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DEVELOPMENT AND VALIDATION OF COLORIMETRIC METHODS FOR THE DETERMINATION OF CINITAPRIDE HYDROGEN TARTARATE IN PURE DRUG AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Two simple, sensitive and highly accurate UV spectrophotometric methods (A and B) have been developed for the determination of Cinitapride Hydrogen Tartarate in pure drug and its pharmaceutical formulations. Method A is based on the diazotization of CHT with nitrous acid to form diazotized CHT, followed by its coupling with β-Naphthol to form red coloured chromogen, which shows absorption maximum at 552 nm and obeys beers law on the concentration range of 1-5 µg/mL. Method B is based on the diazotization of CHT with nitrous acid to form diazotized CHT, followed by its coupling with Chromotropic acid to form pink coloured chromogen which shows maximum absorption at 511 nm and obeys beers law in the concentration range of 4-20 µg/mL. The methods have been successfully applied for the assay of drug in pure and in pharmaceutical formulation. No interference was observed from common pharmaceutical additives. The developed methods were validated by determining its sensitivity, accuracy and precision as per ICH guidelines.

Key words: Cinitapride hydrogen tartarate, Spectrophotometric, Pharmaceutical formulation, Assay, 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] 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 CHT[4-8]. The target of this study was to develop a new, simple economical spectrophotometric method based on diazotization reaction. The absorbance of highly intensive coloured solution was measured at wavelength of maximum absorbance for determination of CHT in bulk drugs and pharmaceutical formulations and validated as per ICH guidelines[9].

EXPERIMENTAL

Materials and methods

Instrument and materials: All absorbance measurements were done on Shimadzu 1700 UV/Visible spectrophotometer with 1 cm matched quartz cells.

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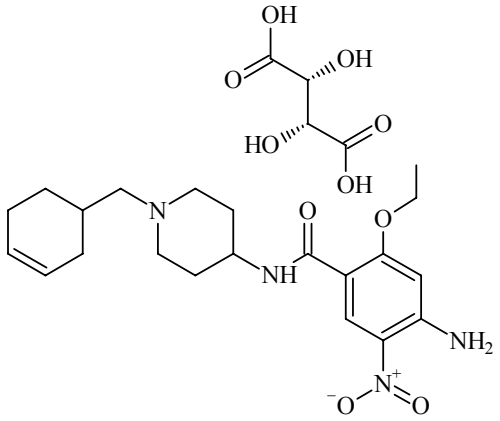


Fig. 1: Structure of cinitapride hydrogen tartarate

Reagents

All the solutions were freshly prepared. All solvent and other chemicals used throughout this study were of analytical grade from SD fine chemicals, Ltd. India.

(i) 2 N Hydrochloric acid: Prepared by diluting 85 mL of hydrochloric acid with distilled water to 1000 mL.

(ii) 5 N Hydrochloric acid: Prepared by diluting 42.5 mL of Hydrochloric acid with distilled water to 100 mL.

(iii) 2% w/v of sodium nitrite: Prepared by dissolving 2 g of Sodium nitrite in 100 mL of distilled water.

(iv) 0.2% w/v sodium nitrite: Prepared by dissolving 0.200 g of Sodium nitrite in 100 mL of distilled water.

(v) 2% Aqueous sodium hydroxide solution: Prepared by dissolving 2 g of sodium hydroxide in 100 mL of distilled water.

(vi) 0.2% w/v β-Naphthol: Prepared by dissolving 0.200 g of β-naphthol in 100 mL solution of 2% sodium hydroxide.

(vii) 2% w/v Ammonium sulphamate: Prepared by dissolving 2 g of ammonium sulphamate in distilled water.

(viii) 0.2% Chromotropic acid: Prepared by dissolving 0.2 g of chromotropic acid solution in 100 mL of 75% of sulphuric acid, which was made by adding 75 mL of concentrated sulphuric acid to 33.3 mL of distilled water.

Preparation of standard solution

A standard stock solution containing 1 mg/mL of CHT was prepared by dissolving 100 mg of CHT in 100 mL of ethanol. From this, a working standard solution containing 100 µg/mL was prepared.

Assay procedure

Method A: Varying aliquots of standard CHT ranging from 0.1-0.5 mL (1 mL = 100 µg/mL) were accurately transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of 2 N HCl was added

followed by 1 mL of 2% w/v sodium nitrite. The solution was kept for 10 mins at 5°C for the completion of reaction. Then 1 mL of 0.1% of β-Naphthol prepared in 2% aqueous NaOH solution was added to each flask and was shaken gently. After 10 min the volume in each flask was made upto 10 mL with distilled water. The absorbance of red coloured chromogen was measured at 552 nm against reagent blank. The coloured chromogen was stable for 2 hrs. Calibration curve was constructed using the measured absorbances. The linearity was found to be between 1-5 μg/mL.

