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Validated RP-HPLC Method with UV Detection for the Determination of Cinitapride in its Formulation and Human Plasma

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A novel, simple method for the determination of cinitapride hydrogen tartrate in human plasma by reversed phase liquid chromatography with UV detection at 260 nm. The method was developed using Hypersil BDS C_{18} (250 × 4.6 mm, 5 µm) column with mobile phase containing a mixture of acetonitrile: buffer (55:45 v/v), pH adjusted to 3 with 0.02 M mixed phosphate buffer. The method is sensitive with limit of quantification of 0.523 ng mL^{-1}. The calibration plot for cinitapride in spiked plasma was linear in the concentration range 25-150 ng mL^{-1}. The chromatographic conditions are flow rate of 1 mL min^{-1}, column temperature 30 ºC and the retention time was 4.233 min for cinitapride. The developed method was validated as per international conference harmonization (ICH) guidelines with respect to specificity, limit of detection, limit of quantitation, precision, accuracy robustness and system suitability. The method is also suitable for the assay of cinitapride which ranged from 97 % to 100 %. The method can be successfully used for analysis of cinitapride in its formulation and human plasma during pharmacokinetic studies.

Key Words: Cinitapride, Reverse-phase chromatography, UV detection, Validation, Plasma.

INTRODUCTION

Cinitapride[1,2], chemically 4-amino-*N*-[3- [cyclohexan-1-yl-methyl]-4-pyridinyl]-2-ethoxy-5-nitrobenzamide (Fig. 1) has the molecular formula $C_{21}H_{30}N_4O_4$ and molecular weight 402.49 g mol. Cinitapride is a drug that has against action to the serotoninergic 5-HT_2 and D_2 dopaminergic receptors that has been indicated in the gastro esophageal reflux and in the functional disorders of gastro kinetic activity, which generates significant increase in the gastric emptiness besides through the serotoninergic system, it stimulate 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[3]. A survey of literature revealed a UV and extractive spectrophotometric estimation of formulation[4-7], polarographic[8], LC -MS/MS methods for its determination in plasma[9] and RP-HPLC[10] in bulk drug. In this paper, we suggest a new method for determination of cinitapride in its formulation and plasma samples. In order to fulfill the aim, the method was first developed for the separation of and determination of cinitapride concentrations by optimizing the experimental parameters and determining linearity for the investigated drug. Then we validated the method for the determination of cinitapride concentration by evaluating recovery, selectivity, linearity, precision and accuracy. Finally, the method was used for determination of cinitapride in plasma samples obtained from gastro intestinal disorder patients and formulations by commercial available tablets.

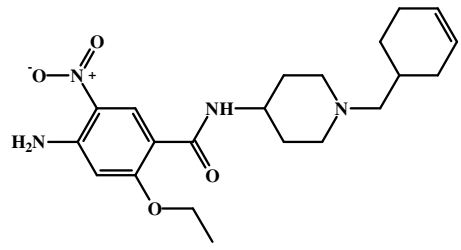

Fig. 1. Chemical structure of cinitapride

EXPERIMENTAL

Cinitapride was provided by Zydus research centre Gujarat, India. Commercial tablets of cinitapride were purchased from the local market. Acetonitrile and methanol, both of HPLC grade were obtained from Merck. Analytical grade 85 % phosphoric acid was purchased from Merck. To prepare buffers and solutions, ultra-pure water was supplied by Milli- Q purification unit and solvents used were of analytical grade.

HPLC was performed with a Shimadzu (Japan) SPD-10 A VP system comprising an LC-10 AT VP pump, an

autosampler and an SPD -10 A VP detector. Data processing was by Shimadzu Class-VP software on a Hewlett-Packard computer. Compounds were separated on a 250 mm × 4.6 mm, 5 µm particle, Hypersil BDS C_{18} column.

Chromatographic conditions: A series of studies were conducted in our laboratory in order to develop a convenient and easy-to-use method for quantitative analysis of cinitapride in plasma. A reverse phase Hypersil BDS C_{18} column, equilibrated with mobile phase acetonitrile: Buffer (55:45 v/v) was used. The active principle was eluted isocratically and the mobile phase flow rate was maintained at 1.0 mL min^{-1}. The effluents were monitored at 260 nm with the detector. The sample was injected using a 10 µL fixed loop and the total run time was 4.233 min.

