Int. J. Chem. Sci.: 12(3), 2014, 871-879
ISSN 0972-768X
www.sadgurupublications.com

DEVELOPMENT AND VALIDATION OF A RAPID RP HPLC METHOD FOR THE DETERMINATION OF CINITAPRIDE HYDROGEN TARTARATE IN PURE AND ITS PHARMACEUTICAL FORMULATION

NAMRATA BASNET*, SYEDA HUMAIRA and SYED SANAULLAH

Department of Quality Assurance, Luqman College of Pharmacy, GULBARGA (K.S.) INDIA

ABSTRACT

A rapid, specific, reverse phase high performance liquid chromatography [RP-HPLC] method has been developed for assaying Cinitapride Hydrogen Tartarate (CHT) in pure and pharmaceutical formulations. The assay involved an gradient elution of CHT in a Symmetry C18 (4.6 × 150 mm) column using a mobile phase composition of acetonitrile and Phosphate buffer pH 2.5 (68 : 32). Detection was carried out by UV at 264 nm. The flow rate was 1 mL/min and the analyte monitored at 264 nm. The assay method was found to be linear from 10 to 60 µg/mL and met all specifications as per ICH guidelines. Statistical analysis revealed that this method can be used in routine quality control studies of Cinitapride Hydrogen Tartarate in pure and its formulations

Key words: Cinitapride hydrogen tartarate, C18 Column, RP-HPLC, Reverse phase, Validation.

INTRODUCTION

Cinitapride Hydrogen Tartarate (CHT), chemically is 4-Amino-N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidyl]-2-ethoxy-5-nitrobenzamide; 4-Amino-N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide[1,2]. It has the molecular formula $C_{25}H_{36}N_4O10$ and molecular weight 552.57 g.mol^{-1}. It is novel prokinetic benzamide-stimulating gastrointestinal motility agent and antiucer agent. It acts as an agonist of the 5-HT1 and 5-HT4 receptors and as an antagonist of the 5-HT2 receptors[3].

Literature survey reveals few methods for the determination of Cinitapride Hydrogen Tartarate[4-8]. The target of this study was to develop a new, simple and rapid HPLC method with gradient elution technique for determination of cinitapride in bulk drugs and pharmaceutical formulations and validate as per ICH guidelines[9].

*Author for correspondence; E-mail: sanashs@rediffmail.com, namratabc@gmail.com

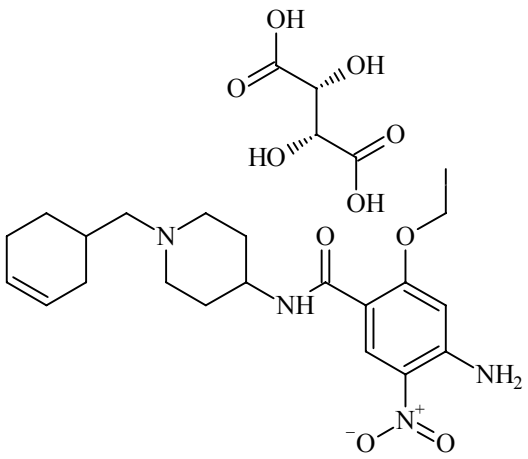

Fig. 1: Structure of cinitapride hydrogen tartarate

EXPERIMENTAL

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of CHT, an gradient peak HPLC instrument with Symmetry C18 (4.6 × 150 mm) column was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable Waters 2487 dual λ absorbance UV detector. A 20 1AL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Standards and chemicals used

Drug Samples (Raw material) of Cinitapride hydrogen tartarate was obtained as a souvenir samples from Chromo Labs, Hyderabad. Formulation as Cintapro (cipla) equivalent to 1 mg of cinitapride hydrogen tartarate was purchased from local pharmacy. Chemicals and solvents such as HPLC graded Acetonitrile was purchased from SD fine Chemicals, Ltd. India. Orthophosphoric acid, potassium dihydrogen phosphate AR GRADE was purchased from Hi media Mumbai, INDIA, HPLC graded water was purchased from Qualigens fine Chemicals, India. All other chemicals used were of analytical grade.

Preparation of the mobile phase

Into a 1000 mL cleaned volumetric flask, HPLC grade Acetonitrile (680 mL), and Phosphate buffer pH 2.5 (320 mL) were (which are filtered through 0.25 mm membrane filters by vacuum filtration) were slowly added, mixed well and sonicated. The solution was

cooled to room temperature and used for chromatography method. (Preparation of phosphate buffer: Weighed 10.54 g of KH_2PO_4 into a 1000 mL volumetric flask, added 775 mL of water and dissolved with HPLC water. Adjusted the pH to 2.5.0 with ortho phosphoric acid. Volume was made upto 1000 mL with HPLC water.)

