



## DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CINITAPRIDE AND PANTOPRAZOLE IN COMBINED DOSAGE FORM

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### ABSTRACT

A new, rapid, precise, selective and sensitive Q-absorbance ratio method is developed for the simultaneous estimation of cinitapride (CNT) and pantoprazole (PNT) in combined dosage form. In the developed method, absorbance was measured at 279 nm (isoabsorptive point) and 289.6 nm ( $\lambda_{max}$  of Pantoprazole). The drugs obeyed the Beer's law in the concentration range of 1-5  $\mu\text{g/mL}$  and 13-65  $\mu\text{g/mL}$  respectively for cinitapride and pantoprazole. Accuracy of the method was determined by recovery studies and was found to be 101.32 % and 98.9 % for Pantoprazole and Cinitapride respectively. The developed method is simple, precise, rapid and selective. It can be used for routine analysis of both drugs in bulk as well as in pharmaceutical formulations.

**Keywords:** Q-absorbance ratio method, Iso-absorptive point, Cinitapride, Pantoprazole.

### INTRODUCTION

Pantoprazole sodium sesquihydrate is official in IP, BP, USP, EP. Pantoprazole sodium sesquihydrate is widely used as anti-ulcer drugs (proton pump inhibitors) through inhibition of hydrogen-potassium adenosine triphosphatase ( $\text{H}^+/\text{K}^+ \text{-ATPase}$ ) in gastric parietal cells. Pantoprazole (PNT) reduces the gastric acid secretion regardless of the nature of stimulation. Chemically PNT is Sodium 5-(difluoromethoxy)-2-[(RS)-[3,4-dimethoxy pyridine-2-yl] methyl ]sulphinyl] benzimidazole-ide-sesquihydrate. Cinitapride (CNT) is a substituted benzamide gastroenteric prokinetic agent acting via complex, but synergistic effects on serotonergic 5-HT<sub>2</sub> (inhibition) and 5-HT<sub>4</sub> (stimulation) receptor and dopaminergic D<sub>2</sub> (inhibition) receptors in the neuronal synapses of the myenteric plexi. Chemically CNT is 4-Amino- N-[1-(3-cyclohexen-1-ylmethyl)-4 piperidinyl]-2-ethoxy-5- nitrobenzamide. Potentiometric titration is the only available official method for the estimation of Pantoprazole in single dosage forms. CNT and PNT combination is not official in any pharmacopoeia, hence no official method is available for the estimation of these two drugs in combined dosage forms.<sup>1,2</sup>

A literature survey regarding quantitative analysis of these drugs revealed that there were several analytical methods for PNT using extractive spectrophotometry<sup>6</sup>, HPLC<sup>7,8</sup> and HPTLC<sup>9</sup>. Extractive spectrophotometry<sup>10</sup>, RP-HPLC<sup>11</sup>, HPTLC<sup>12</sup> methods have been reported for estimation of CNT. There is only first order derivative spectroscopic method<sup>13</sup> is reported for the estimation of these two drugs in combined dosage forms. So in present study simple, sensitive, specific, accurate and precise spectroscopic method is described for the estimation of these two drugs in combined dosage forms.

### MATERIALS AND METHODS

#### Apparatus

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with a pair of 1 cm matched quartz cells. All weighing was done on SHIMADZU analytical balance (model AU-220).

#### Reagents and chemicals

Cinitapride (CNT) was kindly supplied as a gift samples from Zydus Cadila, Ahmedabad, Gujarat (India). Pantoprazole (PNT) was kindly supplied as a gift sample from Acme Pharmaceuticals, Ahmedabad, Gujarat (India). Methanol was supplied from S.D.Fine Chem Ltd., Mumbai. Whatman filter paper no. 41 (Whatman International Ltd., England) Calibrated glass wares were used throughout the work.

#### Marketed formulation

The pharmaceutical formulation containing 40 mg of PNT and 3 mg of CNT was procured from the local pharmacy.

#### Preparation of standard solution

The standard stock solution of CNT and PNT was prepared by dissolving 50 mg of each API in 50 mL of different volumetric flask with methanol to produce 1 mg/mL of each solution. 1 mL of aliquot was taken in 10 mL volumetric flask and diluted with methanol to prepare standard stock solution of 100  $\mu\text{g/mL}$  of each.

#### Selection of analytical wavelength

Standard solutions of CNT (10  $\mu\text{g/mL}$ ) and PNT (10  $\mu\text{g/mL}$ ) were scanned in the range of 200 to 400 nm for the determination of wavelength having maximum absorbance. Cinitapride shows 262.6 nm and Pantoprazole shows 289.6 nm as the wavelength having maximum absorbance. From the overlain spectra, Iso-



absorptive point was found at 279 nm. For the Q-absorbance ratio method, 279 nm and 289.6 nm were selected as analytical wavelengths.

