Research Article

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD TO DETERMINE CINITAPRIDE HYDROGEN TARTARATE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

This study describes development and subsequent validation of a reversed phase high performance liquid chromatographic (RP-HPLC) method for estimation of cinitapride hydrogen tartarate a new prokinetic drug, and anti ulcer agent of the benzamide class It acts as an agonist of the 5-HT_1 and 5-HT_4 receptors and as an antagonist of the 5-HT_2 receptors in raw material and pharmaceutical formulations like tablets. The chromatographic system consisted of inertsil ODS C18 column 150x4.6,5µ column, an isocratic mobile phase composed of acetonitrile and phosphate buffer (30:70 v/v) and UV detection at 264 nm. cinitapride hydrogen tartarate was eluted at 3.737 min with no interfering peak of excipients used for the preparation of dosage forms. The method was linear over the range from 20,40,60,80,100,120 microg/mL in raw drug (R2 = 0.999).. Results were validated statistically according to ICH guidelines in tablets Validation of the method yielded good results concerning range, linearity, precision and accuracy.

Key words: Cinitapride htdrogen tartarate, RP-HPLC, development, validation

INTRODUCTION;

Cinitapride is 4-Amino-N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidinyl]-2-ethoxy- 5-nitrobenzamide hydrogen L-(+)-tartrate with molecular formulae $C_{25}H_{36}N_4O_{10}$,molecular weight 552.57, and is soluble in Acetonitrile and methanol Cinitapride hydrogen tartarate is a gastroprokinetic agent and antiulcer agent of the benzamide class. It acts as an agonist of the 5-HT_1 and 5-HT_4 receptors and as an antagonist of the 5-HT_2 receptors. There is no intact RP-HPLC method reported for the estimation of cinitapride hydrogen tartarate in bulk and tablet dosage forms as per the literature review. The non-availability of any RP-HPLC methods until now for analysis of the present drug cinitapride hydrogen tartarate made it a worth-while objective to pursue the present work. Hence the present work, aim to develop a simple, precise and accurate methods for the estimation of cinitapride hydrogen tartarate in bulk and in pharmaceutical dosage form and to validate the developed methods by RP – HPLC.

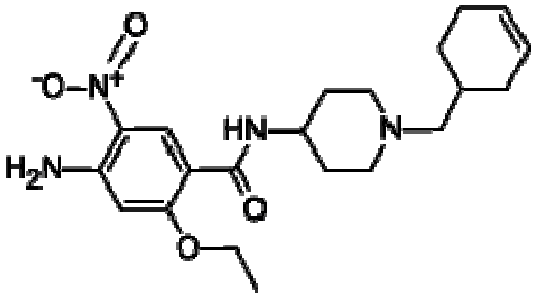

Figure 1: structure of cinitapride hydrogen tartarate

EXPERIMENTAL:

Drug Samples (Raw material) of Cinitapride hydrogen tartarate was obtained as a souvenir samples from Chandra labs, Hyderabad. Formulation as Cintapro (cipla) equivalent to 1mg of cinitapride hydrogen tartarate was purchased from local pharmacy. Chemicals and solvents such as HPLC graded methanol was purchased from E MERC INDIA, Acetonitrile orthophosphoric acid, potassium dihydrogen phosphate AR GRADE was purchased from E MERC INDIA, HPLC graded water was prepared by using Millipore water system.

Chromatographic system and conditions:

Analysis was carried out on Shimadzu HPLC system, 10 AT detector with inertsil ODS C18 column of 150x4.6,5µ. Rheodyne injector with 20µL loop was used. The mobile phase used was prepared by mixing Acetonitrile and phosphate buffer (pH adjusted 3.0 with Orthophosphoric acid) in the ratio of (30:70. The mobile phase is then sonicated using UV-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Preparation of standard solution:

25 mg of cinitapride hydrogen taratrate was weighed accurately and transferred into 25 ml volumetric flask and dissolved in mobile phase, after dissolution the volume was made up to the mark with mobile phase(1000 µg/ml).

Preparation of Calibration graph:

In this progression, from the stock solution solutions containing the concentrations of 20, 40, 60, 80, 100,120 µg/ml of cinitapride hydrogen tartarate was prepared. All the solutions were injected and the chromatograms were recorded at 264 nm. The above concentration range was found to be linear. The peak areas were plotted against concentration and the calibration curve was constructed.

Preparation of sample solution:

Five tablets of formulation (**Cintapro** containing cinitapride hydrogen taratrate equivalent to 1mg of cinitapride) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 1 mg of cinitapride was weighed and made up to 50 ml with mobile phase (100µg/ml). The solution was sonicated for 15 minutes, and filtered through Whatmann filter paper No. 41 and is used for further analysis.

