Development and Validation of a Rapid RP HPLC Method for the Determination of Cinitapride Hydrogen Tartarate in Solid Oral Dosage Forms

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Abstract: In the present study a simple, sensitive rapid and accurate HPLC method with UV detection for the analysis of cinitapride hydrogen tartarate was developed and validated in solid dosage forms. The method utilized gradient elution technique with C18 column (150x4.6 mm I.D, 5 μ m particle size) with mobile phase consisting of 0.1% formic acid in water and acetonitrile. The detection wavelength was at 268 nm, with flow rate of 0.5 mL/min and injection volume of 10 μ L for separation of cinitapride in bulk drugs and pharmaceutical formulations. The gradient elution was developed for better and optimized results. The developed method was validated for precision which includes system precision and method precision, accuracy and linearity studies in the concentration range of 5-100 μ g/mL with correlation coefficient of 0.9987. The accuracy (recovery) was between 97.32 and 100.82%. The proposed method is simple, fast, accurate, precise and reproducible, hence can be applied for routine quality control analysis of cinitapride in pure and pharmaceutical dosage forms.

Keywords: Liquid chromatography, Validation, Cinitapride

Introduction

Cinitapride¹⁻², chemically 4-amino-N-[3-(Cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide has the molecular formula $C_{21}H_{30}N_4O_4$ and molecular weight 402.49 g.mol⁻¹. Cinitapride is a drug that has against action to the serotonergic 5-HT₂ and D₂ dopaminergic

receptors that has been indicated in the gastro-esophageal reflux and in the functional disorders of gastrointestinal motility treatment. The therapeutic effect of cinitapride lies on the capacity of increasing lower esophageal sphincter tone and has strong gastro kinetic activity, which generates significant increases in the gastric emptiness; besides, through the serotonergic system it stimulates the intestinal activity. The use of cinitapride is efficient and safe in treatment of patients with disorders in the gastric emptiness related to gastro esophageal reflux and functional dyspepsia as well as in individuals that present irritable bowel syndrome with constipation and abdominal pain. Literature survey reveals a polarographic³ method for its determination, a fast, sensitive and selective method for measuring plasma cinitapride using LC-MS/MS with positive ion electrospray ionization using multiple reaction-monitoring (MRM) mode to quantify cinitapride in human plasma using respridone as the internal standard⁴, an RP-HPLC method⁵, and UV spectrophotometric method⁶ for the determination of cinitapride in pharmaceutical formulations is also reported. The target of this study was to develop and validate a new, simple and rapid HPLC method with gradient elution technique for determination of cinitapride in bulk drugs and pharmaceutical formulations.

Experimental

Cinitapride was kindly supplied by chromo labs, Hyderabad, India. Formic acid and acetonitrile (HPLC grade) were purchased from Merck, Mumbai, India. All other chemicals used were of analytical grade. Double distilled water was used during the entire HPLC procedure.

Chromatographic conditions and instrumentation

High performance liquid chromatograph was carried out on Shimadzu UFLC prominence with LC20AD quaternary pump, SIL 20 An autosampler and photo diode array detector PDA (SPD-M20A)

The analytical column with 150×4.6 mm *i.d* and 5 μ particle size has been optimized to obtain the best possible results. A mixture of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) was used as mobile phase. The gradient elution programme has been developed and optimized for better, accurate and consistent results. The gradient programme developed for this method is depicted below.

Time, min	Conc., 'A'	Conc., 'B'
0.01	90	10
5.00	70	30
8.00	30	70
16.00	30	70
20.00	70	30
25.00	90	10
30.00	90	10

Preparation of standard stock solution

Accurately weighed 50 mg of cinitapride standard was taken in 100 mL volumetric flask. This was dissolved in few mL of methanol and sonicated for five minutes and diluted up to the mark with same solvent to get 500 μ g/ mL standard stock solution.

Working standard solution

2 mL of the above stock solution was taken in 10 mL of volumetric flask and made up to the volume with methanol to obtain 100 μ g/mL solution. Several aliquots of standard stock solutions 0.1, 0.25, 0.5, 1.0 and 2.0 mL of cinitapride were taken in different 10 mL

volumetric flasks and diluted up to the mark with methanol. Peak area was recorded for all the peaks and calibration graph (Figure 1 & 2) was obtained by plotting peak area *versus* concentration of cinitapride. The plot of peak area of each sample against respective concentration of cinitapride was found to be linear in the concentration range of 5-100 µg/mL and the results are furnished in Table 1. The solutions were injected into the 10 µL loop and the chromatogram was recorded as Figure 1

Table 1. Calibration of the proposed HPLC method for the estimation of cinitapride

Conc. in µg/mL	Peak area ratio
5	344398
12.5	812936
25	1497830
50	2967969
100	6473884

Regression equation $y = 64143.26x - 43697.86$ (Correlation coefficient $R = 0.9987$)

