



VALIDATED EXTRACTIVE SPECTROPHOTOMETRIC ESTIMATION OF CINITAPRIDE IN PURE AND ITS SOLID DOSAGE FORM

B. THANGABALAN^{1*}, A. ELPHINE PRABAHAR², P.VIJAYARAJ KUMAR³

¹SIMS College of Pharmacy, Mangaldas Nagar, Guntur-522 001, India, ²Chalapathi Institute of Pharmaceutical Science, Chalapathi Nagar, LAM, Guntur 522 034, ³Bharat Institute of Pharmacy, Mangalpally Village, Ibrahimpatnam. RR Dist.-501506
Email: *bthangabalan@gmail.com

Received: 26 April 2010, Revised and Accepted: 08 May 2010

ABSTRACT

Two simple, rapid and sensitive extractive spectrophotometric methods have been developed for the assay of cinitapride in pure and pharmaceutical formulations. These methods are based on the formation of chloroform soluble ion-association complexes of cinitapride with Bromocresol Green (BCG) and with Bromothymol Blue (BTB) in potassium hydrogen phthalate buffer pH-4 with absorption maximum at 414 nm and 416 nm for BCG and BTB, respectively. Reaction conditions were optimized to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of cinitapride, which was corroborated by the calculated correlation coefficient values (0.9999 and 0.9998). The systems obeyed Beer's law in the range of 5-40 and 2-10 µg/mL for BCG and BTB, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. No interference was observed from common excipients present in tablets.

Key words: Cinitapride, Extractive spectrophotometric determination, Ion-association complex.

INTRODUCTION

Cinitapride, chemically 4-amino-N-[3-(Cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide is a substituted benzamide gastroenteric prokinetic agent acting via complex, but synergistic effects on serotonergic 5-HT₂ (inhibition) and 5-HT₄ (stimulation) receptor and dopaminergic D₂ (inhibition) receptors in the neuronal synapses of the myenteric plexi¹⁻³. A survey of literature revealed a uv estimation in formulation⁴, polarographic method⁵, LC-MS/MS methods for its determination in plasma^{5,6} and RP-HPLC Method⁷ in bulk drug. This paper describes two simple and sensitive spectrophotometric methods for the determination of cinitapride in pure and its pharmaceutical formulation (tablets).

MATERIAL AND METHODS

Apparatus

The spectrophotometric measurements were carried out using An Elico UV/Visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells.

Reagents

Cinitapride hydrogen tartrate was tested for purity by measuring its melting point and IR spectra and no impurities were found. Methanol and chloroform analytical grade were used.

Assay Procedure for Pure Drug

An aliquot of the solution containing 5-40 µg (for BCG) or 2-10 µg (for BTB) of cinitapride was transferred into a series of 125 mL separating funnels. A volume of 1 mL of potassium hydrogen phthalate buffer of pH 4 for BCG and BTB and 1 mL of 0.1 % BCG or 0.12% BTB were added. Chloroform (10 mL) was added to each separating funnels and then the contents were shaken well and left at room temperature for a minute. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbances of yellow coloured complexes (fig 1 & 2) were measured at 414 nm and at 416 nm for BCG and BTB, respectively, against the corresponding reagent blank. The calibration graphs were plotted.

