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A VALIDATED RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND CINITAPRIDE IN BULK AND COMBINED PHARMACEUITCAL DOSAGE FORMS

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INTRODUCTION

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase,* which carries it through a structure holding another material called the *stationary phase.* The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.[1]

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- Mobile phase: This phase is always composed of "liquid" or a "gaseous component."
- Separated molecules

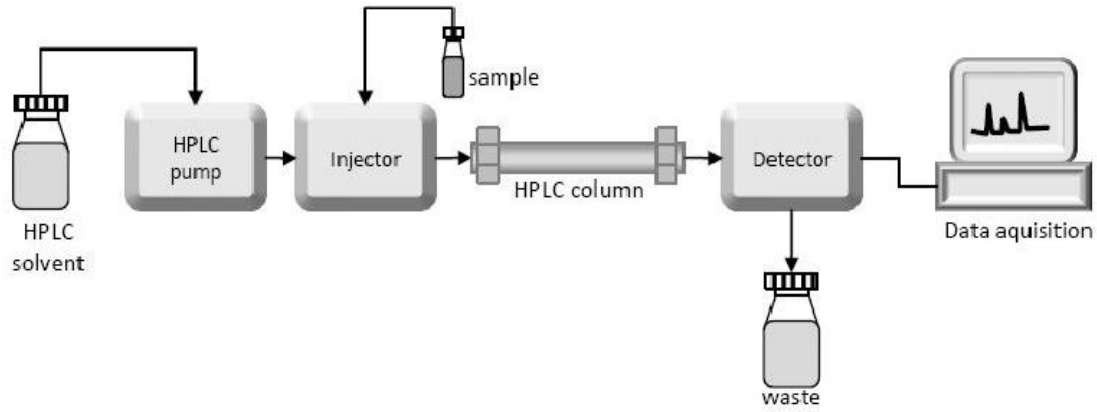
- Column chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) chromatography
- Affinity chromatography
- Paper chromatography
- Thin-layer chromatography

- Gas chromatography
- Dye-ligand chromatography
- Hydrophobic interaction chromatography
- Pseudoaffinity chromatography
- High-pressure liquid chromatography (HPLC)

High-prssure liquid chromatography (HPLC)

1. Normal Phase HPLC
2. Reverse Phase HPLC
3. Size-exclusion HPLC
4. Ion-Exchange HPLC

Instrumentation of HPLC



Drug Profile[22-24]

OMEPRAZOLE DRUG PROFILE

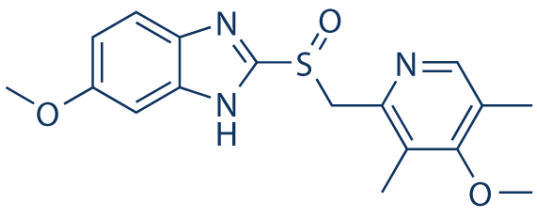
Drug : Omeprazole

Synonym :

- Omeprazole Magnesium
- Omeprazole Sodium
- Prilosec
- Sodium, Omeprazole
- H 168 68

Drug category : Used for the treatment of gastro esophageal reflux disease.

Structure :



Chemical name/ Nomenclature / IUPAC Name: 6-methoxy-2-[(4-methoxy-3, 5-dimethyl pyridin-2-yl) methane sulfinyl]-1H-1, 3-benzodiazole

Molecular Formula : $C_{17}H_{19}N_3O_3S$

Molecular Weight : 345.416 gm/mole.

Official Pharmacopoeia : USP, EP

Adverse effects/Side effects : Common side effects of omeprazole include:

- Headache.
- Abdominal Pain.
- Diarrhea.
- Nausea.
- Vomiting.

Mechanism of action: Hydrochloric acid (HCl) secretion into the gastric lumen is a process regulated mainly by the H(+)/K(+)-ATPase of the proton pump[10], expressed in high quantities by the parietal cells of the stomach. ATPase is an enzyme on the parietal cell membrane that facilitates hydrogen and potassium exchange through the cell, which normally results in the extrusion of potassium and formation of HCl (gastric acid).[9]

Omeprazole is a member of a class of antisecretory compounds, the substituted benzimidazoles that stop gastric acid secretion by selective inhibition of the H+ /K+ ATPase enzyme system. Proton-pump inhibitors such as omeprazole bind covalently to cysteine residues via disulfide bridges on the alpha subunit of the H+/K+ ATPase pump, inhibiting gastric acid secretion for up to 36 hours 11. This antisecretory effect is dose-related and leads to the inhibition of both basal and stimulated acid secretion, regardless of the stimulus**.**

Cinitapride Drug Profile[25-27]

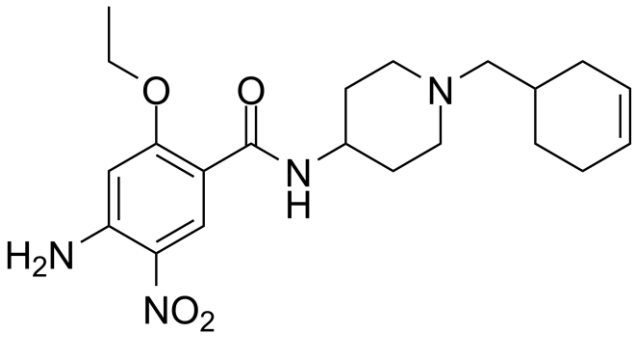
Drug : Cinitapride

Synonym :

- 4-Amino-N-(1-(3-Cyclohexen-1-Ylmethyl)-4-Piperidyl)-2-Ethoxy-5-Nitrobenzamide
- Blaston
- Cidine
- Cinitapride
- Cinitapride Tartrate

Drug category : Cinitapride is a gastroprokinetic agent and antiulcer agent of the benzamide class.

Structure :



Chemical name/ Nomenclature / IUPAC Name: 4-amino-N-{1-[(cyclohex-3-en-1-yl) methyl] piperidin-4-yl}-2-ethoxy-5-nitrobenzamide

Molecular Formula : $C_{21}H_{30}N_4O_4$

Molecular Weight : 402.4873 gm/mole.

Official Pharmacopoeia : USP

Melting point: >197°C (dec.)

pKa (Strongest Basic): 9.74

Log P: 2.79

Adverse effects/Side effects : Major & minor side effects for Cinitapride

- Diarrhea
- Drowsiness
- Extra pyramidal effects
- Itching
- Gynecomastia

Mechanism of action: Cinitapride is a substituted benzamide with 5-HT receptor antagonist and agonist activity.

Contraindications: Low Amount Of Magnesium In The Blood.

LITERATURE REVIEW

Niraimathi. V, et al., (2012): The present study involves the development of a simple, specific, accurate, rapid and cost effective RP-HPLC method assisted with UV detection for the estimation of Cinitapride (CNP) and Omeprazole (OME) in solid oral dosage forms. The method utilized C18 column (250x4.6 i.d 5µ particle size) and a mobile phase consisting of 40:20:40 ratio of Acetonitrile: methanol: phosphate buffer (pH-7). CNP was detected at 262 and OME at 301nm with a retention time of 6.5 and 3.2 minutes respectively. The chromatographic condition and polarity of the mobile phase were optimized. The drugs showed good linearity CNP at 84-132µg/ml and OME at 176-242µg/ml with a correlation coefficient of 1. The recovery percentage was found to be 99-100%. Thus the proposed method is simple, fast, and accurate and could be applied in the routine analysis of CNP and OME in pure and pharmaceutical dosage forms.

AIM AND OBJECTIVE

- Review of literature for Omeprazole and Cinitapride gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other Omeprazole and Cinitapride.
- Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Omeprazole and Cinitapride and single method is available for such estimation by RP-HPLC.

PLAN OF WORK

1. Collection of literature for the selected drug.
2. Extensive literature survey for selection of appropriate solvents to dissolve respective selected drug and preparation of stock solution.
3. Study of drug profile
4. Procurement of samples, standards and other chemicals.
5. Selection of chromatographic conditions
6. Selection of mobile phase
7. Method trials on HPLC by using different solvents and columns.
8. Development of RP-HPLC method which is different from the finished articles.

9. Optimization of the developed method by varying mobile phase conditions, temperature.

EXPERIMENTAL METHODS

Instruments Used

Table: Instruments used.

S.No.	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table: Chemicals used.