Method B: For this method, varying aliquots of standard CHT ranging from 0.4-2 mL (1 mL = 100 μg/mL) were accurately were transferred into a series of 10 mL volumetric flask. Then 1 mL of 5 N HCl was added followed by 1 mL of 0.2% w/v sodium nitrite. The solution was kept for 10 min at 5°C for the completion of reaction. Then 1 mL of ammonium sulphamate 2% w/v was added and shaken vigorously and kept for 2 min. Finally 2 mL of chromotropic acid solution was added and kept for 5 min. After 5 min the volume in each flask was made upto 10 mL with distilled water. The absorbance of red coloured chromogen was measured at 511 nm against reagent blank. The coloured chromogen was stable for 2 hrs. Calibration curve was constructed using the measured absorbances. The linearity was found to be between 4-20 μg/mL.

Pharmaceutical formulations

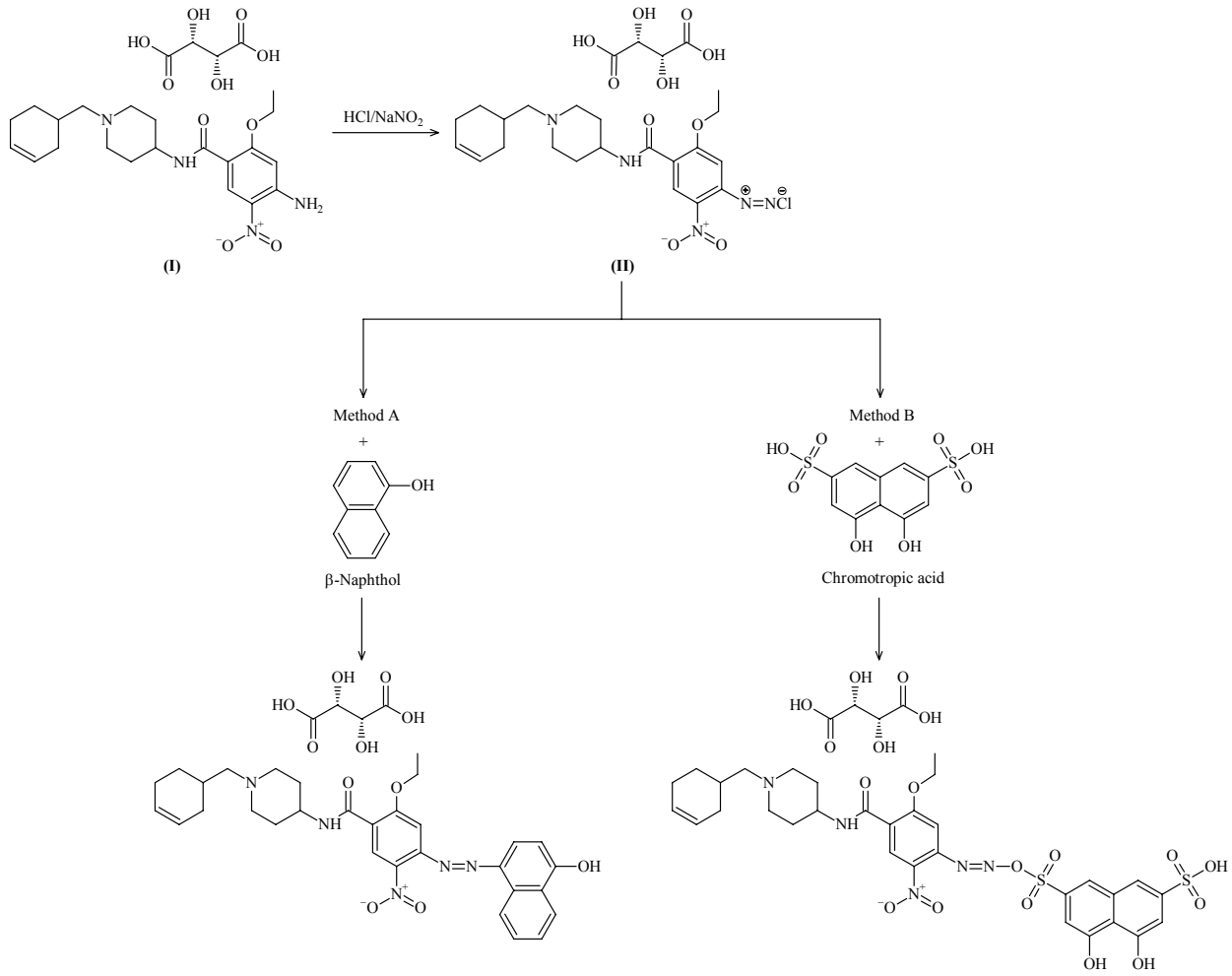
Twenty tablets containing CHT were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 10 mg of CHT was dissolved in a 40 mL of ethanol and sonicated for about 30 min and then filtered. Then the volume was diluted to 100 mL with ethanol and analyzed as given under the assay procedures for bulk samples. The results are represented in Table 2. To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug was added to the previously analyzed formulated samples and these samples were reanalyzed by the proposed methods and were recovery experiments also performed. The percentage recoveries thus obtained were given in Table 2.