### Absorbance Ratio Method<sup>3,4</sup>

From overlain spectra (Fig 1.) 279 nm (Isoabsorptive point) and 289.6 nm  $\lambda_{max}$  for PNT were selected for formation of Absorbance ratio equation of two drugs. The absorbance at 279 nm and 289.6 nm for CNT and PNT were measured. The absorptivity values of each drug at both wavelengths were determined. The absorbance and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$C_x = \frac{(Q_m - Q_y)}{(Q_x - Q_y)} \times \frac{A_1}{a_{x1}} \dots\dots\dots (1)$$

$$C_y = \frac{(Q_m - Q_y)}{(Q_y - Q_x)} \times \frac{A_1}{a_{y1}} \dots\dots\dots (2)$$

$Q_m$ ,  $Q_x$ , and  $Q_y$  were obtained as below:

$$Q_m = A_2/A_1,$$

$$Q_x = a_{x2}/a_{x1},$$

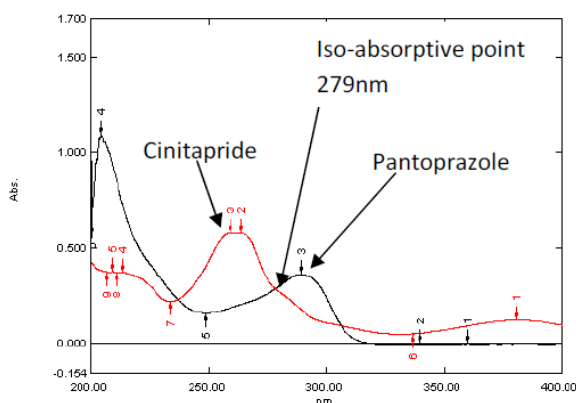
$$Q_y = a_{y2}/a_{y1}$$

Where,  $A_1$  and  $A_2$  were absorbance of sample at 279 nm and 289.6 nm respectively,

$a_{x1}$  and  $a_{x2}$  are absorptivity of CNT at 279 nm and 289.6 nm,

$a_{y1}$  and  $a_{y2}$  are absorptivity of PNT at 279 nm and 289.6 nm.

Validity of above framed equation was checked using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components.



**Figure 1:** Overlain Spectra of cinitapride (10µg/mL) and pantoprazole (10µg/mL)

### Validation of the proposed method<sup>5</sup>

#### Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 1-5 µg/mL for CNT and 13-65 µg/mL PNT. Accurately measured standard stock solutions of CNT (0.2, 0.4, 0.6, 0.8 and 1.0 mL) and PNT (1.3, 2.6, 3.9, 5.2,

6.5 mL) were transferred to a series of 10 mL volumetric flask separately and diluted up to the mark with methanol. The absorbance of solution was measured at 279 nm and 289.6 nm. The calibration curves were constructed by plotting absorbance versus concentration.

#### Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions ( $n = 7$ ) of CNT (3 µg/mL) and PNT (39 µg/mL) without changing the parameters of the proposed method.

#### Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of CNT (2, 3 and 4 µg/mL) and PNT (26, 39 and 52 µg/mL). The results were reported in terms of relative standard deviation (% CV).

#### Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of CNT and PNT by the standard addition method. Known amount of standard solutions of CNT and PNT were added to prequantified sample solutions of CNT (2 µg/mL) and PNT (26 µg/mL). The amounts of CNT and PNT were obtained by applying regression line equations.

#### Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where,  $\sigma$  = the standard deviation of Y-intercept of 5 calibration curves and

S = the mean slope of the 5 calibration curves.

#### Assay of tablet formulation

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 40 mg of PNT and 3 mg of CNT was transferred to a 100 mL volumetric flask. Add 60mL methanol and sonicate it for 10min. The working solution was filtered through whatman filter paper (No. 41) and the volume was made up to the mark with the same solvent. The aliquot portions of above solutions were further diluted with solvent to get final concentration of about 3 µg/mL CNT and 40 µg/mL of PNT and absorbance were measured at 279 nm and 289.6 nm against blank. The concentrations of two drugs in sample were determined by using equations 1 and 2. The results are reported in the table 2.

**RESULTS AND DISCUSSION**

The proposed method was validated as per ICH guideline. Method discussed in the present work provides a convenient and accurate way for simultaneous analysis of CNT and PNT. In Q analysis method, wavelengths selected were 279 nm (isoabsorptive point) and 289.6 nm ( $\lambda_{max}$  of PNT). The plot of absorbance versus respective concentrations of PNT and CNT were found to be linear in the concentration range of 13-65  $\mu\text{g/mL}$  for PNT and 1-5  $\mu\text{g/mL}$  for CNT with correlation coefficient 0.9996 at 289.6 nm and 0.9986 at 279 nm as shown in table 3 and figures 2-4. Precision was calculated in terms of repeatability, intraday and interday variations and % CV (coefficient of variance) was found to be in acceptance range (table 3). The accuracy of method was determined by standard addition method. The % recovery ranges from 100.5-102.1 for CNT and 98.8-99.1 for PNT (table 1).