S.No	Chemical	Brand names
1	Omeprazole	Sura labs
2	Cinitapride	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

METHOD VALIDATION

Preparation of Buffer and Mobile Phase

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-5.2)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 5.2 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase

Accurately measured 250 ml (25%) of Methanol, 750 ml of Phosphate buffer (75%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Trails for Method Development

Trail 1:

Column : Zorbax C18 (4.6mm×250mm) 5µ particle size

Column temperature : 30ºC

Wavelength : 266nm

Mobile phase ratio : Methanol: Water (62:38% v/v)

Flow rate : 0.8ml/min

Injection volume : 10.00µl

Run time : 10 Minutes

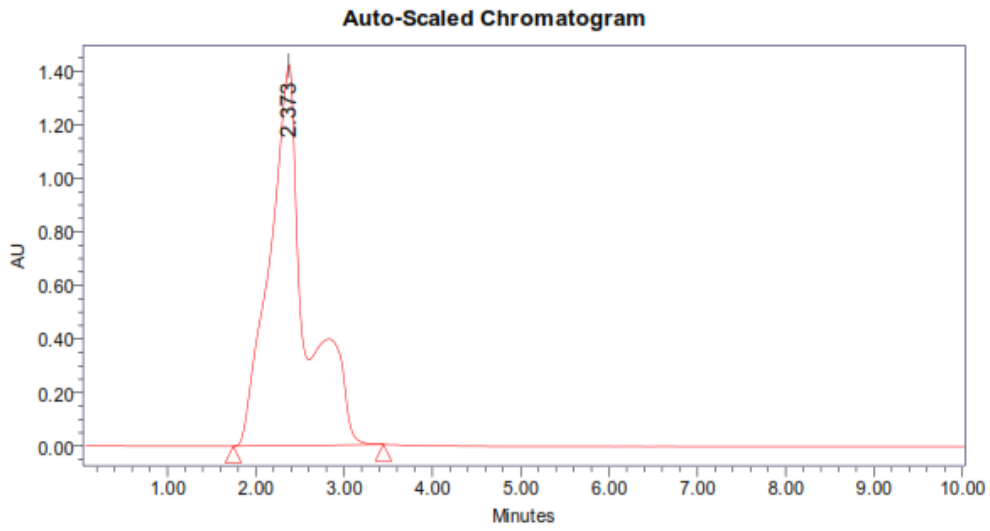


Fig.No.14: Chromatogram for Trail 1.

Table No-14: Peak Results for Trail 1

S.No.	Peak Name	R_t	Area	Height	USP Tailing	USP Plate count
1	Omeprazole	2.373	5263562	165955	1.08	1365

Trail 2:

Column : Develosil ODS C18 (4.6mm×250mm) 5µ

Column temperature : 36ºC

Wavelength : 266nm

Mobile phase ratio : Methanol: Acetonitrile (45:55% v/v)

Flow rate : 1.0ml/min

Injection volume : 20.00µl

Run time : 9.5Minutes

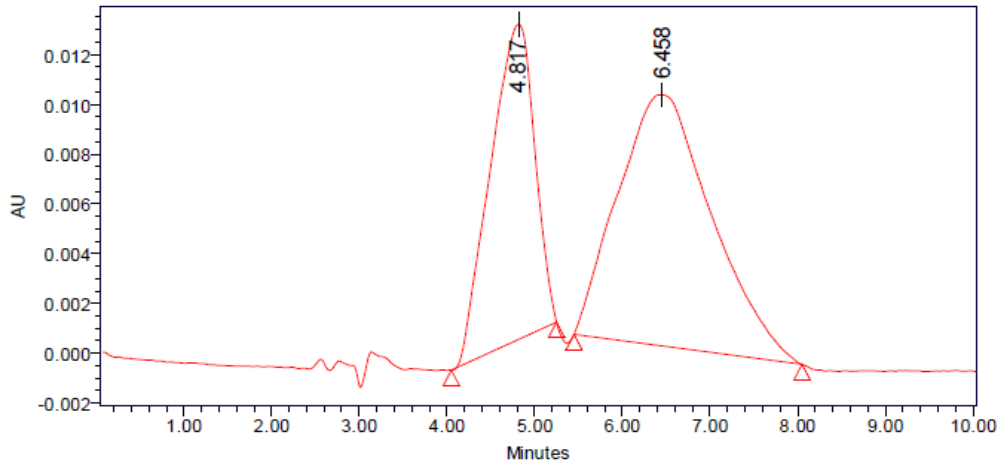


Fig.No.14: Chromatogram for Trail 2.

Table No-14: Peak Results for Trail 2.

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP plate count
1	Omeprazole	4.817	3865852	41565	0.96	3562
2	Cinitapride	6.458	69589451	5668	0.99	4758

Trail 3:

Column : Symmetry C18 (4.6mm×250mm) 5µ

Column temperature : 40ºC

Wavelength : 266nm

Mobile phase ratio : Methanol: Acetate Buffer (35:65% V/V)

Flow rate : 0.9ml/min

Injection volume : 20µl

Run time : 9.5minutes

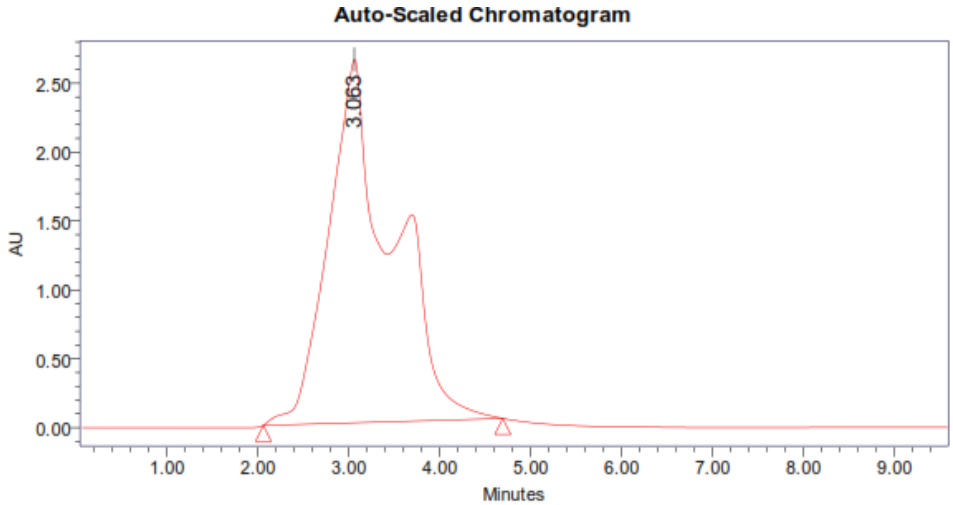


Fig.No.14: Chromatogram for Trail 3.

Table No-14: Peak Results for Trail 3.

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP Plate Count
1	Omeprazole	3.063	4365525	91568	1.14	5651

Trail 4:

Column : Agilent Zorbax (C18) (250mm x 4.6mm, 5µm) column

Column temperature : 35ºC

Wavelength : 266nm

Mobile phase ratio : Methanol: Phosphate Buffer (pH-4.8) (40:60v/v)

Flow rate : 1.0ml/min

Injection volume : 20µl

Run time : 7.5minutes

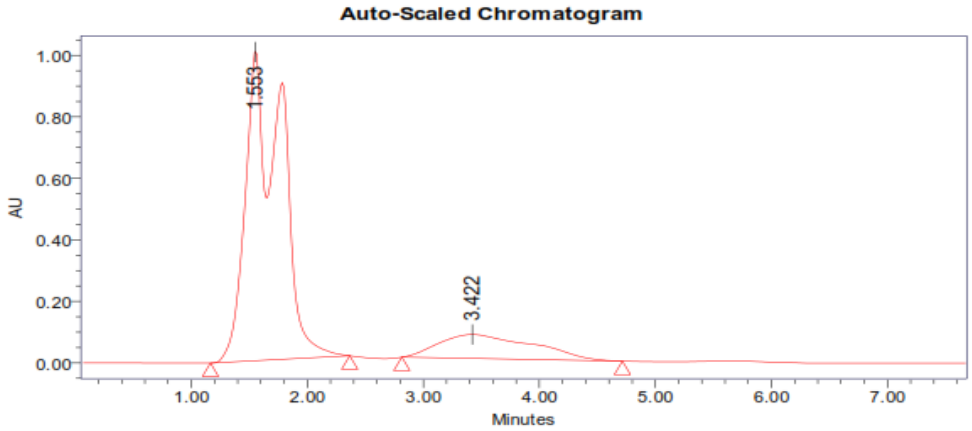


Fig.No.14: Chromatogram for Trail 4.

Table No-14: Peak Results for Trail 4.

S. No.	Peak Name	R_t	Area	Height	USP Tailing	USP Plate Count
1	Omeprazole	1.553	189856952	6985454	2.68	5628
2	Cinitapride	3.422	6452154	52465	1.07	6454

Optimized Chromatogram (Standard)

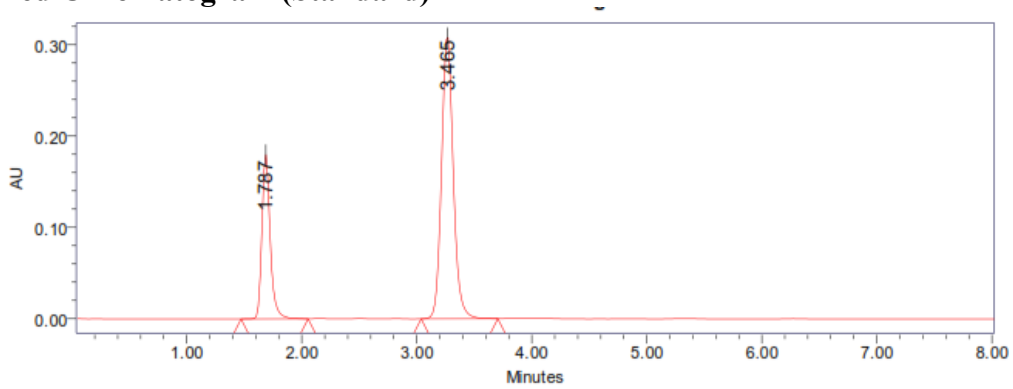


Fig.No.18: Optimized Chromatogram (Standard).

