

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF CINITAPRIDE IN PURE AND IN ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

Two simple, sensitive, accurate and economical spectrophotometric methods (A and B) were developed for the determination of cinitapride in pure and in pharmaceutical formulation. Method A is based on the reaction of the nitro group of the drug with potassium hydroxide in dimethyl sulphoxide medium to form a colored product, which shows absorbance maximum at 401 nm. Method B is based on the diazotization of cinitapride with nitrous acid to form diazotized cinitapride, followed by its coupling with β -naphthol to form a red colored chromogen which shows maximum absorption at 552 nm. Beer's law is obeyed in the concentration range of 10–50 $\mu\text{g mL}^{-1}$ for method A and 1–5 $\mu\text{g mL}^{-1}$ for method B. Correlation coefficient was found to be 0.999 for both methods. The methods have been successfully applied for the assay of the drug in pure and in pharmaceutical formulation. No interference was observed from common pharmaceutical additives. The developed methods were validated respect to linearity, precision, LOD, LOQ and accuracy.

Key words: Cinitapride, spectrophotometry, pharmaceutical formulation, validation.

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 UV⁴ and extractive spectrophotometric method⁵ and hydrotropic estimation⁶ in formulation, polarographic method⁷, LC-MS/MS methods for its determination in plasma^{7,8} and RP-HPLC Method⁹ in bulk drug. In the present work, the method A is based on the reaction of the nitro group of the drug with potassium hydroxide in dimethyl sulphoxide medium to form a colored product and method B is based on the diazotization of cinitapride with nitrous acid to form diazotized cinitapride, followed by its coupling with β -naphthol to form a red colored chromogen.

EXPERIMENTAL

Materials and methods

T60 uv/visible spectrophotometer with 1 cm matched quartz cells was used for spectrophotometric analysis. Pure Cinitapride hydrogen tartrate was a gift sample from Zydus Research Centre, (Gujarat, India). Commercial tablets of cinitapride were purchased from the local market.

Reagents

All chemicals used were of analytical grade. A 0.05M KOH (Merck, India) solution was prepared in alcohol. 2N HCl was prepared by dissolving 17 mL of con. HCl in 100 mL distilled water. 2 % sodium nitrite was prepared by dissolving 100 mg in 50 mL distilled water. 0.1 % β -naphthol was prepared in 2 % aqueous sodium hydroxide.

Preparation of standard solution

100 mg of drug was weighed and dissolved in 100 mL of methanol (1000 $\mu\text{g mL}^{-1}$).

PROCEDURE

Method A

Varying aliquots of standard cinitapride solution equivalent to 10–50 $\mu\text{g mL}^{-1}$ were accurately measured by means of micro pipette and transferred into a series of 10 mL volumetric flasks. Then, 0.6 mL of 0.05 M KOH solution was added into each flask and diluted to 10.0 mL with DMSO. The contents of each flask was mixed well at room temperature and the absorbance was measured at 401 nm against the reagent blank prepared similarly within the stability time period

of 2 h. Calibration curve was plotted by taking concentration in $\mu\text{g mL}^{-1}$ of cinitapride in x axis and absorbance in y axis. The concentration of cinitapride was calculated from a calibration curve.

Method B

Varying aliquots of standard cinitapride solution equivalent to 1–5 $\mu\text{g mL}^{-1}$ were accurately transferred into a series of 10 mL volumetric flasks. To each flask, 1.0 mL of 2 N hydrochloric acid and 1.0 mL of 2 % w/v of sodium nitrite was added and a reaction time of 10 min at 0–5°C was given for the completion of reaction. Then 1.0 mL of 0.1 % w/v of β -naphthol in 2% aqueous NaOH solution was added to each flask with gently shaking and after 10 min, the volume in each flask was made up to 10 mL with distilled water. The absorbances of red colored chromogen were measured at 552 nm against the reagent blank. The colored chromogen was stable for 3 h. The amount of cinitapride present in the sample solution was calculated from a calibration curve.

Assay of cinitapride in pharmaceutical formulation

Twenty tablets were weighed accurately and triturated to fine powder. The powder equivalent to 10 mg cinitapride 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

Nitro compounds are known to give interesting colors with alkali in different polar media. It has been suggested that the reactions of nitro aromatic compounds with alkali in acetone, alcohol, *N,N*-dimethylformamide or DMSO^{10, 11} yield nitroquinoid ions. Cinitapride contains a nitro group attached to the benzene ring, which reacts with KOH in DMSO medium to produce coloured nitroquinoid ion which absorbs maximally at 401 nm (fig. 1). The aromatic primary amine group present in cinitapride gets diazotised and coupled with β -naphthol to form a red coloured chromogen which shows maximum absorption at 552 nm (fig. 2). Optical characteristics of proposed methods were given in Table 1.