Result and discussions:

Method development and optimisation

Column chemistry solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent in the mobile phase), additive strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized, so there was no interference with the cinitapride peak from solvent. After each change of mobile phase the column was equilibrated by the passage of least twenty volumes of the new mobile phase. To investigate the appropriate

wavelength for the determination of cinitapride, UV-visible spectra in the range of 200-400 nm were acquired from a solution of the drug in the mobile phase. From the UV spectra obtained the wavelength selected for monitoring the drug was 264 nm. It was observed there was no interference from the mobile phase or baseline disturbance at 264 nm. Therefore it was concluded that 264nm was the most.

Chromatography:

Symmetrical peaks are obtained for cinitapride.Typical chromatograms obtained from a bulk and from a solution of drug are illustrated in fig 2 (a & b). The retention time was 3.737 min was 5 min.

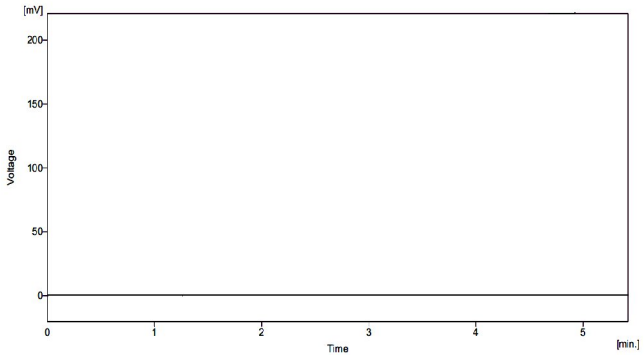

Figure 2 (A &B)

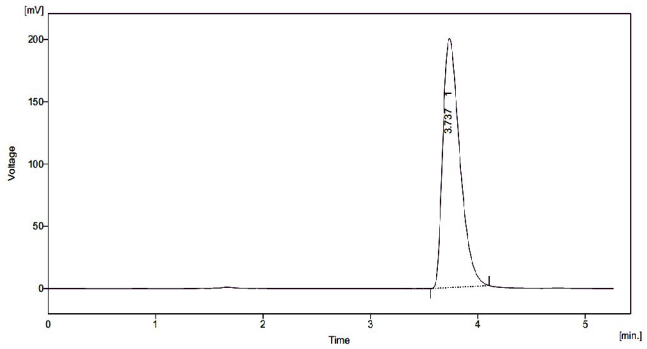

Figure 2 a) Typical chromatogram obtained from blank and b) cinitapride solution

METHOD VALIDATION:

Linearity

The linearity of the method was tested using the calibration solution described above. plot of concentration against responses were linear in the range of 20-120µg ml^{-1}(figure3).the mean regression equation was Y=31.73x-36.26.The correlation coefficient was 0.999

Limit of Detection (LOD):

The limit of detection (LOD) is defined as the lowest concentration of the analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal to noise ratio (SNR) is 3:1.LOD of cinitapride hydrogen tartaratre is 1.410µg/ml.

Limit of Quantation (LOQ):

The limit of quantitation (LOQ) is defined as the lowest concentration of the analyte that can be readily quantified with acceptable precision and accuracy .It is usually regarded as the amount for which the signal to noise ratio (SNR) is 10:1.LOQ of cinitapride hydrogen tartaratre is 4.274µg/ml.

Accuracy:

The accuracy of the method was evaluated by determination of recovery of cinitapride hydrogen tartarate at three levels of concentrations. The sample solutions were spiked with cinitapride hydrogen tartarate standard solutions corresponding to 80, 100, and 120% of nominal analytical concentrations. (80µg/ml, 100µg/ml and 120µg/ml). The results showed good recovery within limits (98% – 102%). The results were discussed in the table 1.

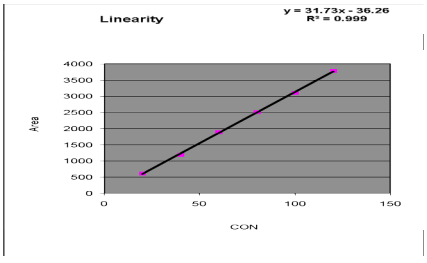

Figure3.calibration plot

Table1. Accuracy of the method

Sample id	Concentration	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	80%	101.3	100.9121	0.066	0.067
2	80%	100.1			
3	80%	101.3			
4	100%	99.90	99.9785	0.055	0.055
5	100%	99.89			
6	100%	99.91			
7	120%	100.10	100.6043	0.0	0.051
8	120%	100.53			
9	120%	100.13			

Precision:

The precision of the method was evaluated by injecting five samples of 100 mcg per ml solutions into the HPLC system as per test procedure. % Relative standard deviation of results should not be more than 2.0 %.The results obtained are listed in table2

Injection number (100 mcg/ml)	Retention time	Area
1	3.847	3143.993
2	3.87	3174.717
3	3.857	3143.858
4	3.837	3149.569
5	3.87	3163.513
Avg	3.8562	3155.13
SD	0.014446	13.56411
%RSD	0.374629	0.429907

Table-2 precision of the method

Specificity:

Specificity of the method was established by injecting all excipients used for manufacturing in six times. It was observed that there was no interference of the placebo with the principle peaks and hence the method is specific as well as stability indicating for the determination of drug.