Table No.18: Optimized Chromatogram (Standard).

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Omeprazole	1.787	545265	7462	1.09	7564
2	Cinitapride	3.465	7768545	43652	1.12	8695

Optimized Chromatogram (Sample)

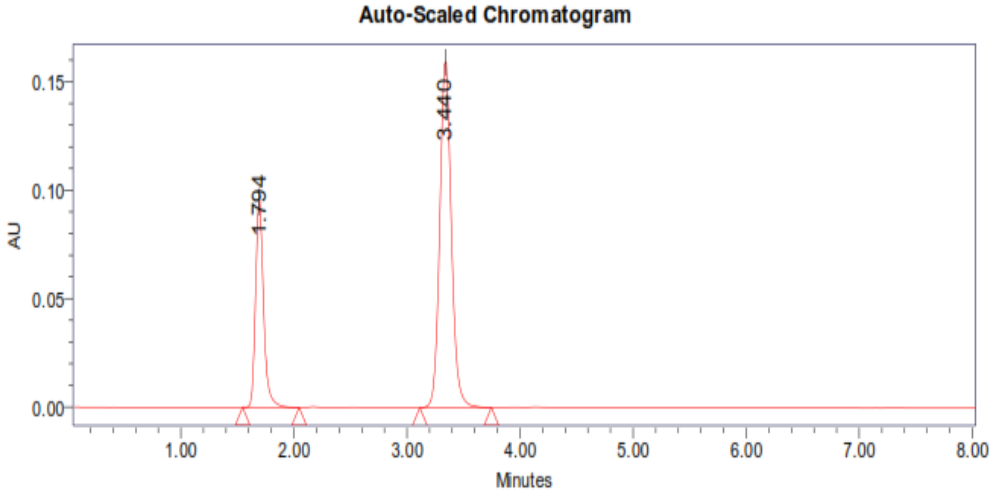


Fig.No.19: Optimized Chromatogram (Sample).

Table No. 19: Optimized Chromatogram (Sample).

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Omeprazole	1.794	558659	7584	1.10	7659
2	Cinitapride	3.440	7856985	44658	1.13	8743

METHOD VALIDATION

Blank

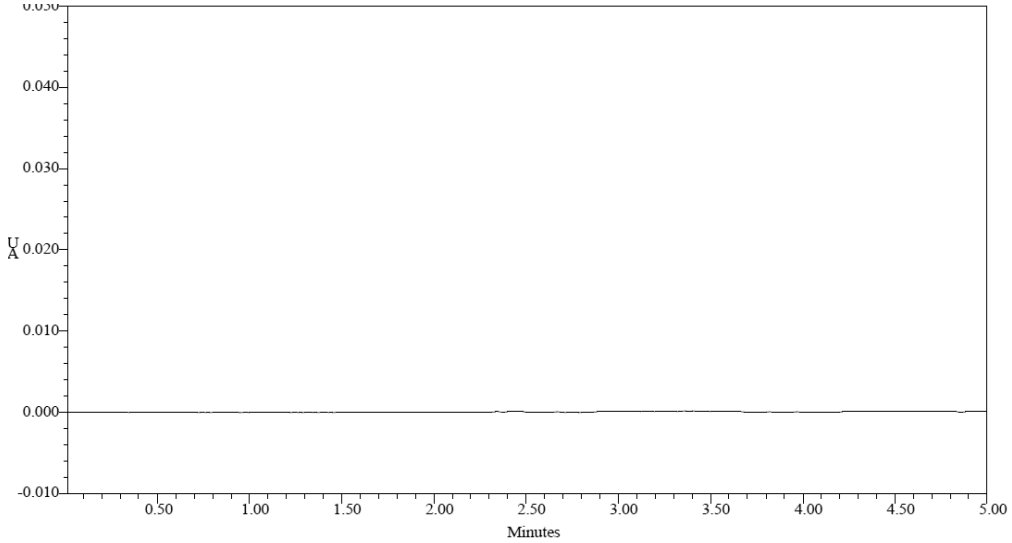


Fig.No.20: Chromatogram showing blank (mobile phase preparation)

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 quantities Omeprazole and Cinitapride in drug product.

Assay (Standard)

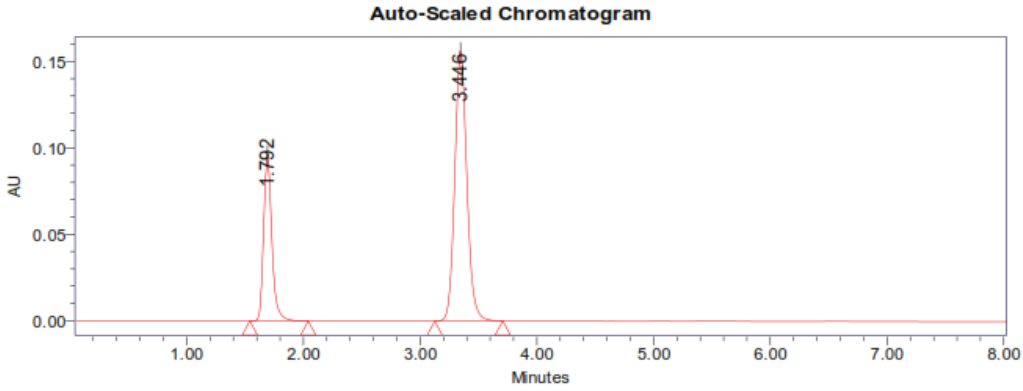


Fig.No.21: Chromatogram showing assay of standard injection -1.

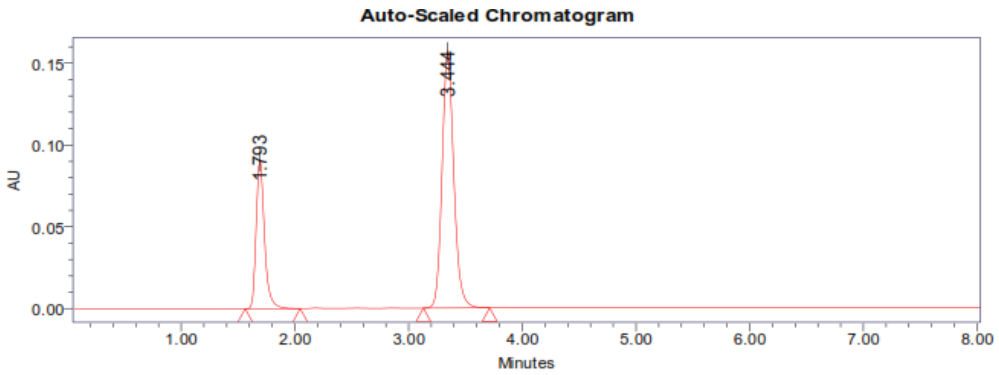


Fig.No.22: Chromatogram showing assay of standard injection -2.

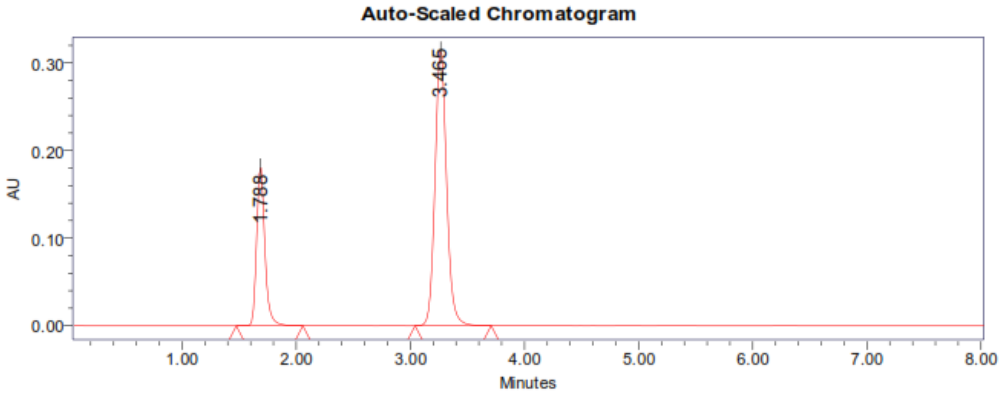


Fig.No.23: Chromatogram Showing Assay of Standard Injection -3.