Figure 1. Typical chromatogram of cinitapride

Figure 2. Calibration curve of cinitapride

Assay

Two commercial brand tablets were chosen for testing suitability of proposed method to estimate cinitapride in pharmaceutical dosage forms. Twenty tablets were weighed accurately and powdered. A quantity equivalent to 50 mg of cinitapride was weighed accurately and transferred to 100 mL volumetric flask. About 30 mL of methanol was added and kept in ultrasonic bath for 20 min. This solution is filtered through membrane filter and volume was made up to the mark with methanol to get 500 µg/mL concentration. Solution obtained was diluted with methanol to obtain in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and chromatogram was recorded. The amount of cinitapride present in the tablet formulation was determined by comparing the peak area from the standard. The results are furnished in Table 2

Table 2. Assay and recovery studies

Formulation	Label claim	Amount found, mg	%Amount found	%Recovery
Brand -1	2.5	2.49	99.72	100.12
Brand-2	5.0	4.98	99.70	100.10

Validation of the proposed method

Selectivity of the method was assessed on the basis of elution of cinitapride using above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters has been validated for the determination of cinitapride. The results are furnished in Table 3.

Table 3. System suitability parameters

S No	Parameter	Result
1	Linearity, µg/mL	5-100 µg/mL
2	Correlation coefficient	0.9987
3	Theoretical plates	5877
4	Tailing factor	0.98
5	Asymmetry factor	1.09
6	LOD, µg/mL	0.048
7	LOQ, µg/mL	0.678
8	Percentage recovery	98.58-100.24

Specificity study

The evaluation of the specificity of the method was determined against a placebo. The interference of the claimed excipients present in the pharmaceutical dosage form was derived using placebo solution.

Linearity

A 5-point calibration curve was obtained in the concentration range of 5-100 µg/mL for cinitapride hydrogen tartarate. The peak area versus concentration data were evaluated by linear regression analysis. It was found that correlation coefficient and regression analysis are within the limits.

Precision

The method precision has been studied and results obtained for five preparations are tabulated (Table 4) along with calculations for mean, standard deviation and % relative standard deviation. The system precision was studied by preparing the standard solution at test concentration *i.e.*, 100 µg/mL and injected repeatedly for 5 times. The obtained results are shown in Table 5

Table 4. Method precision

Run	%Area for cinitapride
1	6605666
2	6604918
3	6642417
4	6606552
5	6621343

Mean= 6616179.2, *Standard Deviation*= 21437.391, *%RSD* 0.3239

Table 5. System precision

Run	% Area for cinitapride
1	6513992
2	6492545
3	6611946
4	6492343
5	6513675

Mean=6524900.2, *Standard Deviation* =63654.74416, *%RSD* 0.97

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for cinitapride were predicted based on the parameters of standard error of estimate and slope, calculated from linearity of the response data of cinitapride.

Accuracy

An accuracy study was performed by adding known amounts of cinitapride to the sample solution. The actual and measured concentrations were compared. Recovery of the method was evaluated at 3 different concentration levels (corresponding to 50,100 and 150% of the test preparation concentration). For each concentration level, 3 sets were prepared and injected in duplicate. The results are furnished in Table 6

Table 6. Accuracy studies

Description	150%	100%	50%
Wt of sample taken	38.02	25	12.05
Run 1 (area)	2279309	1519538	379884
Run 2 (area)	2275642	1513358	387654
Run 3 (area)	2274352	1499869	376588
Mean area	2276434.3	1510921	381375.3
Std.deviation	2571.73	10058.28	5681.73
%RSD	0.11	0.66	1.48
Amount Recovered	37.67	24.42	12.15
%Recovery	99.07	97.68	100.82

Results and Discussion

By applying the proposed method, the retention time of cinitapride in a typical chromatogram was found to be 15.03 min, which indicates a good baseline. Linearity range was observed in concentration of 5-100 µg/mL. The regression equation of cinitapride concentration over its peak area ratio was found to be $y=64143.26x-43697.86$ ($r=0.9987$). The developed method was validated for precision which includes system precision and method precision. The method is ascertained to be having good reproducibility and repeatability. The %RSD for method precision was observed to be 0.32 wherein the method precision was observed to be 0.97. The asymmetry factor was found to be 1.09, which indicated asymmetry nature of the peak. The number of theoretical plates was found to be 5877, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.048 and 0.678, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of 0.1% formic acid in water and acetonitrile were tested. The use of mobile in the ratio 1:1 resulted in peak with good shape and resolution. The high percentage of recovery of cinitapride ranging from 97.68 to 100.82 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

Conclusion

A new analytical method has been developed to be routinely applied to determine cinitapride in its pharmaceutical dosage forms. The results of the study indicate that proposed HPLC method was simple, precise, highly accurate, specific and less time consuming.

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