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# Simultaneous Estimation Of Cinitapride And Pantoprazole Sodium By Rp-Hplc In Their Marketed Formulation

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**Abstract:** A reversed-phase-liquid chromatographic (RP-HPLC) method was developed for the determination of Cinitapride and Pantoprazole Sodium in their marketed formulation. A reversed-phase C-18 column (250 mm × 4.60 mm i.d., particle size 5 µm) with mobile phase consisting of acetonitrile : water : Triethylamine (80:20:0.05) was used. The flow rate was 1.2 ml/ min and effluents were monitored at 260 nm. The retention times of Cinitapride and Pantoprazole Sodium were found to be 5.26 ± 0.10 min and 1.72 ± 0.10 min, respectively. The method was validated in terms of linearity, range, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The method showed good linearity in the range of 12-28 µgm/ml for Cinitapride and 24-56 µgm/ml for Pantoprazole Sodium. The % recoveries of Cinitapride and Pantoprazole Sodium were found to be between 99.52 - 99.85% and 99.38 – 99.74 % respectively. The percentage RSD for the method precision was found to be less than 2%. The proposed method was successfully applied to the estimation of Cinitapride and Pantoprazole Sodium in combined capsule dosage forms.

**Keywords:** Cinitapride, Pantoprazole Sodium, RP-HPLC.

## INTRODUCTION

Cinitapride (CNT) chemically 4-amino-*N*-[3-(Cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ethoxy-5-nitro benzamide has the molecular formula $C_{21}H_{30}N_4O_4$ (Figure 1). Cinitapride is a drug that has against action to the serotoninergic 5-$HT_2$ and $D_2$- dopaminergic receptors that has been indicated in the gastro-esophageal reflux and in the functional disorders of gastrointestinal motility treatment.[1,2] Cinitapride is not official in any Pharmacopoeia. Pantoprazole Sodium (PSS) chemically Sodium-[5-(Difluoromethoxy)-2-[[(3, 4-di methoxy-2-pyridinyl)-methyl]-sulfinyl] 1H-benzimidazolide sesquihydrate has the molecular formula $C_{16}H_{14}F_2N_3NaO_4S * 1.5\ H_2O$ (Figure 2). It is official in Indian Pharmacopoeia.[3] The proton-pump inhibitor Pantoprazole inhibit gastric acid by blocking the H+/K+-adenosine triphosphatase enzyme system (the proton pump) of the gastric parietal cell1. It is used for short-term treatment of erosion and ulceration of the esophagus. Literature survey reveals spectrophotometry [4], Colourimetry [5,6] HPLC [7,8] methods have been reported for estimation of Pantoprazole in bulk as well as pharmaceutical dosage forms. Literature survey reveals Spectrophotometry [9], HPLC [10, 11] method in pharmaceutical dosage form But no method had yet been reported for simultaneous estimation of these two drugs using HPLC in pharmaceutical dosage forms. Therefore, the present work was aimed to develop and validate a new RP- HPLC method for simultaneous estimation of Cinitapride and Pantoprazole Sodium in pharmaceutical dosage forms.

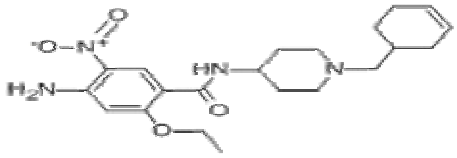

**FIGURE 1. Structure of Cinitapride (CNT)**

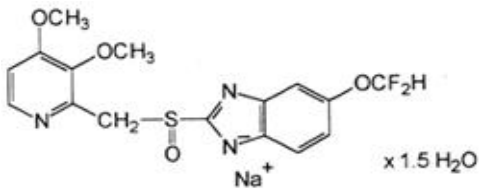

**FIGURE 2. Pantoprazole Sodium (PSS)**

## EXPERIMENTAL

### CHEMICALS AND REAGENTS

Reference standards of CNT and PSS were procured as gift samples from Euro Asian Chemical Pvt. Ltd. and Sun Pharmaceuticals Ltd. Respectively. Cintodac Capsules (3 mg Cinitapride and 40 mg Pantoprazole) were procured from the local market. HPLC grade acetonitrile, water and triethylamine were obtained from Polychem Limited, Mumbai, India.

### INSTRUMENTATION

Separation was performed with a Shimadzu SPD-20 A, Equipped with a Rheodyne injector valve with a 20.0 µl loop and a UV/VIS detector.

### CHROMATOGRAPHIC CONDITIONS

A Phenomenex C-18 column (250 mm * 4.60 mm, 5 µ) was used for the separation.The isocratic mobile phase was consisted of Acetonitrile **:** Water **:** Triethylamine 80:20:0.05(v/v). The mobile phase was sonicated for 15 min and filtered through a 0.20 µM membrane filter
Paper. Flow rate of mobile phase was 1.2 ml/min. The UV–visible detector was set at 260nm.