The results of the above methods are compared with results obtained with UV spectrophotometric method. In UV method, solution of CHT in ethanol either pure or formulation (100 μg/mL) was prepared. Aliquots of CHT ranging from 0.25-1.25 mL. (1 mL = 100 μg) were transferred into a series of 10 mL volumetric flasks. The volume in each flask was made upto 10 mL with ethanol and the absorbances were measured at 265 nm against solvent blank. The obtained absorbance values when plotted against the concentration of CHT give the calibration graph.

RESULTS AND DISCUSSION

The acidic solution of CHT formed coloured diazotized-coupled complexes with β-naphthol and chromotropic acid which are soluble in ethanol. This property of the drug was followed for development of colorimetric methods for analysis of drug. CHT as a positively charged amino compound in acidic medium, formed red coloured diazotized-coupled complexes with β-naphthol and chromotropic acid. The absorption curves showed maxima at 552 nm and 511 nm, respectively. The colorless reagent blanks under similar conditions showed no absorption

The optical characteristics such as absorption maxima, Beer´s law limits, molar absorptivity and Sandell´s sensitivity are presented in Table 1. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) and results are summarized in Table 1. The high molar absorptivities of the resulting colored complexes indicate the high sensitivity of the methods.



Reaction scheme of CHT

Table 1: Optical characteristics of proposed method

Parameter	Proposed method	
	Method A (β-Naphthol)	**Method B (Chromotropic acid)**
λ_{max} (nm)	552	511
Beer's law limits (µg/mL)	1-5	4-20
Molar absorptivity (L mol^{-1} cm^{-1})	1.15000 x 10^5	2.1717 x 10^5
Sandell's sensitivity (µg/cm^2 -0.001 absorbance units)	0.005	0.001
Regression equation (Y = a + bC)	Y = 0.270 x -0.000238	Y = 0.0498 x - 0.0029
Slope (b)	0.2069	0.05
Intercept (a)	-0.0002	-0.002
Correlation coefficient (r)	1.000	0.9999

*Y = a+bX, where Y is the absorbance and X concentration in µg/mL
Sa = Standard deviation of intercept,
Sb = Standard deviation of slope

The optimum conditions for colour development for methods A and B was established by varying the parameter one at a time and keeping the others fixed and observing the effects of the product on the absorbance of the coloured species and incorporated in the procedures. Recovery studies were close to 100% that indicates good accuracy of the method. The results showed that these methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for dosage forms (Table 2) confirm the suitability of these methods for pharmaceutical formulations. The absorption spectrum and calibration graph of CHT with method A and B are shown in Fig. 1, 2 and 3, 4 respectively.