Preparation mobile phase and stock solution

Mobile phase: 1.625 g Potassium dihydrogen phosphate (KH_2PO_4) and 0.3 g of potassium hydrogrn phosphate (K_2HPO_4) dissolve in water made up the volume to 1000 mL with water. Then pH was adjusted with orthophosphoric acid at 3.0 ± 0.5 ºC. 450 mL of above buffer mixed with 550 mL acetonitrile and degassed by filtering through 0.45 membranes. 13.80 mg of cinitapride hydrogen tartrate sample (99.7 %) dissolved mobile phase in 100 mL volumetric flask.

Preparation of standard stock solution: The stock solution of cinitapride (100 mcg mL^{-1}) was prepared by dissolving 13.80 mg cinitapride hydrogen tartrate sample (99.7 %) which equivalent to 10 mg cinitapride with mobile phase in a standard 100 mL volumetric flask. The final concentration brought to 100 ng/mL.

Preparation of plasma samples: A standard stock solution 100 mcg mL^{-1} of cinitapride was prepared in CH_3OH and then diluted in order to obtain six working solutions from 20 to 120 ng mL. Aliquots of 1 mL blank human plasma were spiked with 50 µL each of working solutions to yield the plasma standards corresponding to 0.5, 1.0, 2.0, 5.0, 10.0, 50.0 ng mL^{-1}. The calibration standard solutions were vortex-mixed for 2.0 min and allowed to equilibrate at room temperature for 15.0 min.

Clinical plasma samples were obtained from gastrointestinal disorder patients receiving an oral administration of 5 mg cinitapride. Then the plasma standards and the clinical samples were stored at -20 ºC.

Analytical procedure: Sample preparation and analysis were conducted at room temperature. Cinitapride working standard (100 mcg mL^{-1}) was added to 15 mL graduated centrifuge tubes in volumes of 20, 40, 60, 80, 100, 120 ng/mL. Drug free human plasma was added to complete volume to 10 mL vortex-mixed for 30 s to yield final calibration standard concentrations of 0.0 (No cinitapride added) 10, 25, 50, 75, 100, 125, 150, 200 ng mL^{-1} (Fig. 2).

For preparation of samples for injection on to HPLC system, 1 mL of plasma was added in glass stoppered tube and vortex-mixed for 15 s. The sample was then alkalinized by addition of 100 µL of 1 N NaOH, vortex-mixed for 30 s and 5 mL of mobile phase was added. This mixture was vortex-mixed for 1 min and centrifuged at 2400 xg for 10 min. The supernatant organic layer was quantitatively transferred to another 10 mL glass centrifuge tube and the contents were evaporated to dryness

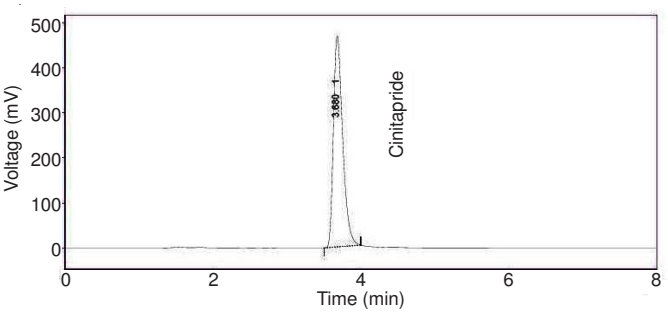

Fig. 2. Typical blank chromatogram of cinitapride

at room temperature under a steam of pure nitrogen. The residue was reconstituted in 250 µL of mobile phase, Vortex-mixed for 30 s, transferred to 1.5 mL eppendort tubes and centrifuged at 8500 xg for 5 min to precipitate any particulate matter. Aliquots of 80 µL were injected on to the column.

RESULTS AND DISCUSSION

Proposed method was validated as per ICH guidelines: Under these chromatographic conditions cinitapride in a plasma matrix was resolved with retention time 4.233 min. The method was found to be suitable because reproducible results were obtained on each sample run on validation of the above method using as the following results were obtained.

Linearity: The linearity of the calibration plot for cinitapride in human plasma at concentrations 25, 50, 75, 100, 125, 150 ng mL^{-1} was excellent (r = 0.9983) over the concentration range investigated. A typical calibration plot obtained during plasma analysis could be described by linear equation, y = 36.819X + 152.72, where y is peak area and X is concentration (ng mL^{-1}). The calibration plot was constructed as the results obtained are presented in Table-1 (Figs. 3 and 4).