### PREPARATION OF STANDARD STOCK SOLUTION

100 mg CNT and 112.75 mg PSS ( Equivalent to Pantoprazole 100 mg) were accurately weighed and transferred to 100 ml volumetric flasks separately and dissolved in the mobile phase to give stock solution of 1000 µg/ml and 1000 µg/ml CNT and PSS respectively. The above standard stock solution were further diluted to obtain working Stock solution 200 µg/ml and 400 µg/ml CNT and PSS respectively.

### PREPARATION OF SAMPLE SOLUTION

Twenty capsules (Cintodac, Zydus Cadila Healthcare Ltd) were weighed. The powder from twenty capsules were collected and weighed. The Powder equivalent to 3 mg of CNT and 40 mg of Pantoprazole was transferred to a 100 ml volumetric flask and dissolved in mobile phase. The solution was ultrasonicated for 30 min and filtered through 0.20 micron membrane filter. From this 20 ml of stock solution was transferred to 100 ml volumetric flask. And 3.4 ml of accurately prepared solution of Standard stock solution of Cinitapride (1000 µg/ml) was spiked, and then make up the volume with the mobile phase. The solutions were further diluted with mobile phase to obtain concentration of 20 µg/ml of CNT and 40 µg/ml of PSS and were subjected to HPLC analysis. From the peak area of CNT and PSS, the amount of drugs in samples was computed.

### PREPARATION OF MOBILE PHASE

Mix Acetonitrile, Water and trithylamine in ratio of 90:10:0.05(v/v/v). Sonicate it for 30 minutes and filter through 0.2 µ size membrane filter.

## METHOD VALIDATION

**SPECIFICITY:** Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interference was detected at the retention time of CNT and PSS in sample solution.

**LINEARITY AND RANGE*:*** Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions of different concentrations and analyses were performed five times. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration.

**ACCURACY:** The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. Samples were spiked with 80, 100, and 120% of the standard and analyzed. The experiment was performed in triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

**PRECISION*:*** The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision was considered at different levels, i.e. Method, System, Interday and Intraday. Repeatability was studied by carrying out system precision And Method Precision. System Precision

was determined from results for six replicate of synthetic mixture and Method Precision is for formulation Mixture.
Intraday precision of the developed method was evaluated by analyzing samples of three different concentration of CNT (16, 20, 24 µg/ml) and PSS (32, 40, 48 µg/ml) in triplicates on same day.
Interday was determined from the same concentration of three consecutive days. Results from determinations of precision were expressed as % RSD. It should not greater than 2%.

**LIMIT OF DETECTION AND LIMIT OF QUANTITATION*:*** The LOD and LOQ were separately determined on the basis of standard calibration curve. The standard deviation of the peak area of the standard solution (n = 3 determination) was used to calculate LOD and LOQ. Following formulae were used; LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the intercept and S is the slope of the calibration curve.

**ROBUSTNESS:** For demonstrating the robustness, some of experimental conditions were purposely altered and evaluated.

- Change mobile phase composition by ± 2 ml of (absolute) organic solvent.
- Change in detection wavelength ± 10 nm

% RSD should not greater than 2%.

## RESULTS AND DISCUSSION

### METHOD DEVELOPMENT

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. Several mobile phase compositions were tried to resolve the peaks of CNT and PSS. The optimum mobile phase containing Acetonitrile **:** Water **:** Triethylamine 80:20:0.05 (v/v) was selected because it could resolve the peaks of CNT (RT = 5.26 ± 0.10 min) and PSS (RT = 1.72 ± 0.10 min) with a resolution factor of 14.0 Quantification was achieved with UV detection at 260 nm on the basis of peak area at 1.2 ml/min flow rate. A typical HPLC chromatogram obtained during simultaneous determination of CNT and PSS is given in (Figure 3).

### METHOD VALIDATION

**LINEARITY AND RANGE*:*** Different concentrations (24, 32, 40, 48, 56 µg/ml of PSS and 12, 16, 20, 24, 28µg/ml) of the mixture of two drugs were prepared for linearity studies. The calibration curves obtained by plotting peak area against concentration showed linear relationship over a concentration range of 12-28 µg/ml for CNT and 24-56 µg/ml or PSS. The linear regression equations for CNT and PSS were found to be y = 85.42x + 12.87 and y = 23.97x – 54.37 respectively. The regression coefficient values ($r^2$) were found to be 0.997 and 0.999 respectively indicating a high degree of linearity. Calibration curves of CNT and PSS are shown in Figure 4 and 5 respectively**.** Regression characteristics of the proposed HPLC method are given in (Table 1).