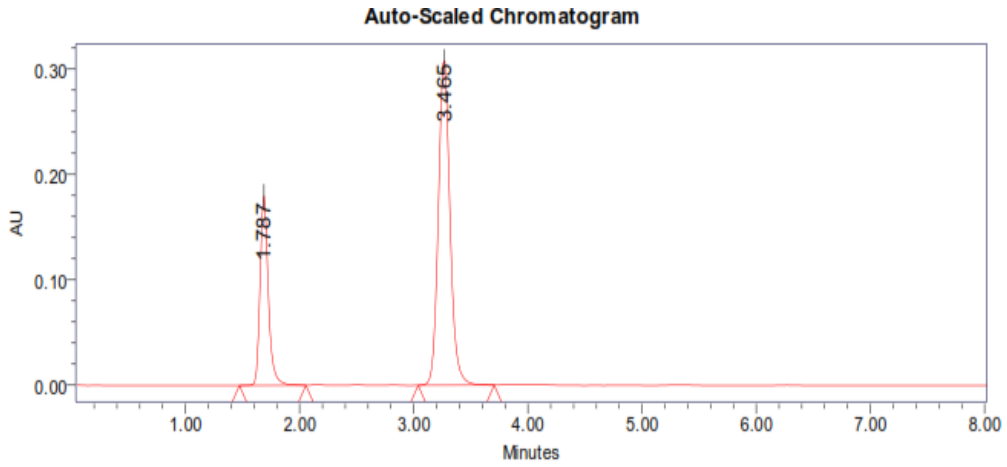


Fig.No.24: Chromatogram showing assay of standard injection -4.

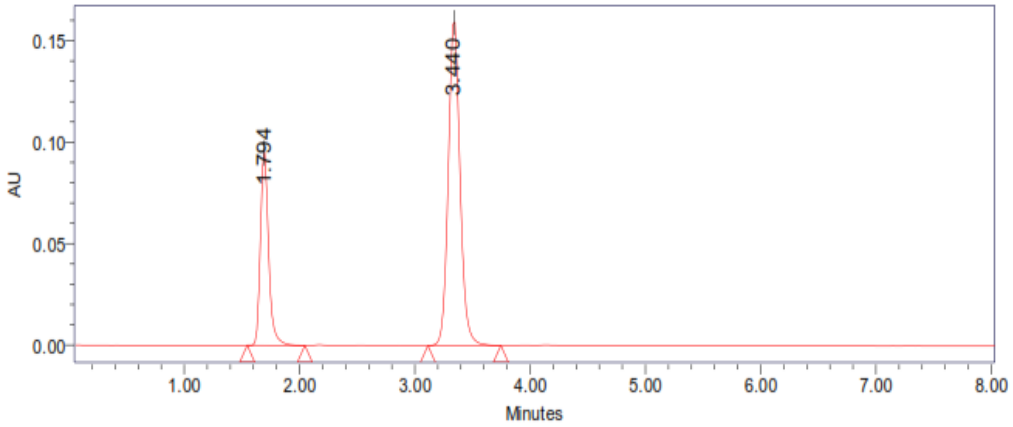


Fig.No.25: Chromatogram showing assay of standard injection -5

Table No. 20: Peak results for assay standard of Omeprazole

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Omeprazole	1.788	545698	7458	7595	1.09
2	Omeprazole	1.792	548765	7469	7548	1.10
3	Omeprazole	1.793	548965	7428	7563	1.09
4	Omeprazole	1.788	548783	7495	7592	1.10
5	Omeprazole	1.787	548752	7461	7543	1.09
Mean			548192.6			
Std. Dev.			1397.209			
% RSD			0.254876			

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 No.21: Peak results for assay standard of Cinitapride.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Cinitapride	3.438	7785698	43652	8652	1.12
2	Cinitapride	3.446	7786354	43698	8674	1.13
3	Cinitapride	3.444	7786942	43587	8692	1.13
4	Cinitapride	3.465	7785464	43698	8649	1.12
5	Cinitapride	3.465	7785986	43568	8625	1.12
Mean			7786089			
Std. Dev.			581.3667			
% RSD			0.007467			

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.

Assay (Sample)

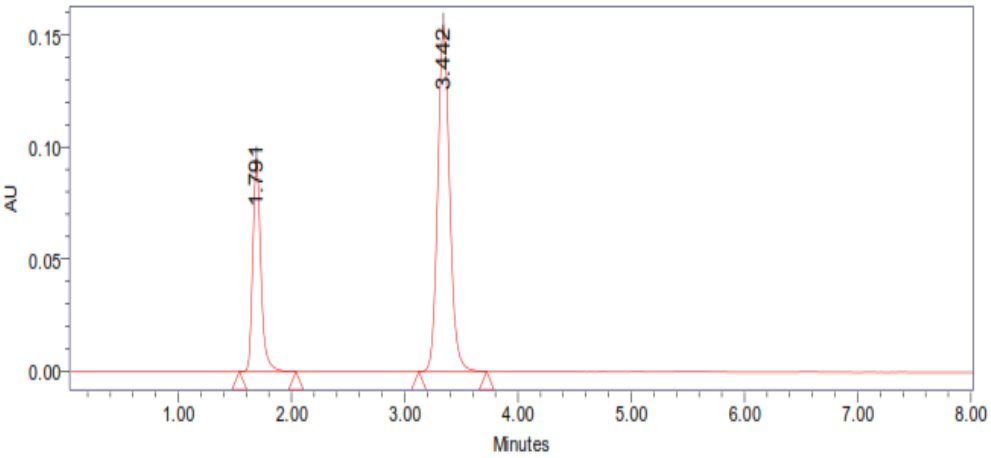


Fig.No.26: Chromatogram showing assay of sample injection-1.

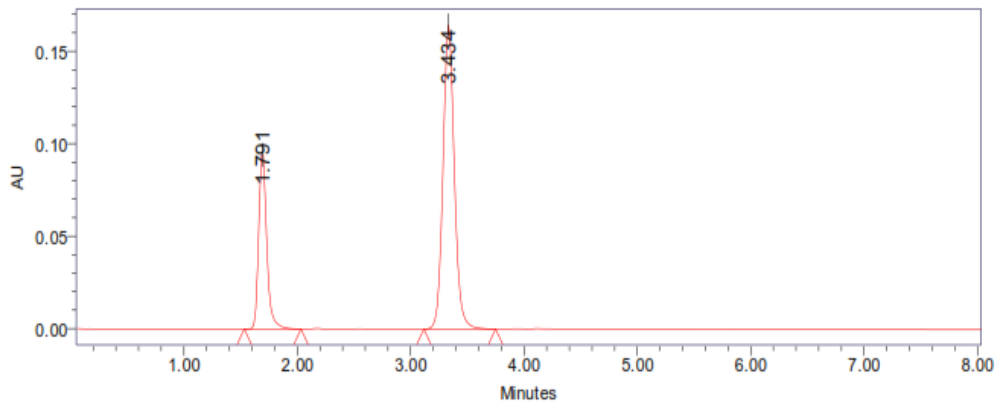


Fig.No.27: Chromatogram showing assay of sample injection-2.

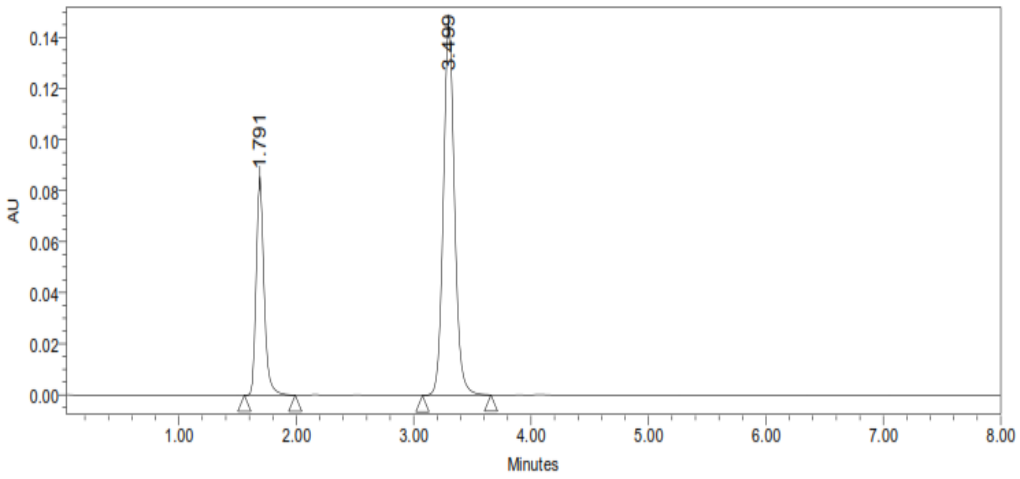


Fig.No.28: Chromatogram showing assay of sample injection-3.