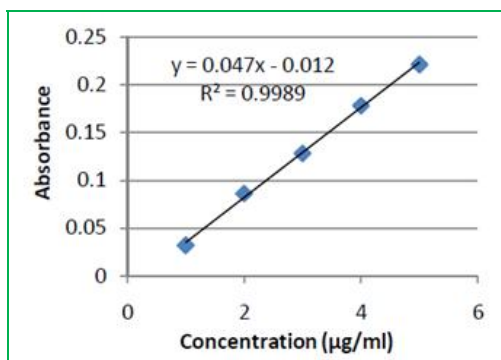


Figure 2: Calibration curve of standard CNT at 262.6 nm

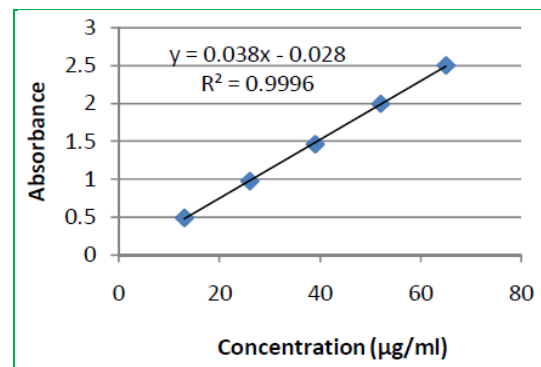


Figure 3: Calibration curve of standard PNT at 289.6 nm

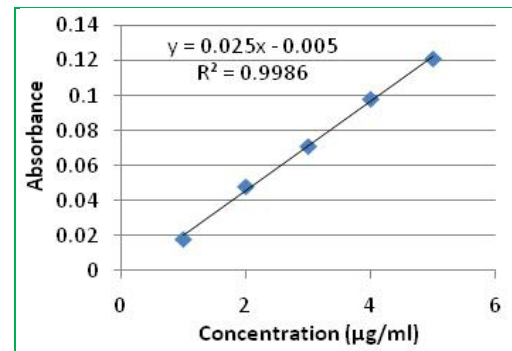


Figure 4: Calibration curve of standard CNT at 279 nm

This method can be successfully used for simultaneous estimation of CNT and PNT in their combined capsule dosage form. Marketed capsules were analysed and results obtained were within the range of 98-102% (table 2).

Table 1: Recovery studies

Concentration ( $\mu\text{g/mL}$ )		Spiked level ( $\mu\text{g/mL}$ )		Percent recovery % $\pm$ SD (n=3)	
CNT	PNT	CNT	PNT	CNT	PNT
2	26	1	13	101.35 $\pm$ 0.20	98.93 $\pm$ 0.29
2	26	2	26	102.10 $\pm$ 0.05	99.13 $\pm$ 0.25
2	26	3	39	100.5 $\pm$ 0.09	98.85 $\pm$ 0.89

Table 2: Results of simultaneous estimation of CNT and PNT in marketed formulation

Marketed Formulation	Labelled mg/tablet		Obtained % of label claim	
	CNT	PNT	CNT	PNT
Cintodac	3	40	99.1 $\pm$ 0.20	99.6 $\pm$ 0.25

Table 3: Validation parameters

Parameter	Q-absorbance ratio method		
	PNT	CNT	(Iso)
	289.6 nm	289.6 nm	279 Nm
Linearity Range ( $\mu\text{g/mL}$ )	13-65	1-5	1-5
Slope	0.038	0.017	0.025
Intercept	0.028	0.002	0.005
Correlation coefficient	0.9996	0.9979	0.9986
Precision (%CV)			
Repeatability	1.699	1.658	1.818
Interday (n=3)	1.0-3.4	0.7-3.2	1.0-2.7
Intraday (n=3)	0.6-2.1	1.1-2.9	1.2-2.4
LOD ( $\mu\text{g/mL}$ )	0.520	0.223	0.256
LOQ ( $\mu\text{g/mL}$ )	1.821	0.815	0.890

Where, LOD = limit of detection, Iso = Isoabsorptive point, LOQ = limit of quantification



**CONCLUSION**

The low value of relative standard deviation for repeated measurement indicates that the method is precise. The value of % recovery is approximately 100%, which indicates that the method can be used for estimation of these two drugs in combined dosage forms without any interference due to the other components present in the formulations. Hence this study presents simple, accurate, precise and rapid spectroscopic analytical method for the simultaneous estimation of these two drugs in combined dosage form.

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