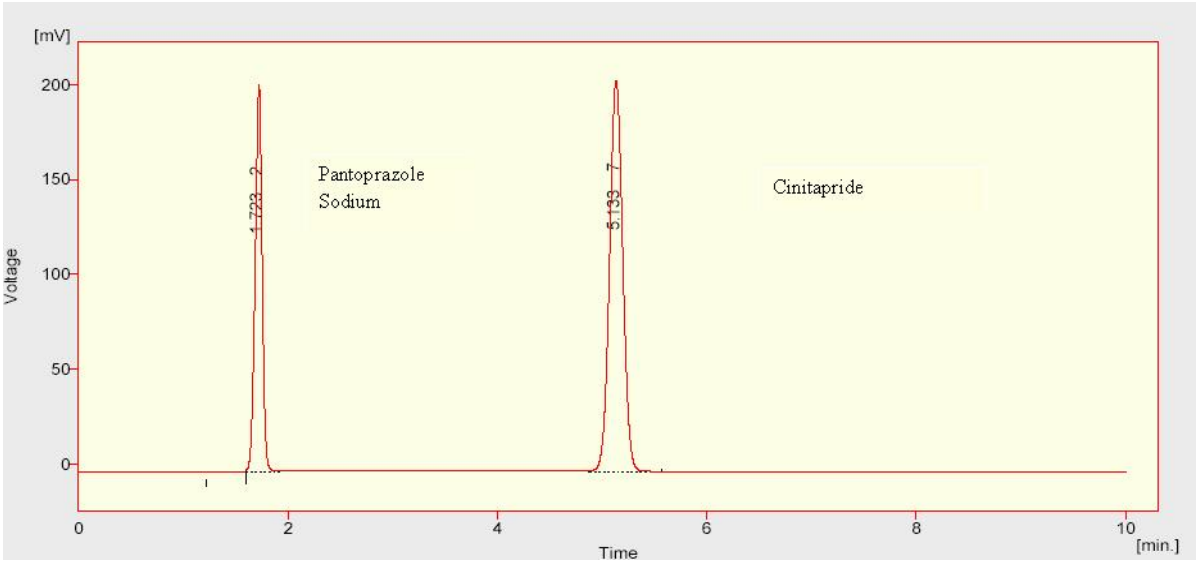


**Figure 3. HPLC chromatogram obtained during simultaneous determination of CNT and PSS**

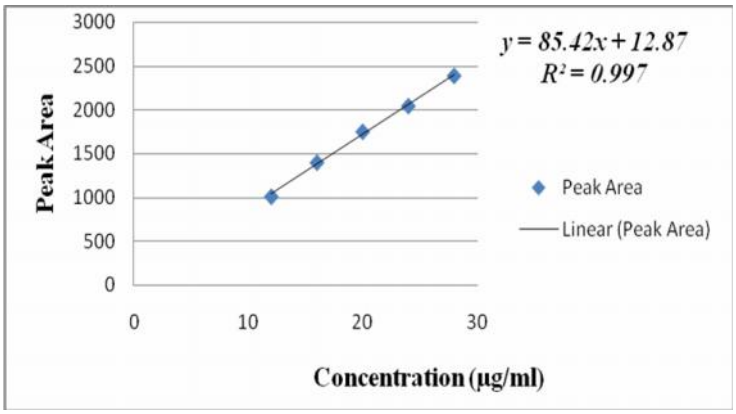


**FIGURE 4: Calibration Curve of Cinitapride**

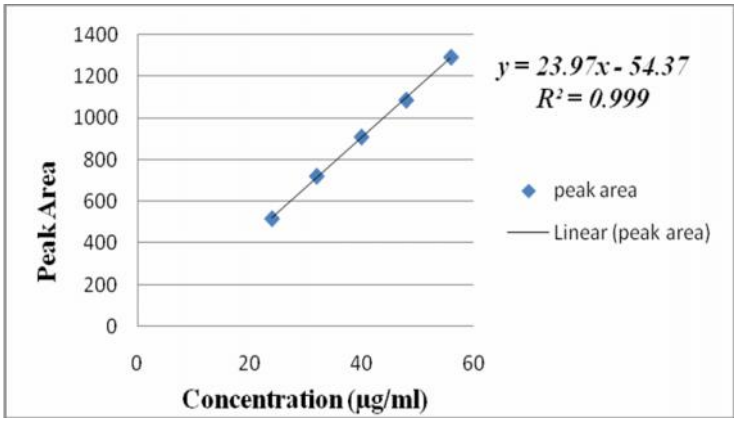


**FIGURE 5: Calibration Curve of Pantoprazole Sodium**

**Table 1. Regression characteristics of the proposed HPLC method**

| Linearity Experiment | CNT | PSS |
|---|---|---|
| Range (µg/ml) | 12-28 | 24-56 |
| Regression coefficient ($r^2$) | 0.997 | 0.999 |
| Slope | 85.42 | 23.97 |
| Intercept | 12.87 | 54.37 |