Table No.22: Peak results for Assay sample of Omeprazole.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Omeprazole	1.794	556985	75895	1.10	7698	1
2	Omeprazole	1.791	558742	75468	1.10	7682	2
3	Omeprazole	1.791	559683	75426	1.11	7649	3

Table No.23: Peak results for Assay sample of Cinitapride.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Cinitapride	3.440	7856859	44586	1.14	8759
2	Cinitapride	3.442	7826594	44658	1.15	8726
3	Cinitapride	3.434	7854879	44859	1.14	8794

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

The % purity of Omeprazole and Cinitapride in pharmaceutical dosage form was found to be 100.154%

LINEARITY

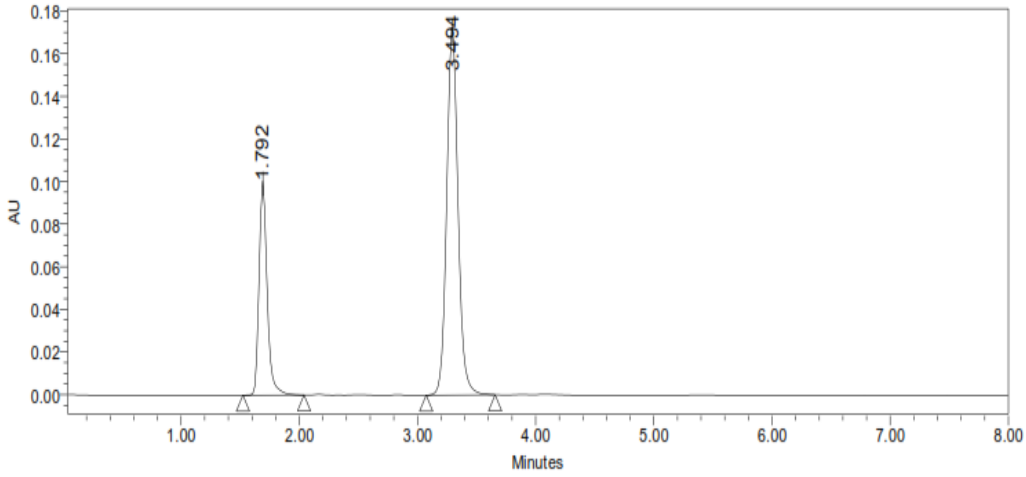


Fig.No.34: Chromatogram showing linearity level-1.

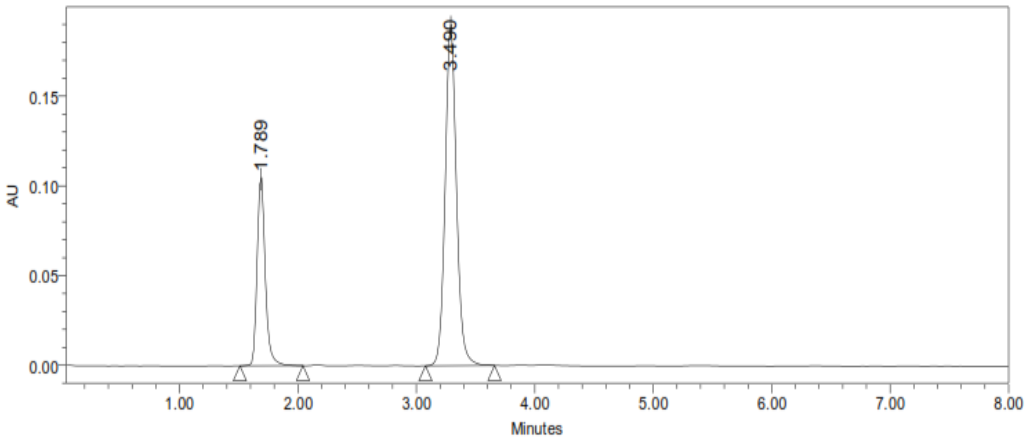


Fig.No.35: Chromatogram showing linearity level-2.

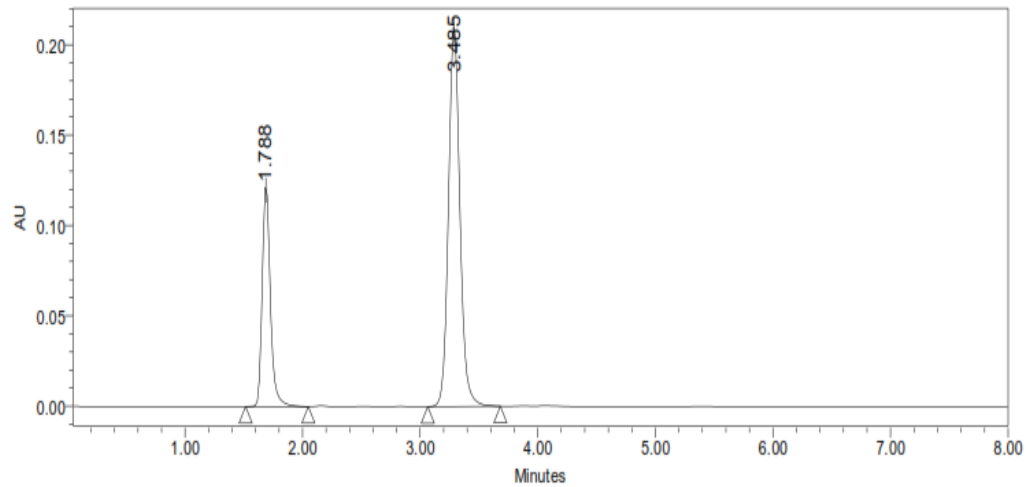


Fig.No.36: Chromatogram showing linearity level-3.

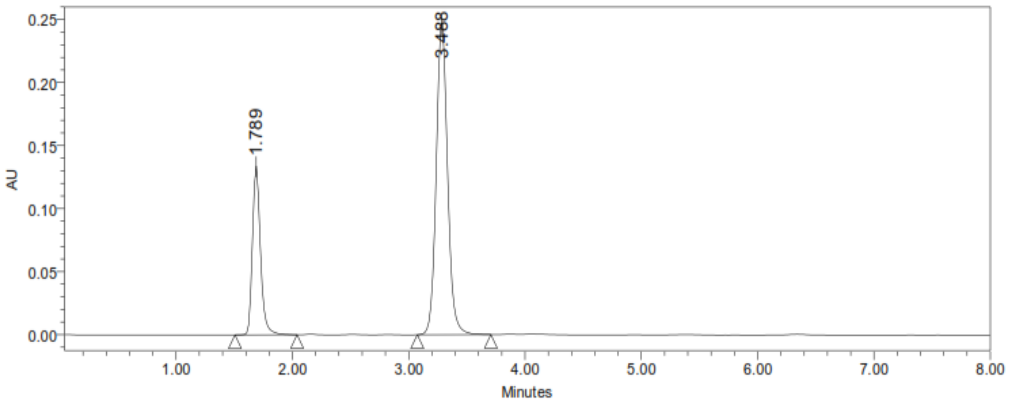


Fig.No.37: Chromatogram showing linearity level-4.

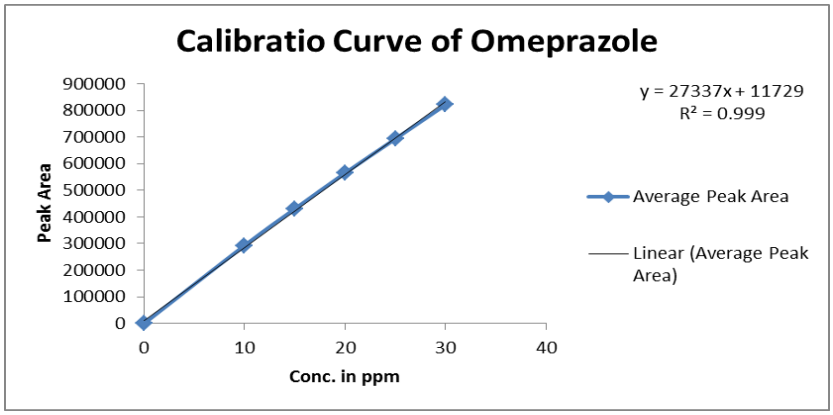


Fig.No.38: Chromatogram showing linearity level-5.

Table No. 25: Chromatographic Data For Linearity Study For Omeprazole.

Concentration µg/ml	Average Peak Area
10	292985
15	430752
20	565265
25	693487
30	821584

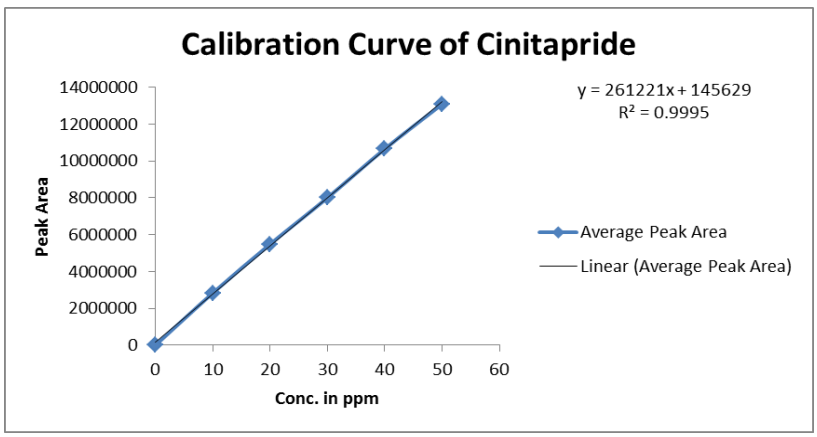


Fig.No.39: Chromatogram showing linearity level.

LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Omeprazole is a straight line.

$$Y = mx + c$$

Slope (m) = 27337

Intercept (c) = 11729

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 11729. These values meet the validation criteria.

Table No. 26: Chromatographic Data For Linearity Study For Cinitapride.

