102	658985	67854		1.06	6859	1
2	Cinitapride	3.537	8659852	845798	4.68	1.18	8643	1
3	Omeprazole	2.105	657542	67259		1.07	6874	2
4	Cinitapride	3.552	8652874	846354	4.69	1.19	8596	2
5	Omeprazole	2.112	658935	67823		1.06	6982	3
6	Cinitapride	3.560	8659875	849653	4.68	1.18	8569	3

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3.3. LINEARITY

Table 4: Linearity study of Omeprazole :

Concentration µg/ml	Average Peak Area
10	1215225
20	2135937
30	3020839
40	4078841
50	5058145

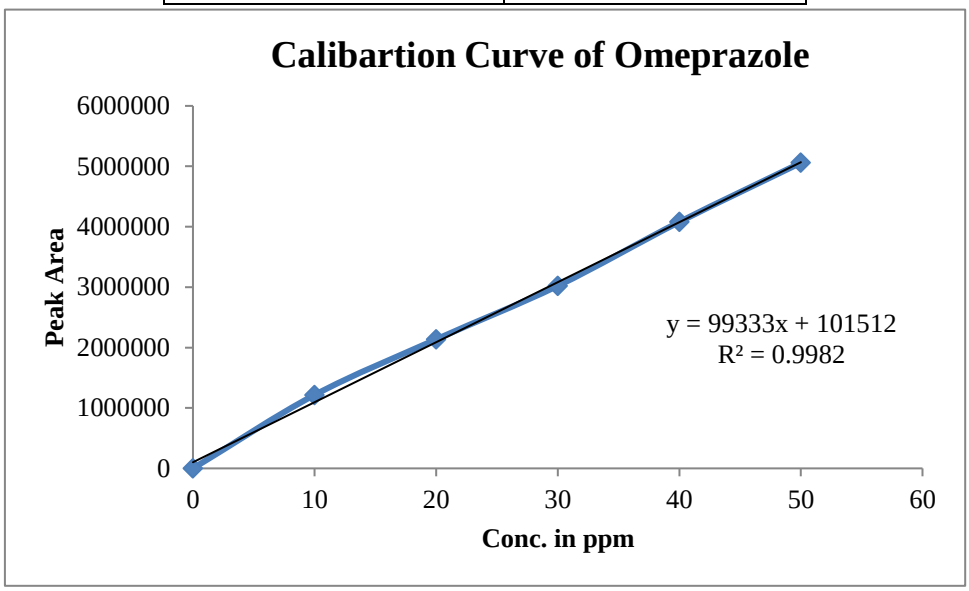

Figure1: Calibration graph for Omeprazole

Table 4: Linearity study of Cinitapride

Concentration µg/ml	Average Peak Area
5	230247
10	462332
15	659905
20	892989
25	1101075

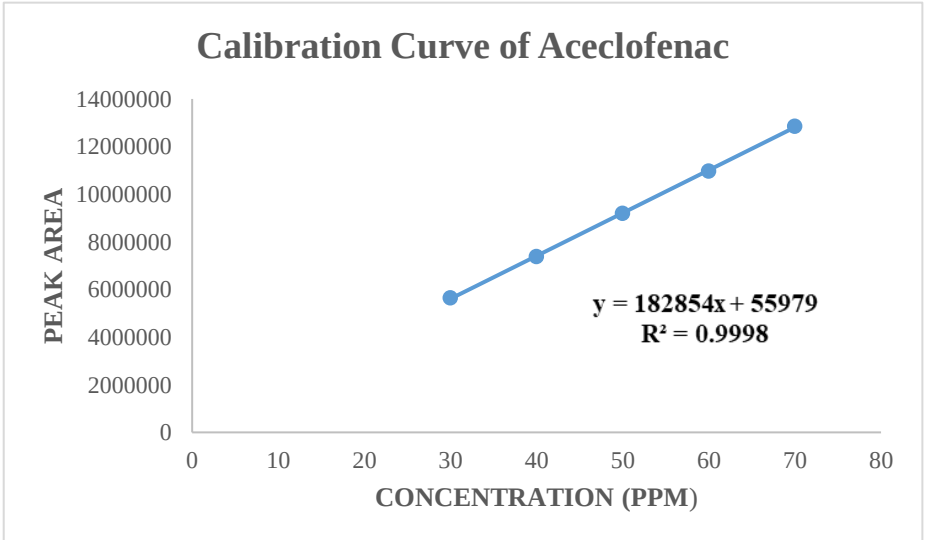

Figure2: Calibration graph for Cinitapride

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Table 5: Results of repeatability for Omeprazole