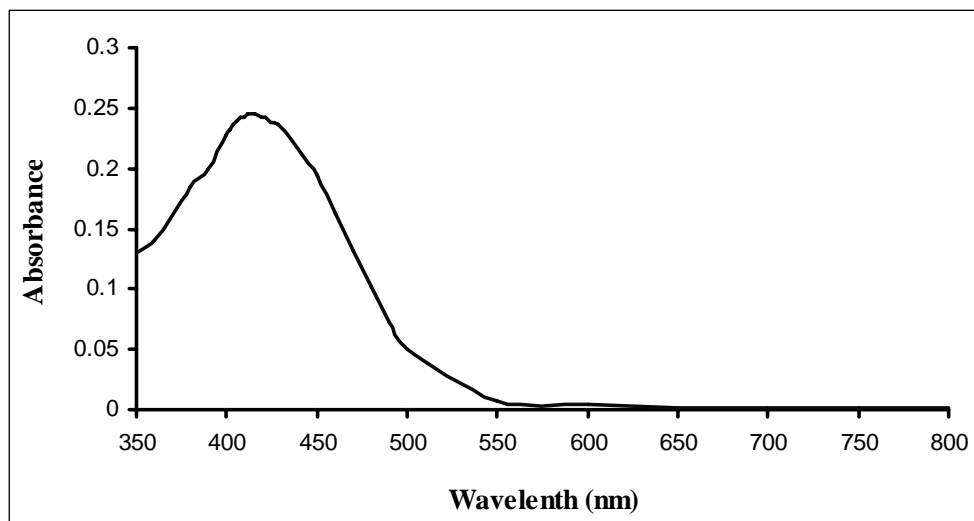


Fig. 1: Absorption spectra of Cinitapride -BCG complex extracted into 10 ml chloroform:

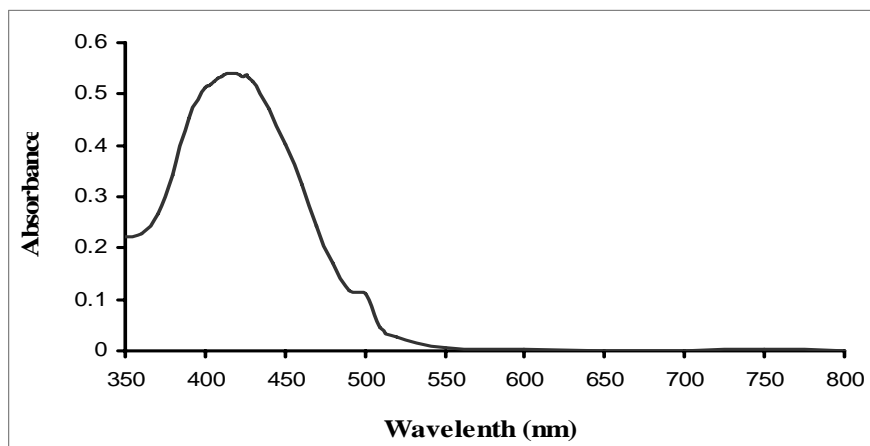


Fig. 2: Absorption spectra of Cinitapride -BTG complex extracted into 10 mL chloroform.

Assay procedure for tablets

Twenty tablets were weighed accurately and triturated to fine powder. The powder equivalent to 10 mg CTP was weighed and transferred to 100 mL volumetric flask. To this 50 mL of methanol was added and sonicated for 15 minutes, then filtered through Whatman No. 42 filter paper. The residues were washed thoroughly with methanol and volume made-up to get $100 \mu\text{g mL}^{-1}$ and suitable aliquots were analyzed using the methods given above.

RESULTS AND DISCUSSION

It was observed that the anionic dyes such as BCG and BTB form ion-association complexes with the positively charged drug. Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by weak electrostatic forces of attraction. Cinitapride reacts with BCG and BTB in acidic buffer to give chloroform soluble ion-association complexes, which exhibit absorption maxima at 414 and 416 nm for BCG and BTB, respectively. Under the experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of cinitapride.

Optimization of reaction conditions

Optimum reaction conditions for quantitative determination of ion-pair complexes were established via various preliminary experiments. It was observed that the effective extraction of the complex depends on the type of buffer used and its pH. Potassium hydrogen phthalate buffer pH-4 was found to be suitable for this method.

The effects of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of cinitapride and varied amounts of the reagent separately. Maximum colour intensity of the complex was achieved with 1 mL of 0.1% BCG or 1 mL of 0.12% BTB. Several organic solvents were tried for effective extraction of the coloured species from aqueous phase. Chloroform was found to be the most suitable extractant since only

one extraction was shown adequate to achieve a quantitative recovery of the complex. No appreciable change in the absorbance or colour of the product was observed even if the order of addition of the reactants was varied.