Concentration µg/ml	Average Peak Area
10	2828756
20	5485784
30	7999859
40	10656542
50	13085985

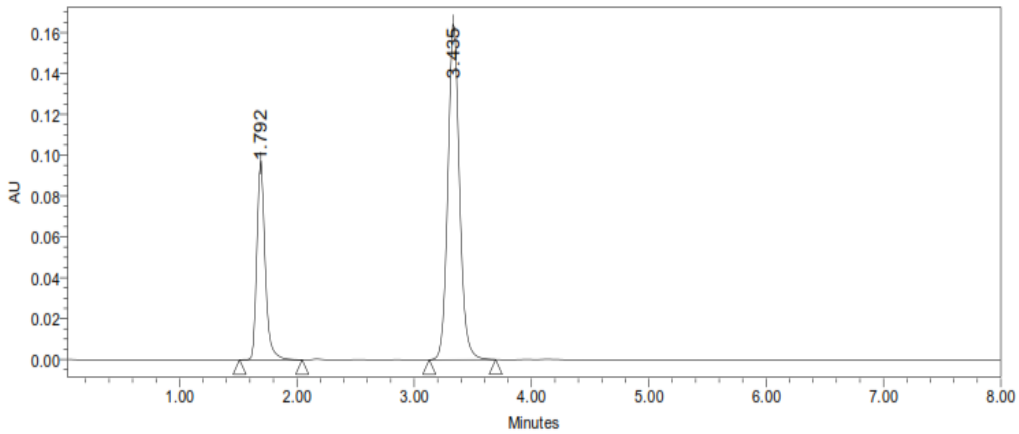


Fig.No.40: Chromatogram showing linearity level.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

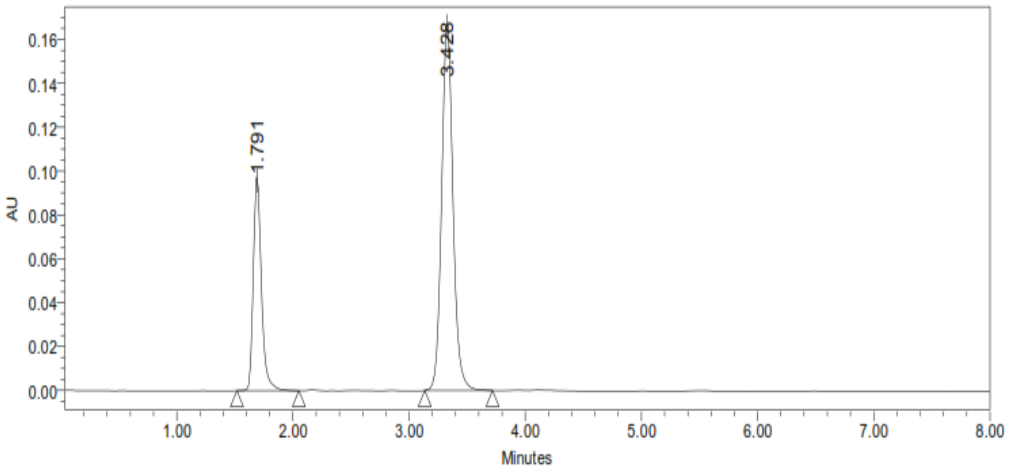


Fig.No.41: Chromatogram showing precision injection -1.

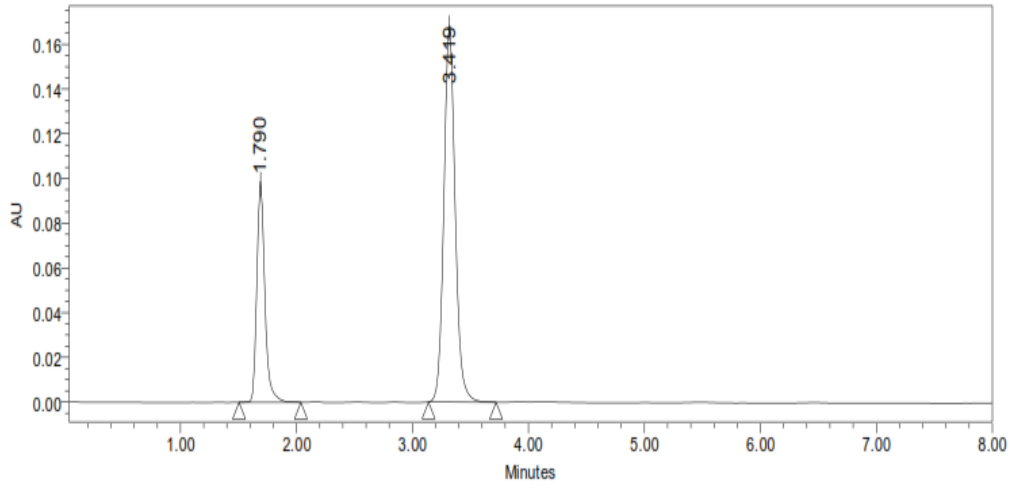


Fig.No.42: Chromatogram showing precision injection -2.

Table 27: Results of Repeatability for Omeprazole.

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Omeprazole	1.792	548698	7458	7569	1.10
2	Omeprazole	1.791	548955	7485	7546	1.10
3	Omeprazole	1.790	548745	7469	7592	1.09
4	Omeprazole	1.790	549856	7463	7519	1.10
5	Omeprazole	1.789	546587	7495	7535	1.09
Mean			548568.2			
Std.dev			1202.217			
%RSD			0.2191554			

Table No. 28: Results of Repeatability for Cinitapride:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Cinitapride	3.435	7768958	43659	8659	1.12
2	Cinitapride	3.428	7765984	43856	8647	1.13
3	Cinitapride	3.419	7785469	43658	8675	1.12
4	Cinitapride	3.414	7785498	43549	8652	1.12
5	Cinitapride	3.408	7769852	44526	8692	1.13
Mean			7775152			
Std.dev			9539.236			
%RSD			0.122689			

Intermediate precision

Day 1.

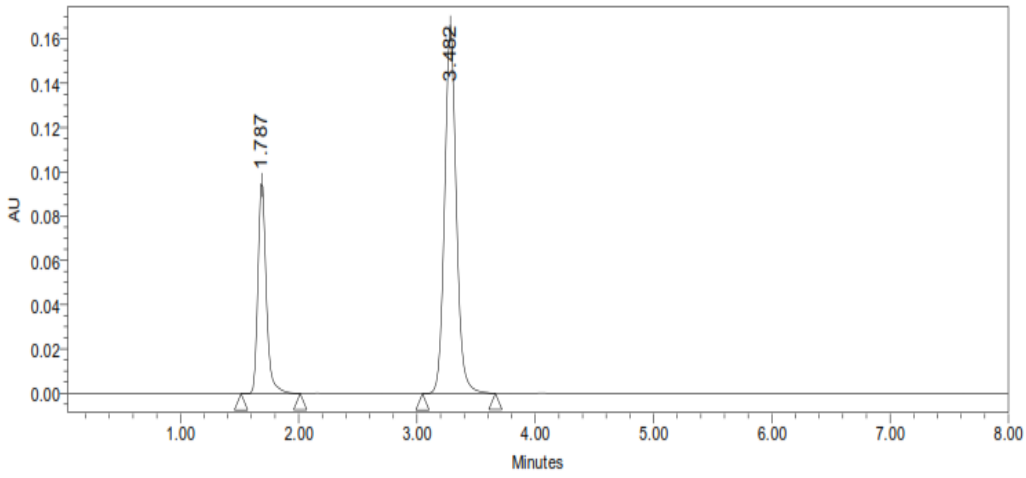


Fig.No.46: Chromatogram showing Day1 injection -1.

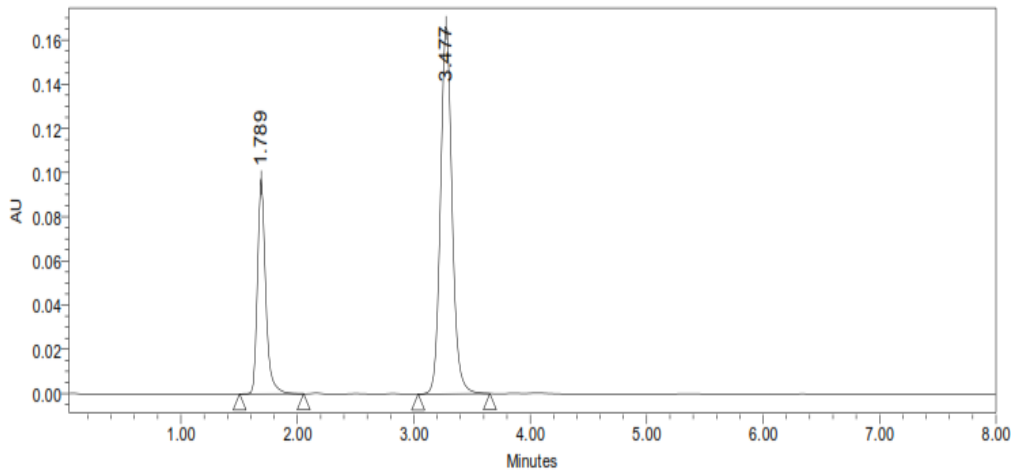


Fig.No.47: Chromatogram showing Day1 injection -2.