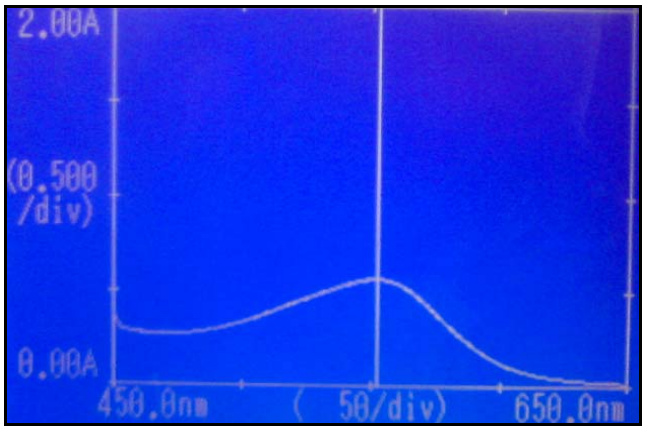


Fig. 1: Absorption spectrum of method A: CHT with β-Naphthol at 552 nm

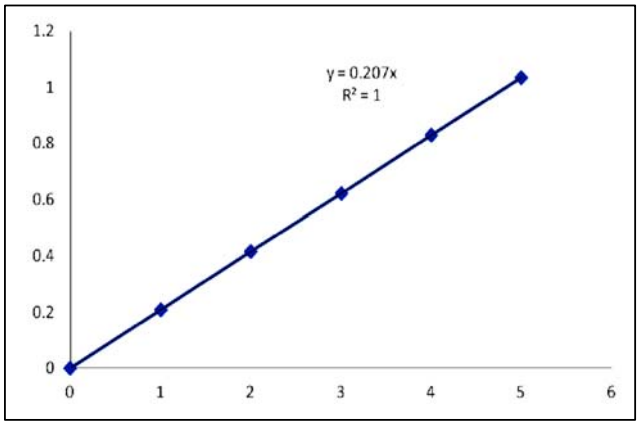


Fig. 2: Calibration graph of method A: CHT with β-Naphthol at 552 nm

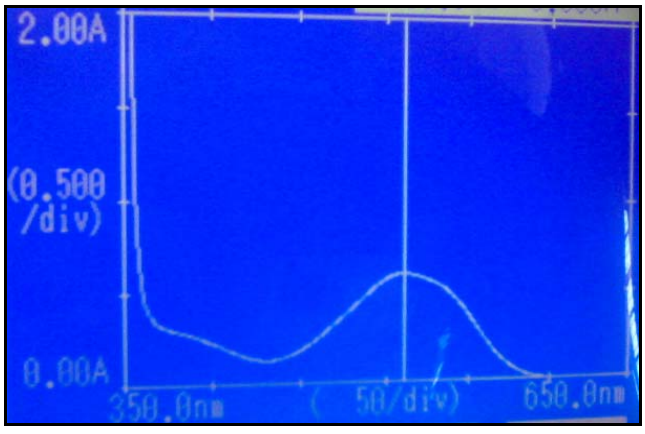


Fig. 3: Absorption spectrum of method B: CHT with Chromotropic Acid at 511 nm

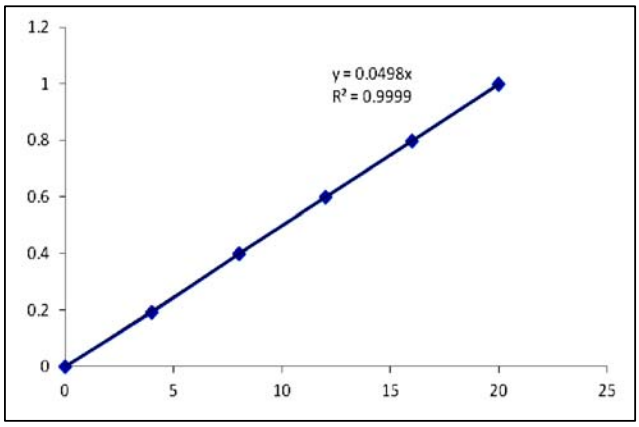


Fig. 4: Calibration graph of method B: CHT with Chromotropic Acid at 511 nm

In order to justify the reliability and suitability of proposed methods, known quantities of pure CHT was added to its various reanalyzed formulations and the mixtures were analyzed by the proposed methods. The results of recovery experiments are also summarized in Table 2. The additives and excipients usually present in pharmaceutical preparations did not interfere.

Table 2: Evaluation of CHT in pharmaceutical formulations

Brand	Labeled amount (mg/tab)	Amount obtained (mg)				
		Proposed method		Reference method UV	% Recovery*	
		A	B		A	B
Kinpride	1	0.9984 ± 0.289	0.9944 ± 0.249	0.9917 ± 1.135	100.045	100.030
Cintapro	1	0.9977 ± 0.215	0.9977 ± 0.235	1.0021 ± 1.19	100.09	100.45

*Average of 8 determination based on the label claim

The proposed methods are found to be simple, sensitive, selective, economical, accurate and precise and can be used in the determination of CHT in bulk and its pharmaceutical formulations in routine manner.

CONCLUSION

Thus, the proposed method is simple, sensitive, accurate and reproducible and can be used for the routine analysis of CHT in bulk and in pharmaceutical formulations.

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