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Omeprazole	3.003	654426	61521	8474	1.1
2	Omeprazole	3.005	659862	61937	8262	1.2
3	Omeprazole	3.007	650837	62018	8117	1.1
4	Omeprazole	3.008	651433	61893	7917	1.2
5	Omeprazole	3.005	652752	61867	8011	1.1
Mean			653862			
Std. Dev			3626.323			
% RSD			0.554601			

Table-6: Results of method precision for Cinitapride:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cinitapride	3.851	3028371	381736	6881	3.851
2	Cinitapride	3.852	3009188	380138	9363	3.852
3	Cinitapride	3.854	3067464	386615	7844	3.854
4	Cinitapride	3.853	3076611	380183	9746	3.853
5	Cinitapride	3.851	3011912	379471	7883	3.851
Mean			3038709			
Std. Dev			31463.69			
% RSD			1.035429			

3.4. ACCURACY:

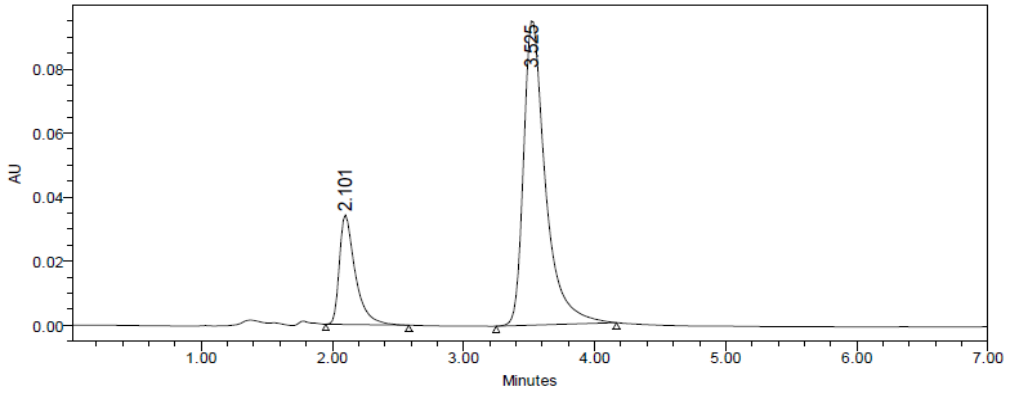

Fig-3: Chromatogram showing accuracy-50% injection

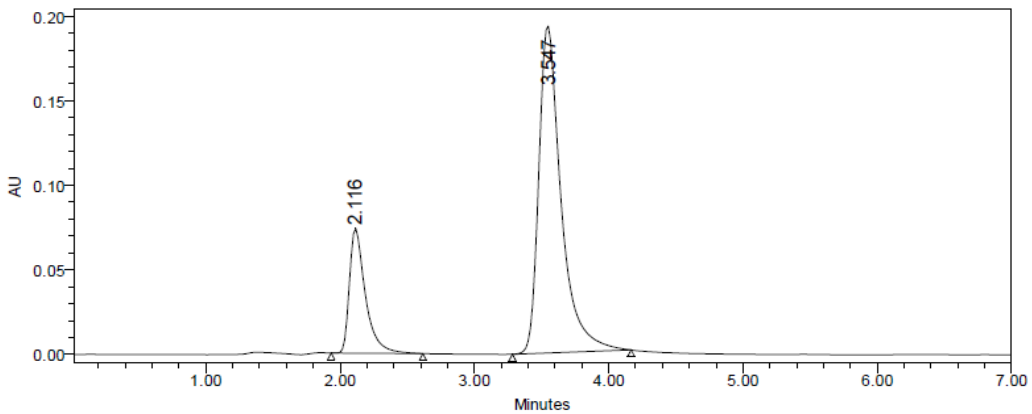

Fig 4: Chromatogram showing accuracy-100% injection

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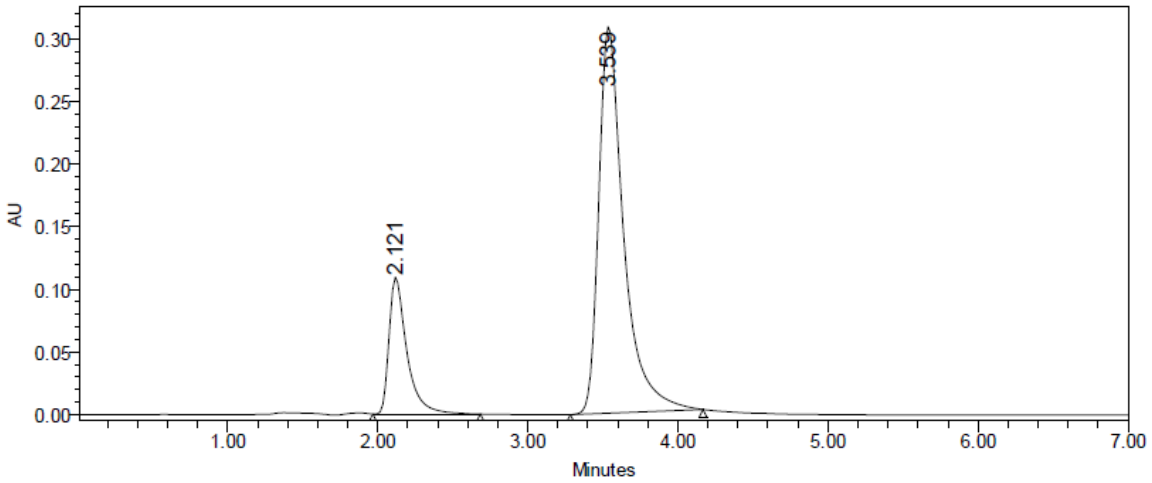

Fig-5: Chromatogram showing accuracy-150% injection

3.5. LIMIT OF DETECTION

Omeprazole := 1.6µg/ml

Cinitapride:= 0.8µg/ml

3.6. LIMIT OF QUANTITATION

Omeprazole := 5.6µg/ml

Cinitapride= 2.4µg/ml

3.7. ROBUSTNESS

Table7-: Results for Omeprazole Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	429069	3.853	5224	1.59
Less Flow rate of 0.9 mL/min	472673	4.426	6328	1.58
More Flow rate of 1.1 mL/min	392497	3.415	6217	1.54
Less organic phase	391379	4.291	6996	1.61
More organic phase	391703	3.583	6120	1.50

Table 8-: Results for Cinitapride Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	658211	3.006	8793	1.2
Less Flow rate of 0.9 mL/min	621077	3.441	7269	1.3
More Flow rate of 1.1 mL/min	642190	2.663	9446	1.2
Less organic phase	542402	3.185	8126	1.1
More organic phase	642112	2.867	5854	1.3

4. CONCLUSION

The method was rigorously validated in accordance with International Conference on Harmonisation (ICH) guidelines. Key validation parameters assessed included specificity, linearity, precision, accuracy, and robustness. The developed RP-HPLC method demonstrated excellent resolution and peak symmetry for both omeprazole and cinitapride, ensuring accurate and reliable quantification. This method provides a robust analytical tool for the simultaneous determination of these drugs in bulk and pharmaceutical formulations, facilitating effective quality control and supporting the consistent quality of pharmaceutical products. The RP-HPLC method developed and validated in this study offers a precise and reliable approach for the simultaneous determination of omeprazole and cinitapride. The method, characterized by its effective separation and excellent resolution on a Hypersil C18 column, meets the stringent validation requirements set forth by ICH guidelines.

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