Preparation of standard stock solution

About 10 mg of CHT was weighed accurately and dissolved in 100 mL of mobile phase in a 100 mL volumetric flask and diluted up to the mark with mobile phase to get the concentration of 100 µg/mL. Resultant solution was filtered through Whatman filter paper No. 41.

Working standard solution

Several aliquots of standard stock solutions 1, 1.5, 3, 4.5 and 6 mL of CHT were taken in different 10 mL volumetric flask and diluted upto the mark with mobile phase. Peak area was recorded for all the peaks and calibration graph were obtained by plotting peak versus concentration of CHT. The plot of peak area of each sample against respective concentration of CHT was found to be linear in the concentration range of 10-60 µg/mL.

Sample preparation

Two commercial brand tablets were chosen for testing suitability of proposed method to estimate CHT in pharmaceutical dosage forms. Twenty tablets were weighed accurately and powdered. A quantity equivalent to 10 mg of CHT was weighed accurately and transferred to 100 mL volumetric flask. About 40 mL of mobile phase was added and kept in ultrasonic bath for 30 min. This solution was filtered through membrane filter and volume was made up to the mark with methanol to get 100 µg/mL concentration. Solution obtained was diluted with mobile phase 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 CHT present in the tablet formulation was determined by comparing the peak area from the standard.

Methodology

The HPLC system was stabilized for thirty min. by passing mobile phase, detector was set at 264 nm and flow rate of 1.0 mL/min was maintained to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Eight replicates of each standard solutions 10, 15, 30, 45, and 60 µg/mL were injected. Calibration graph was plotted by concentration of CHT on X-axis and peak area on Y-axis. The amount of drug present in sample was computed in calibration graph.

Pharmaceutical formulations

Prepared dilution of pharmaceutical formulation was injected and the procedure described under bulk samples was followed. The amount of drug present in sample was computed in calibration graph. Chromatographic conditions for estimation of CHT as described in Table 1.

Table 1: Optimized chromatographic conditions for CHT

Api concentration	30 µg/mL
Mobile phase	Acetonitrile: Phosphate buffer (pH 2.5) (68:32 v/v)
Wavelength	264 nm
Column	C18 Column
Concentration	30 µg/mL
Retention time	2.227
Run time	4 min
Area	1397626
Th. Plates	2066
Tailing factor	1.528
Pump pressure	13.0 MPa

RESULTS AND DISCUSSION

The objective of the present work was to develop simple, precise and reliable HPLC method for the analysis of CHT in bulk and pharmaceutical dosage form. This was achieved by using the most commonly employed column C18 with U.V. detection at 264 nm.

The representative chromatogram indicating CHT is shown in Fig. 1.

Parameter fixation

In developing this method, a systemic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

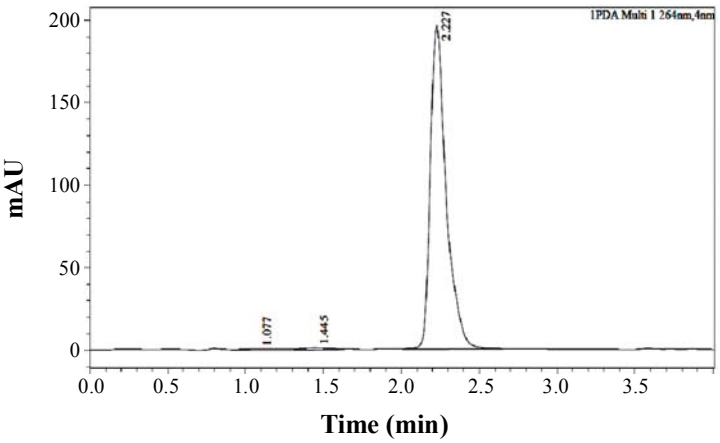

Fig. 1: Chromatogram of CHT

Stationary phase characteristics

Based on nature and solubility characteristics of CHT, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C18 column was found to be optimum.

Mobile phase characteristics

In order to get sharp peak with base line separation from interfering peaks, a number of experiments were carried out by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under gradient conditions, mixtures of solvents like acetonitrile with different buffers in different combinations were tested as mobile phase. A mixture of Acetonitrile: pH 2.5 Orthophosphate buffer (68 : 32) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined, resolved and almost free from tailing.

Validation of the proposed method

As an integral part of analytical method development is validation, the proposed method was validated as per ICH guidelines.

Linearity

It is the ability of the method to Elict test results directly propotional to analyate concentration within a given range. Linearity was performed by preparing standard solutions of CHT at different concentration levels. Twenty micro liters of each concentration was

injected into the HPLC system. The peak responses were read at 264 nm and the corresponding chromatograms were recorded. Linearity plots of concentration over areaswere constructed individually. Linearity results were obtained in the concentration range of 10-60 µg/mL. The results are presented in Table 2.