TABLE-1
CALIBRATION DATA FOR CINITAPRIDE IN ACETONITRILE–PHOSPHATE BUFFER 55:45 (v/v) AFTER SPIKING OF PLASMA WITH STOCK SOLUTIONS

S. No	Drug concentration in spiked plasma (ng mL^{-1}) X	Peak area[a]	Regressed value of peak area, Y	Standard deviation
1	25	1107.251	1258.536	32.18
2	50	2006.235	2132.284	28.73
3	75	2867.588	2986.620	15.28
4	100	3738.211	3481.141	43.84
5	125	4866.730	4729.997	30.24
6	150	5660.083	5721.710	53.87

N[a] = 5, *i.e* results are means from five observations

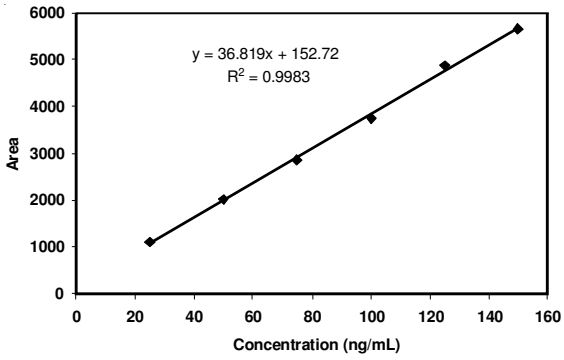

Fig. 3. Calibration plot of cinitapride in spiked human plasma

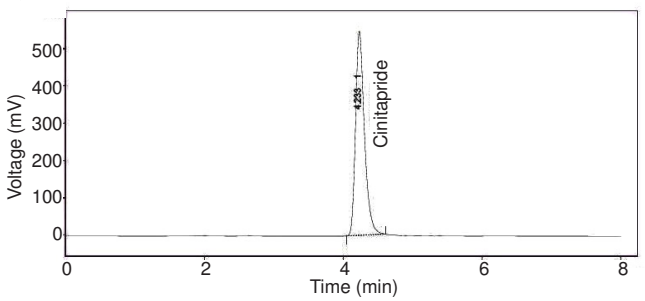

Fig. 4. Typical chromatogram of cinitapride in spiked human plasma

Specificity: Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed method was checked in the presence of its impurities and plasmatic concentrations. For this purpose, the spiked samples of cinitapride eluted peaks at different retention times. All the impurities and plasma concentrations are well resolved from one another and cinitapride peak indicating the specificity of the proposed method to quantify cinitapride.

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The relative standard deviation (RSD) obtained for cinitapride was 0.37 %. The intra and interday variability or precision data of summarized in Table-2. The intraday precision of the developed RP-HPLC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. The RSD of the assay results, expressed as percentage of the label claim, was used to evaluate the method precision. The interday precision was also determined by assaying the tablet in replicate for day consecutive three days. The results indicated the good precision of the developed method (Table-2).

TABLE-2
INTRA AND INTERDAY ASSAY PRECISION DATA (n = 9)

Actual concentration of cinitapride (mcg mL^{-1})	Measured concentration (ng mL^{-1}) ± RSD (%)	
	Intraday	Interday
3.27	3.26 ± 0.42	3.14 ± 0.75
7.53	7.57 ± 0.38	7.44 ± 0.89
15.11	15.07 ± 0.54	15.81 ± 0.42

Data expressed as mean for measures concentration values

Accuracy: Accuracy of the method was checked by recovery study using standard addition method, at three different concentration levels, that is multi level recovery study. The pre analyzed sample spiked with extra 25, 50, 75 % standard cinitapride was analyzed by proposed method. Recovery of standard drug added was found to be 98.95-100.39 % for cinirapride with the value of % CV less than two indicating proposed method is accurate for the estimation of cinitapride. Results of recovery study are shown in Table-3. The absolute recovery was calculated by comparing area under the peaks obtained from standard working solutions with the peak areas from standard extracts are shown in Table-4.