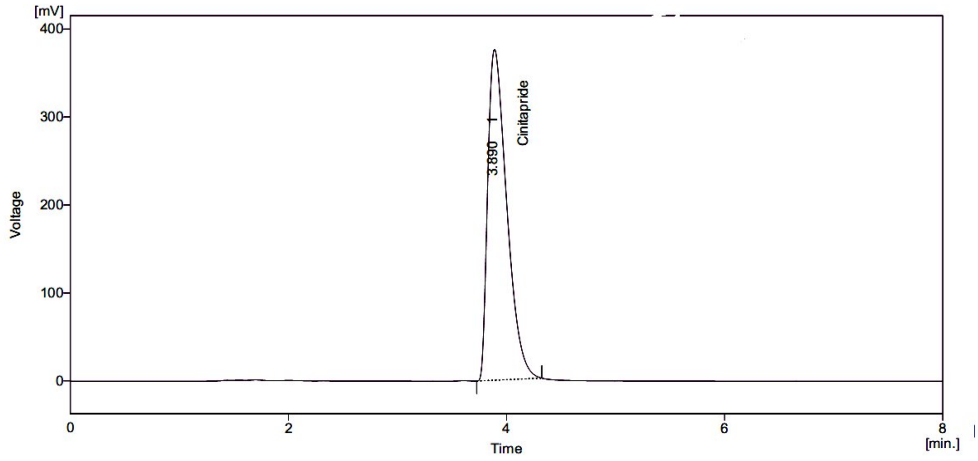

Figure4. Chromatogram obtained from tablet sample

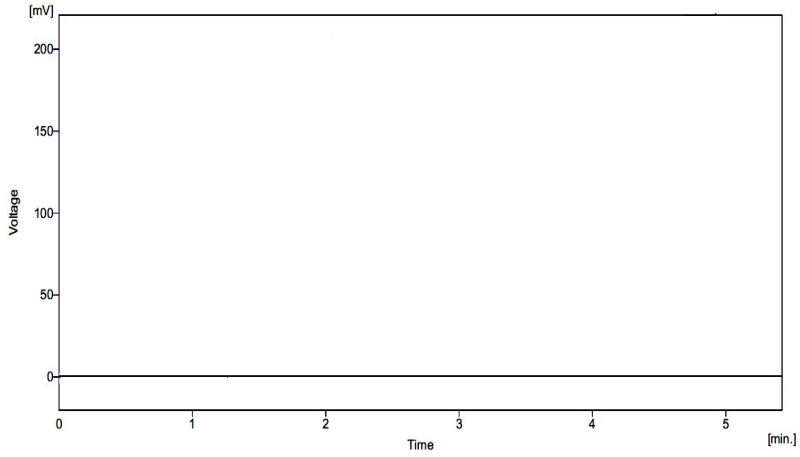

Figure5: chromatogram obtained from placebo

Assay:

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae. Recovery studies were carried out.

Calculate the percentage purity of cinitapride present in tablet using the formula:

$$\text{Percentage purity} = \frac{\text{Spl}_{\text{area}} \times \text{Std}_{\text{dil}} \times \text{Avg wt} \times P}{\text{Std}_{\text{area}} \times \text{Spl}_{\text{dil}} \times \text{L.C} \times 100} \times 100$$

Where,

P = (%) potency of cinitaprideg standard use

L.C = Label claim

Avg wt = Average weight of tablets

Sample i.d	Concentration(mcg/ml)	Standard peak area	Sample peak area	Percentage recovery
1	100	3173.706	4530.759	100.4%
2	100	3239.246	4549.03	
3	Average	3207.869	4539.895	

Table-3

CONCLUSION:

It can be concluded that the proposed method was simple, selective, sensitive, accurate, precise and rapid for the estimation of cinitapride hydrogen tartarate in a short analysis time. The method was proved to be superior to most of the reported method. The mobile phase are simple to prepare and economical. The sample recovery in the formulation were in good agreement with their respective label claims and they suggested non interference of formulation in the estimation hence this method can easily be adopted as an alternative method to the reported one for the routine determination of cinitapride hydrogen tartarate depending upon the nature of their ingredient present `in the sample.

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