Table 2: Linearity results of CHT

Conc. in µg/mL	Area
10	289609
15	571034
30	1397626
45	2248730
60	2192192

Precision

Precision is the degree of repeatability of an analytical method under normaloperational conditions. Precision of the method was performed as intraday precision and inter day precision.

Intraday precision

To study the intraday precision, six replicate standard solutions (30 µg/mL) of CHT were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.173, which are well within the acceptable criteria of not more than 2.

Interday precision

To study the interday precision, six replicate standard solutions (30 µg/mL of CHT was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.664, which are well within the acceptable criteria of not more than 2.0.

Specificity

The effect of wide range of excipients and other additives usually present in the formulation of CHT in the determinations under optimum conditions were investigated. infact, may have no observation at this UV maximum chromatographic parameters maintained are specific for CHT.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010 A HT), and Sphinco HPLC by different operators using different columns of similar type like Hypersil C18, Symmetry C18. They didn't show any significant change.

Limit of detection and limit of quantification

A calibration curve was prepared using concentrations in the range of 10-60 µg/mL (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of detection limit and quantitation limit.

The results are reported in Table 3.

Table 3: Limit of detection and limit of quantification of CHT

LOD	0.02 µg/mL
LOQ	0.07 µg/mL

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. The standard addition method was performed at 80%, 100% and 120% level of 30 µg/mL. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery was calculated and the results are presented in Table 4. Satisfactory recoveries ranging from 99% to 101% were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 4: Recovery results

CHT					
% Recovery	**Target conc. (µg/mL)**	**Spiked conc. (µg/mL)**	**Final conc. (µg/mL)**	**Conc. obtained**	**% of Recovery**
	15	12	27	23.97	99.86
80	15	12	27	23.99	99.98
	15	12	27	23.93	99.69

Cont…

CHT					
% Recovery	Target conc. (µg/mL)	Spiked conc. (µg/mL)	Final conc. (µg/mL)	Conc. obtained	% of Recovery
100	15	15	30	30.18	100.60
	15	15	30	29.96	99.85
	15	15	30	29.94	99.79
120	15	18	33	35.99	99.98
	15	18	33	36.07	100.20
	15	18	33	36.05	100.13

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which means that the RP-HPLC method developed is robust. The results of robustness are given in Table 5.

Table 5: Robustness results

Parameter	Change	Area	% of change
Standard	…….	1397626	……
MP	Acetonitrile : Buffer pH 2.5 63:27	1392216	0.39
	68:32	1395561	0.15
pH	2.3	1389978	0.55
	2.7	1393076	0.33
Wavelength	266 nm	1388684	0.64
	262 nm	1410826	0.94

Formulation

Formulation	Brand name	Prepared conc.	Area	% Assay	Amount found
CHT	Kinpride	30 µg/mL	1754070	100.60	1.006
	Cintapro	30 µg/mL	1750373	99.79	0.9979

REFERENCES

1. M. J. O'Neil, The Merck Index- An Encyclopaedia of Chemicals, Drugs & Biologicals, 13th Ed., Merck and Co Inc, Whitehouse Station (NJ, USA), 2297 (2006).
2. S. C. Sweetman, Inc. Martindale, The Complete Drug Reference, 35th Ed., London: Pharmaceutical Press (2002) p. 1220.
3. Chemical Book, http://www.chemicalbook.com
4. S. Humaira, A. Dey, S. A. Raju and S. Sanaullah Applications of Colorimetric Methods for the Determination of Cinitapride Hydrogen Tartarate in Drug Formulation, Int. J. Pharm. Pharm. Sci., **2(1)**, 134-136 (2010).
5. B. Tangabalan, A Elphine Prabahar, R. Kalaichelvi and P. Vijay Kumar, UV Spectrophotometric Method for Determination of Cinitapride in Pure and its Solid Dosage Form E-J. Chem., **6(1)**, S21-S24 (2009).
6. B. Thangabalan, A. Elphine Prabahar and P. Vijayaraj Kumar, Validated Extractive Spectrophotometric Estimation of Cinitapride in Pure and its Solid Dosage Form, Int. J. Pharm. Pharm. Sci., **2**, 153-155 (2010).
7. N. M. Jagani, J. S. Shah and P. B. Patel, Development and Validation of Dual Wavelength Method for Simultaneous Estimation of Omeprazole and Cinitapride in Combined Capsule Dosage Form, Int. J. Res. Pharm. Biomed. Sci., **3(2)**, 762-767 (2012).
8. G. Nagarajan, P. Nagesh, B. V. Ramana, N. R. Prassana and C. Triveni. Development of a RP-HPLC Method for Simultaneous Estimation of Omeprazole and Cinitapride in Bulk and Capsule Dosage Form, Int. Res. J. Pharm., **4(2)**, 131-135 (2013).
9. International Conference on Harmonization, (Q2 (R1) Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, IFPMA, Geneva (2005).

Revised : 10.05.2014 *Accepted : 11.05.2014*