Validation of the methods

Detection and quantification limits

The LOD values were found to be 0.7777 and $0.2202 \mu\text{g mL}^{-1}$ for cinitapride with BCG and with BTB, respectively. The LOQ values were observed to be 2.3565 and $0.6673 \mu\text{g mL}^{-1}$ for cinitapride with BCG and with BTB, respectively. These values indicate that the BTB method is more sensitive than BCG method.

Quantification

The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are shown in Table 1. Regression analyses of Beer's law plots at their respective λ_{max} values revealed a good correlation. Graphs of absorbances versus concentration showed zero intercept, and are described by regression equation, $Y = bX + c$ (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of each selected drug in $\mu\text{g/mL}$). Results are summarized in Table 1.

Accuracy, precision and recovery

The validation of the methods for the assay of cinitapride was examined by determining precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of relative standard deviation (R.S.D.) indicate good precision of the methods. The results of analysis of dosage forms are given in Table 2. The results were reproducible as evident from low R.S.D. values. To study accuracy of the methods, recovery studies were carried out by the standard addition method. The results are given in Table 2. The average percent recoveries obtained were quantitative, indicating good accuracy of the methods. (Table 2)

Table 1: Optical characteristics of proposed method

Parameters	BCG	BTB
λ_{max} (nm)	414	416
Beer's law limit ($\mu\text{g mL}^{-1}$)	5 - 40	2 - 10
Sandell's sensitivity ($\mu\text{g cm}^{-2}/0.001$ absorbance unit)	0.0405	0.02242
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	1.384×10^4	4.622×10^4
Limit of detection, $\mu\text{g mL}^{-1}$	0.7809	0.2202
Limit of quantification, $\mu\text{g mL}^{-1}$	2.3665	0.6672
Regression equation ($Y = a + bc$)		
a)		
b)	0.0245	0.848
Intercept(a)	0.0013	0.0013
Correlation coefficient (r^2)	0.9999	0.9998

Table 2: Assay results, recovery and precision studies

Method	Labeled amount (mg/ tablet)	(% label claim* \pm S.D	% Recovery*	Precision**		
				Repeatability	Inter-day	Intra-day
BCG	1	100.37 \pm 0.9374	100.1724	0.9223	0.8962	0.8132
BTB	1	100.24 \pm 0.3282	100.0679	0.8203	0.7071	0.7237

* Average of six determinations. **SD of five determinations.

CONCLUSIONS

Unlike the gas chromatographic and HPLC procedures, the instrument is simple and affordable. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of cinitapride in pure form and in pharmaceutical preparations.

REFERENCES

1. Roberts D J. The pharmacological basis of the therapeutic activity of clebopride and related substituted benzamides. *Curr. Ther. Res.* 1982; 31: S1-S44.
2. Fernandez A G and Massingham R. Peripheral receptor populations involved in the regulation of gastrointestinal motility and the pharmacological actions of metoclopramide-like drugs. *Life Sci.* 1985; 36: 1-14.
3. Massingham R, Bou J and Roberts D J. A comparison of the stimulatory effects of metoclopramide and cinitapride in the guinea-pig isolated ileum. *J. Auton. Pharmacol.* 1985; 5: 41-53.
4. Thangabalan B, Elphine Prabahar A, Kalaichelvi R and Vijayaraj Kumar P. UV spectrophotometric method for determination of cinitapride in pure and its solid dosage form. *E-J. Chem.* 2009; 6(S1): S21-S24.
5. Gonzalez M I, Gonzalez P C and Blanco Lopez M A, Polarographic determination of cisapride and cinitapride. *Analytica. Chimica. Acta.* 1998; 368: 175-181.
6. Roy S M N, Yetal S M, Chavan S V, Pradhan V R and Joshi S S, Determination of free levels of cinitipride in human plasma by liquid chromatography-tandem mass spectrometry. *E-J. Chem.* 2008; 5: 453-460.
7. Roy S M N, Mangaonkar K V, Desai A Y and Yetal S M, RP-HPLC method for the determination of cinitapride in the presence of its degradation products in bulk drug. *E-J. Chem.* 2010; 7: 311-319.