Optimization of variables and method development

In method A the effect of KOH on the intensity of the color developed at constant cinitapride concentration (20 $\mu\text{g mL}^{-1}$) was examined in the range 0.05–0.8 mL of 0.05 M KOH, the maximum absorbance was attained with 0.6 mL of 0.05 M KOH; above this volume the absorbance remained unchanged. Therefore, 0.6 mL of 0.05 M KOH

was used in all further measurements. In method B the concentration of different reagents used for method development was optimised by performing a series of experiments and the spectroscopic conditions were fixed given under procedure part.

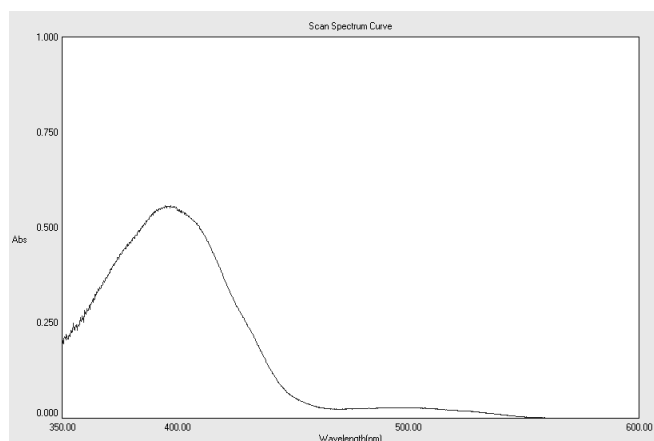


Fig. 1: Absorption spectra of coloured product of cinitapride. (50 $\mu\text{g mL}^{-1}$ cinitapride + 0.6 mL of 0.05M KOH in DMSO medium)

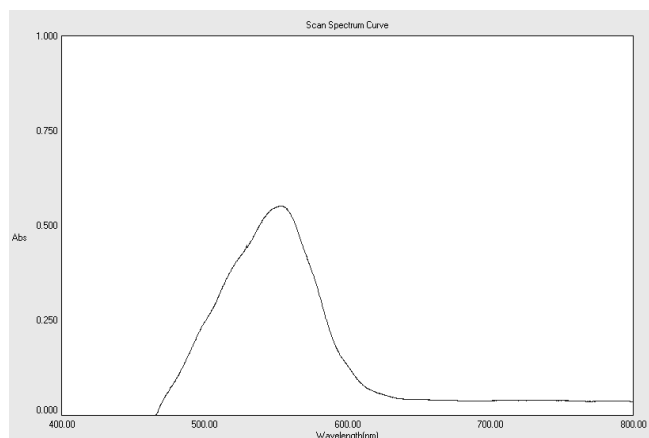


Fig. 2: Absorption spectra of coloured product of cinitapride. (3 $\mu\text{g mL}^{-1}$ cinitapride + 1.0 mL 2 N HCl + 1.0 mL of 2 % sodium nitrite + 1.0 mL of 0.1% β - naphthol in 2% aqueous NaOH and made upto 10 mL with distilled water.)

Table 1: Optical characteristics of proposed methods.

Parameters	Method A	Method B
λ_{max} (nm)	401	552
Beer's law limit ($\mu\text{g mL}^{-1}$)	10 - 50	1 - 5
Sandell's sensitivity ($\mu\text{g cm}^{-2}/0.001$ absorbance unit)	9.717 x	4.724 x
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	5.695 x	1.171 x
Limit of detection, $\mu\text{g mL}^{-1}$	1.158	0.0796
Limit of quantification, $\mu\text{g mL}^{-1}$	3.474	0.2389
Regression equation ($Y = a + bc$)		
Slope (b)	0.0106	0.2053
Intercept(a)	-0.0055	0.0095
Correlation coefficient (r^2)	0.999	0.999

Method validation

The developed methods were validated respect to linearity, precision, LOD, LOQ and accuracy. The accuracy of the proposed methods was checked by performing recovery experiments through standard addition technique. For this purpose, a known amount of pure cinitapride was added to pre-analysed pharmaceutical formulation and then determined by the recommended procedures. The results are shown in Table 2. No interference from the common

excipients was observed. The proposed methods were tested on tablet formulations and the results are presented in Table 2.

Table 2: Assay results, recovery and precision studies.

Method	Labeled amount (mg/tablet)	(% label claim* \pm S.D)	%Recovery*	Precision S.D	
				Inter-day (n=18)	Intra-day (n=6)
A	1	100.05 \pm 0.421	99.84-100.30%	0.761	0.421
B	1	99.88 \pm 0.493	99.82-100.40%	0.719	0.493

*Average of six determinations.

CONCLUSIONS

The proposed methods are found to be more sensitive, simple and accurate. The proposed methods do not require any pretreatment of the drug and tedious extraction procedure prior to its analysis. The methods can be used for routine quality control analysis of cinitapride in pharmaceutical formulation.

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