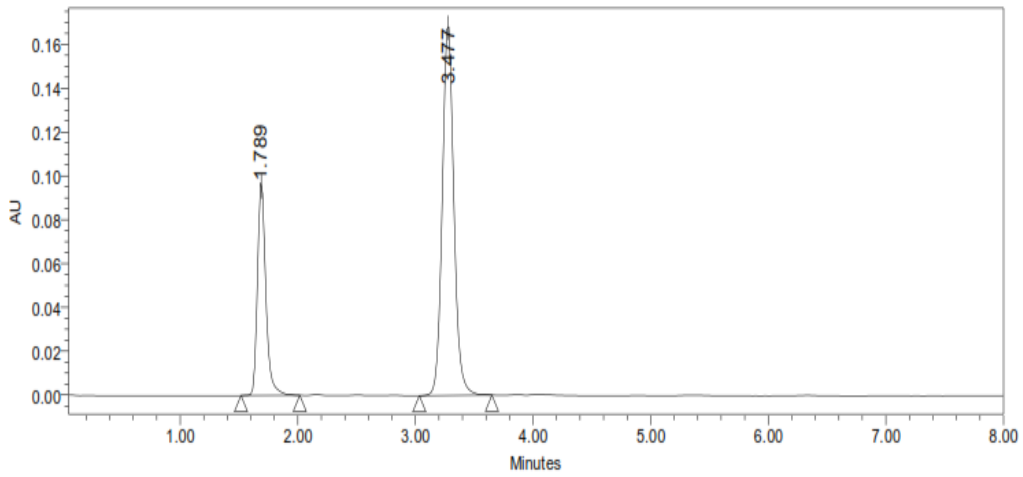


Fig.No.48: Chromatogram showing Day1 injection -3.

Day 2:

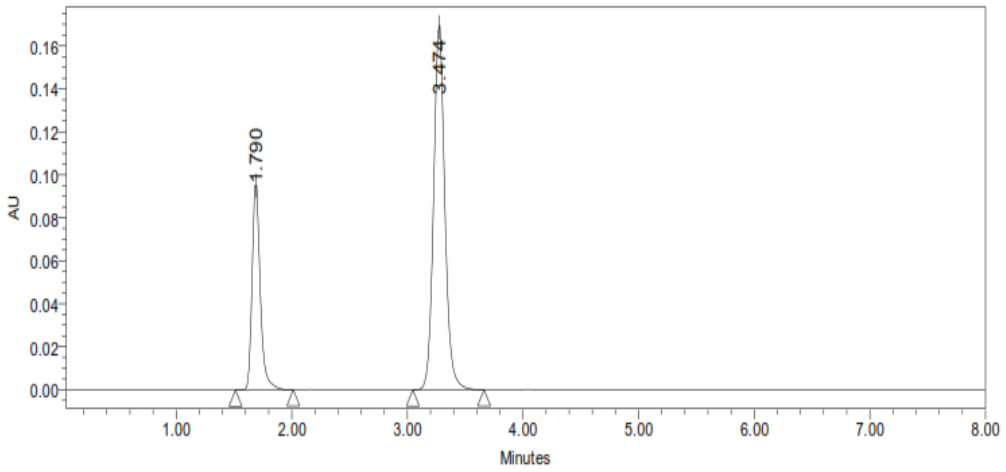


Fig.No.52: Chromatogram showing Day 2 injection -1

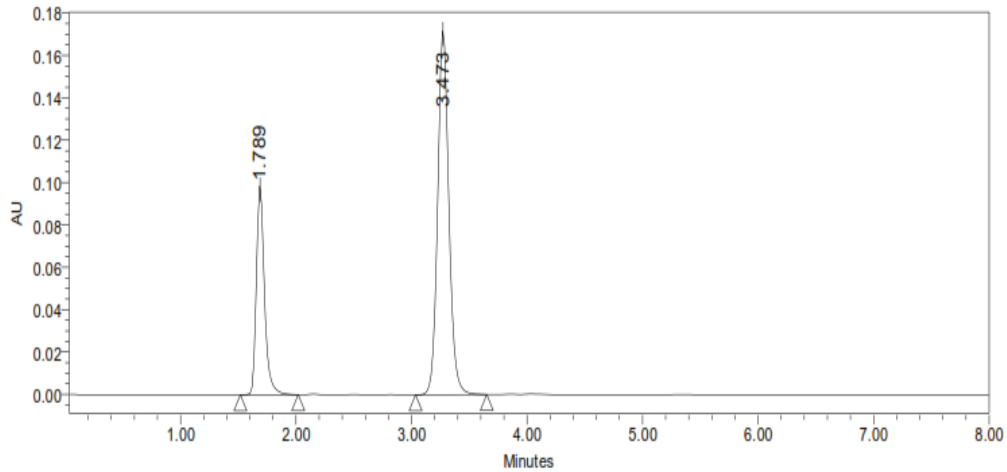


Fig.No.53: Chromatogram showing Day 2 injection -2.

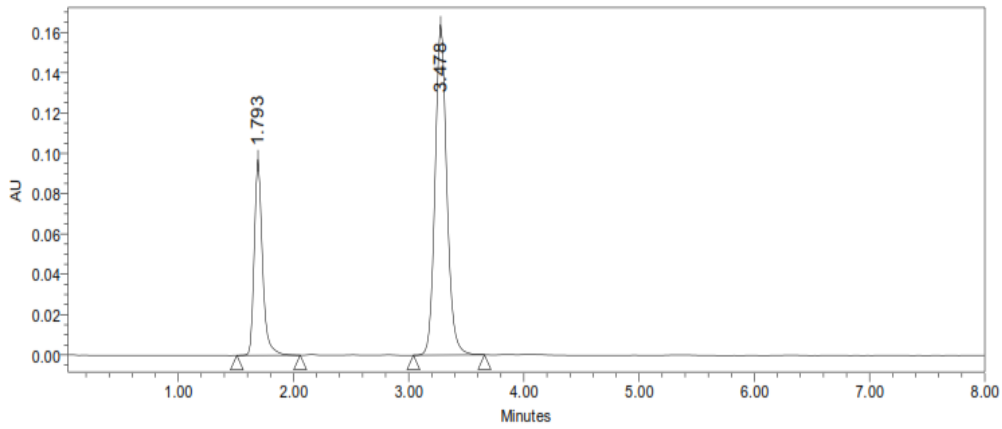


Fig.No.54: Chromatogram showing Day 2 injection -3.

6.3.4: ACCURACY

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

Accuracy 50%

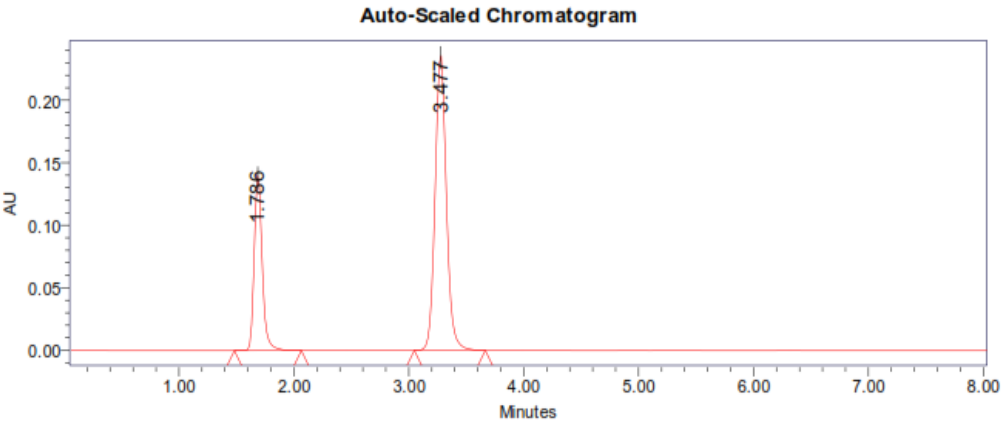


Fig.No.58: Chromatogram showing accuracy-50% injection-1.

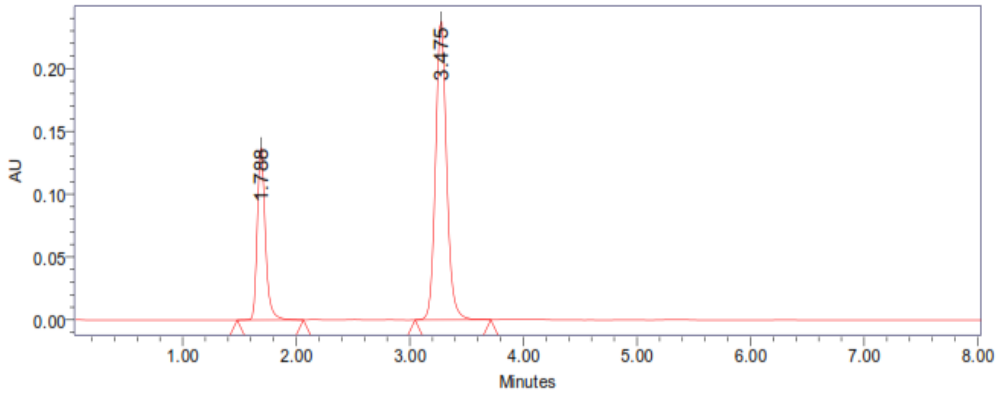


Fig.No.59: Chromatogram showing accuracy-50% injection-2.