**ACCURACY*:*** Recovery studies were carried out by applying the standard addition method. Known amounts of standard CNT and PSS corresponding to 80%, 100%, and 120% of the label claim were added to sample of capsule dosage form separately. The average % recoveries for CNT and PSS in marketed formulation were found to be between 99.52 – 99.85 % and 99.38 – 99.74 % respectively. The results revealed that there was no interference of excipients. The results of accuracy are shown in Table-2.

**PRECISION:** The system and method precision showed a % RSD of 0.71% of CNT & 0.70% for PSS and 1.20 % of CNT & 1.23% for PSS respectively. The intraday precision having %RSD of (1.08-1.17 %) of CNT and (1.05 – 1.27 %) for PSS. Likewise, the interday precision showed a %RSD (1.27 – 1.45 %) of CNT and (1.16 – 1.50 %) for PSS.

**LIMIT OF DETECTION AND LIMIT OF QUANTITATION:** The limit of detection and limit of quantification were found to be 0.52 and 1.58 µg/ml for CNT and 0.91 and 2.77 µg/ml for PSS. The values indicate that the method is sensitive.

**ROBUSTNESS:** The proposed method was found to be robust enough(% RSD < 2.) to withstand such slight changes and allow routine analysis of the sample. The result of robustness is shown in Table-2.

**Table-2 Summary of Validation parameters for proposed method**

| Parameters | | RP-HPLC Method | |
|---|---|---|---|
| | | CNT | PSS |
| Conc. Range | (µg/ml) | 12-28 | 24-56 |
| Accuracy | 80% | 99.73 ± 0.55 | 99.53 ± 0.70 |
| | 100% | 99.52 ± 0.68 | 99.38 ± 0.77 |
| | 120% | 99.85 ± 0.95 | 99.74 ± 0.60 |
| Precision | System | 0.71 | 0.70 |
| | Method | 1.20 | 1.23 |
| | Intraday | 1.08-1.17 | 1.05-1.27 |
| | Interday | 1.27-1.45 | 1.16-1.50 |
| LOD | (µg/ml) | 0.52 | 0.91 |
| LOQ | (µg/ml) | 1.58 | 2.77 |
| Robustness | Change in the organic phase (78:22:0.05 v/v/v) | 1.39 | 1.25 |
| | Change in the organic phase (82:18:0.05 v/v/v) | 1.65 | 1.57 |
| | Change in the Wavelength (250 nm) | 1.42 | 1.11 |
| | Change in the Wavelength (270 nm) | 1.57 | 1.38 |

**Analysis of Marketed Formulation**

Analysis of marketed Capsule (CINTODAC-Capsule Zydus Cadila Healthcare Ltd.) was carried out using optimized mobile phase and HPLC conditions. The % drug content of capsule obtained by the proposed method for CNT and PSS was found to be 99.65 and 99.98, respectively. This showed that the estimation of dosage forms was accurate within the acceptance level of 95% to 105%. The results are given in Table 3.

**SYSTEM SUITABILITY PARAMETERS**

For system suitability parameters, seven replicate injections of mixed standard solution were injected and parameters such as the resolution, theoretical plate and asymmetry factor of the peaks were calculated. The results are shown in Table 4.

**CONCLUSION**

A novel RP- HPLC method has been developed for the simultaneous estimation of CNT and PSS in marketed formulations. The method gave good resolution for both the drugs with a short analysis time below 6 minutes. The developed method was validated. It was found to be novel, simple, precise, accurate, and sensitive. The good % recovery in capsule forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of CNT and PSS in combined dosage form. It can be also used in the quality control in bulk manufacturing.

**Table 3. Analysis of marketed Formulation**

| Drug | Label Claim (mg) | Quantity Found (mg) | % Amount Found ± SD |
|---|---|---|---|
| **CNT** | 3 | 2.99 | 99.98 % ± 1.03 |
| **PSS** | 40 | 39.87 | 99.65% ± 1.83 |

**Table 4. System suitability Parameter**

| Parameter | Pantoprazole Sodium | Cinitapride |
|---|---|---|
| **Resolution** | 14.0 | |
| **Asymmetry Factor** | 0.87 | 0.90 |
| **Theoretical Plates** | 2832 | 5005 |

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