TABLE-4
ABSOLUTE RECOVERY OF CINITAPRIDE AFTER EXTRACTION FROM HUMAN PLASMA

Concentration cinitapride (μg mL^{-1})	Recovery[a]	CV (%)
5	97.92	1.721
10	98.60	1.121
15	97.85	0.515
20	99.72	0.693

a = Mean of five determinations.

System suitability: A system suitability test according to USP was performed on the chromatograms obtained from standard and test solution to check different parameters and the results obtained from six replicate injections of the standard solutions are summarized in the Table-5.

TABLE-5
SYSTEM SUITABILITY PARAMETERS

Retention time, R_t (min)	4.233
Theretical plates	5316
HETP (h, mm)	0.216
Resolution (R_s)	0.00
Assymmetry (A_s)	1.613
Linearity range (ng mL^{-1})	25-150
Limit of quantification (ng mL^{-1})	0.523
Limit of detection (ng mL^{-1})	1.68
Regression equation (y^*=a +bc)	
Slope (b)	0.7945
Intercept (a)	5003.5
Correlation coefficient (r)	0.9961
Method Precision CV % (n = 7)	0.37

Robustness: Robustness of the method was studied by changing the composition of organic phase by ± 5 %, mobile phase flow rate (±1.0 mL/min), pH (±3.0) and column temperature (±30 ºC) and also by observing the stability of the drug for 24 h at ambient temperature in mobile phase.

Ruggedness: Ruggedness was performed on the second HPLC column of the same type by injecting the standard solution of cinitapride. Relative error was calculated by the mean peak height for the substance to obtain by changing pH value of the mobile phase from 3 to 3.2. Ruggedness was carried out by changing the instrument and analyst.

TABLE-3
RECOVERY DATA FOR THE PROPOSED RP–HPLC METHOD (n = 5)

Drug	Amount of sample taken (ng mL^{-1})	Amount standard spiked (ng mL^{-1})	Amount standard recovered (ng mL^{-1})	Recovery (%)	CV (%)
Cinitapride					
1	20	5	4.95	98.95	0.659
2	20	10	9.99	99.95	0.693
3	20	15	15.06	100.39	0.828

Solution stability: Stability of cinitapride in solution and in biological matrix was determined at different temperatures. No significant changes in the concentration of cinitapride at 30 ºC was observed during a weak time. The test performed in triplicate for one low and one high concentration of the compound ensures that no degradation occurred during the step, before loading the plasma in to an extraction column. No degradation was noticed over a 24 h interval.

Sensitivity: Initially 1 mg mL^{-1} cinitapride was injected. This solution was further diluted to achieve the signal-to-noise (S/N) ratio of 3:1 and 10:1 for determining limit of detection (LOD) and limit of quantitation (LOQ) respectively. The S/N ratio was for cinitapride was calculated using waters Empowerz software. According to ICH guidelines, Cinitapride LOD found to be 1.68 ng mL^{-1} and LOQ was found to be 0.523 ng mL^{-1}.

Application of the method: The content of ten tablets (Cinmove 5 mg and Cinmove 10 mg cinitapride for table) were crushed, powdered, weight out and the average weight of on tablet was determined. An accurate weight euivivalent to 10 mg cinitapride was dissolved in 20 mL methanol with shaking for 5.0 min and filtered. The filtrate was diluted to 100 mL with by distilled water in a 100 mL measuring flask to give 100 µg mL^{-1} stock solutions. An aliquot of the diluted drug solution was treated as proposed method. The proposed RP-HPLC method was applied for the determination of cinitapride in its pharmaceutical formulations (Table-6). The recovery of the data and the agreement between the label claim and the amount found were excellent. This confirms the suitability of the proposed method for the routine quality control determination of the component in pharmaceutical formulations.

TABLE-6
RESULTS OF ANALYSIS OF CINITAPRIDE IN TABLETS

Drug	n	Amount claimed (mg per tablet)	Amount found (mg per tablet)	Mean recovery (%)	RSD (%)
Cinmove	5	10	10.12	101.25	0.75
Cinmove	10	20	20.05	100.50	1.15

Conclusion

A simple, rapid, reproducible and sensitive RP-HPLC method has been developed for analysis of cinitapride in human plasma. The short chromatographic run time of only 4.233 min makes this method suitable for processing of many samples in limited time for ICH guidelines. The method was validated for analysis of cinitapride in human plasma over range 10-200 ng mL^{-1}.

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