Accuracy 100%

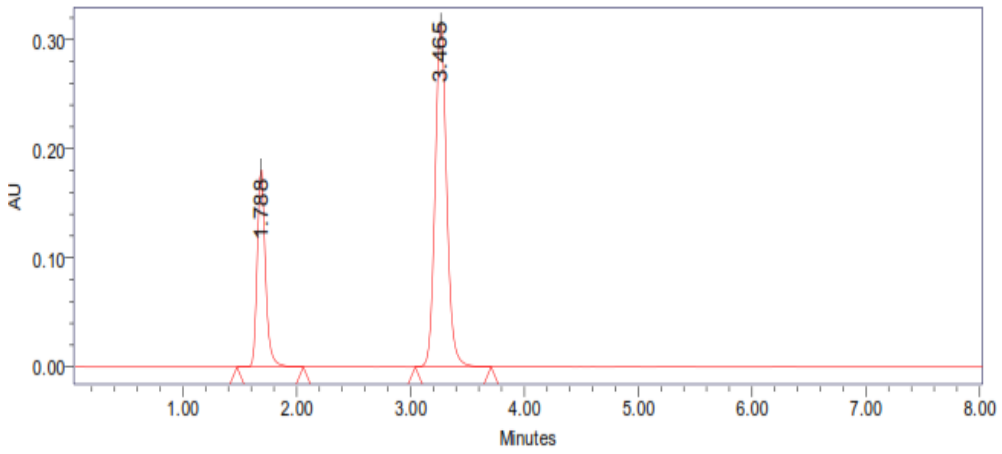


Fig.No.61: Chromatogram showing accuracy-100% injection-1.

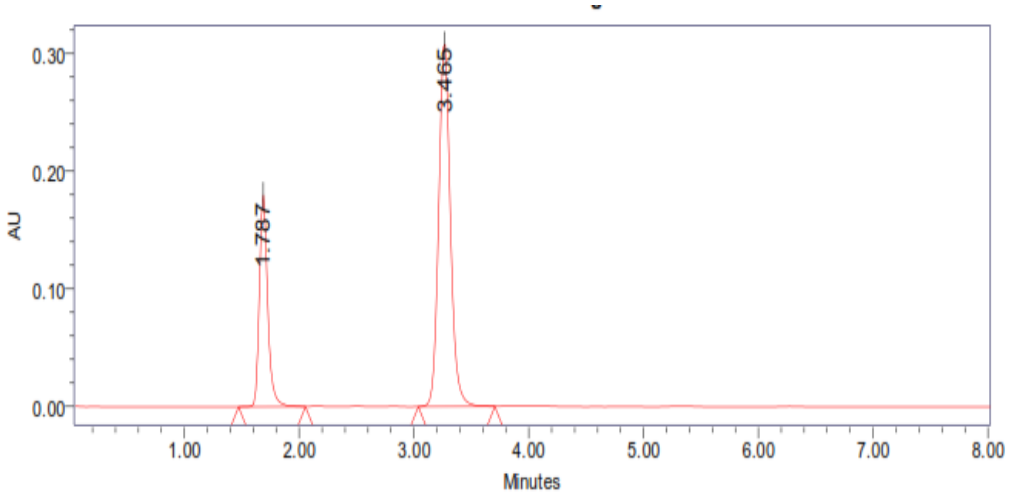


Fig.no.62: Chromatogram showing accuracy-100% injection-2.

Accuracy 150%

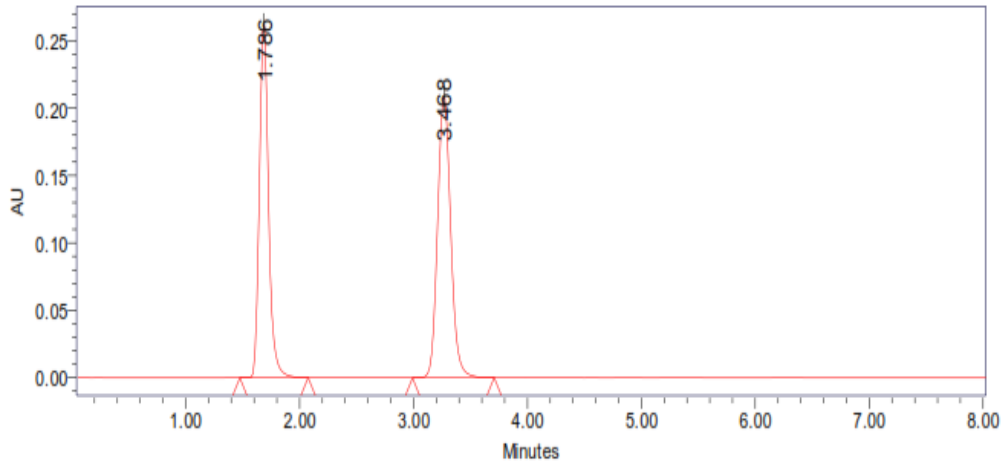


Fig.No.64: Chromatogram showing accuracy-150% injection-1.

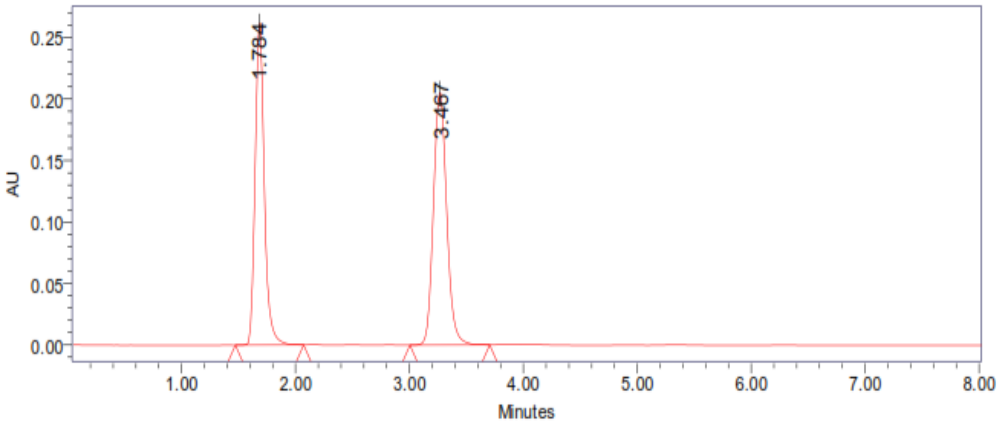


Fig.No.65: Chromatogram showing accuracy-150% injection-2

Table No. 36: The accuracy results for Omeprazole.

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

LIMIT OF DETECTION FOR OMEPRAZOLE AND CINITAPRIDE

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.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Omeprazole

=0.86µg/ml

Cinitapride

=1.28µg/ml

Quantitation Limit for Omeprazole and Cinitapride

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

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Omeprazole

=2.58µg/ml

Cinitapride

= 3.84µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Omeprazole and Cinitapride. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Omeprazole and Cinitapride were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate coun.

Table No. 38: Results for Robustness –Omeprazole.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	545265	1.787	7564	1.09
Less Flow rate of 0.8mL/min	625486	1.867	7856	1.13
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	526548	1.744	7425	1.12
Less organic phase (about 5 % decrease in organic phase)	536548	1.831	7265	1.06
More organic phase (about 5 % Increase in organic phase)	514875	1.874	7169	1.08

Table No. 39: Results for Robustness-Cinitapride.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	7768545	3.465	8695	1.12
Less Flow rate of 0.8mL/min	7985695	3.721	8948	1.13
More Flow rate of 1.0mL/min	7458642	3.097	8452	1.12
Less organic phase (about 5 % decrease in organic phase)	7685421	6.242	8365	1.10
More organic phase (about 5 % Increase in organic phase)	7569864	2.402	8254	1.09

SUMMARY AND CONCLUSION

A new method was established for simultaneous estimation of Omeprazole and Cinitapride by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Omeprazole and Cinitapride by using Agilent Zorbax (C18) (250mm x 4.6mm, 5µm) column, flow rate was 1ml/min, mobile phase ratio was (25:75 v/v) Methanol: Phosphate Buffer (pH-5.2 was adjusted with orthophosphoric acid), detection wave length was 266nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 1.787mins and 3.465mins. The % purity of Omeprazole and Cinitapride was found to be 100.154%. The system suitability parameters for Omeprazole and Cinitapride such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Omeprazole and Cinitapride was found in concentration range of 10µg-30µg and 10µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.291% and 100.163%, %RSD for repeatability was 0.219 and 0.122. The precision study was precise, robust, and repeatable. LOD value was 0.86 and 1.28, and LOQ value was 2.58 and 2.84 respectively.

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