

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PANTOPRAZOLE AND CINITAPRIDE IN ANTIULCER FORMULATION

JITESH S. JADHAV, SWETAL P. VASSA, HEMANT KUMAR JAIN*

Department of Quality Assurance Techniques, STES's Sinhgad College of Pharmacy, Vadgaon (Bk), Off. Sinhgad Road, Pune 411041, Maharashtra, India. Email: hemantkjain2001@yahoo.co.in

Received: 22 Jun 2012, Revised and Accepted: 29 July 2012

ABSTRACT

The present work describes a reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Pantoprazole and Cinitapride in capsule formulation. Chromatography was performed on a Chromatopak Peerless LC-C₁₈ column (250 mm × 4.6 mm) with mobile phase containing methanol: 0.02 M potassium dihydrogen phosphate (98: 02 % v/v) at pH 7. The flow rate was 0.6 mL/min and the eluent was monitored at 280 nm. The selected chromatographic conditions were found to effectively separate Pantoprazole (run time 4.8 ± 0.05 min) and Cinitapride (run time 6.0 ± 0.05 min). Linearity for Pantoprazole and Cinitapride was found in the range of 240-560 and 18-42 µg/mL, respectively. The proposed method was found to be simple, accurate, precise, economical and specific. Therefore, it can be used for simultaneous analysis of these drugs in capsule formulation.

Keywords: Pantoprazole, Cinitapride, RP-HPLC.

INTRODUCTION

Pantoprazole, 2-((4,5-dimethoxy pyridine-2-yl)methylsulfinyl)-6-(difluoromethoxy)-1H-benzodimidazole, widely used as anti-ulcer drugs (proton pump inhibitors) through inhibition of hydrogen-potassium adenosine triphosphatase (H⁺/ K⁺ - ATPase) in gastric parietal cells. Pantoprazole reduces the gastric acid secretion regardless of the nature of stimulation hence hyperacidity can be controlled by it^{1,2}. Cinitapride, 4-amino-N-(1-((cyclohex-3-enyl)methyl)piperidin-4-yl)-2-ethoxy-5-nitrobenzamide, is used as gastroprokinetic and anti-ulcer agent. It acts via inhibition of serotonergic 5-HT₂ and dopaminergic D₂ receptors and stimulation of serotonergic 5-HT₁ receptors in the neuronal synapses of the myenteric plexi^{1,2}, thereby enhancing gastrointestinal peristalsis, motility and increasing lower esophageal sphincter tone. This increased gastrointestinal motility can facilitates the movement of acid contents further down in the intestine preventing reflux esophagitis and in this way control nausea and vomiting. Thus, the pharmacology of Pantoprazole and Cinitapride corroborates their use in combined dosage form to treat various gastro intestinal disorders in particular for hyperacidity frequently associated with gastro intestinal dysmotility¹.

Spectrophotometric and chromatographic methods are reported for estimation of Pantoprazole alone and in combination with other drugs³⁻⁷. Spectrophotometric and chromatographic methods are reported for estimation of Cinitapride⁸⁻¹⁰. However, there is no report yet for determination of combination of Pantoprazole and Cinitapride by proposed method. The aim of the present work was the development of a RP-HPLC method for simultaneous estimation of Pantoprazole and Cinitapride and its validation according to ICH guidelines¹¹.

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade Pantoprazole was received as a gift sample from Fedun Pharma, Palghar (E), Thane, India and Cinitapride was received as a gift sample from Symed Labs Ltd., Hyderabad, India. The capsule formulation Cintodac (manufactured by Cadila Healthcare Limited, Ponda, Goa, India) with a labeled claim of 40 mg Pantoprazole and 3 mg Cinitapride, respectively, was purchased from local drug stores. The HPLC grade of solvents methanol and water was used in this investigation.

Instrumentation

The HPLC system (Shimadzu, LC2010 CHT, Kyoto, Japan) consisted of a quaternary system with automatic injection facility, loop

capacity 20 µL, UV-visible detector and LC Solution version 1.25 software. The column used was Chromatopak Peerless LC-C₁₈ (250 mm × 4.6 mm).

Chromatographic conditions

The column temperature was kept ambient with following optimized conditions. The flow rate of mobile phase was maintained 0.6 mL/min. The detection was carried out at 280 nm. Injection volume was taken 20 µL and run time was kept 10 min.

Preparation of buffer

Potassium dihydrogen phosphate solution (0.02 M) was prepared by dissolving 2.7225 g in 1000 mL of HPLC grade water and pH of this solution was adjusted to 7.0 with triethylamine then the solution was filtered through 0.22 µm nylon filter and sonicated.

Preparation of mixed standard solutions

The mixed standard solution was prepared by dissolving 42 mg of Cinitapride in 50 mL volumetric flask containing methanol and volume was made up to mark. From this solution 5 mL was added into 100 mL volumetric flask containing 56 mg of Pantoprazole, then volume was made up to the mark with methanol. The solution was ultrasonicated for 5 min, at room temperature. Final concentration of Pantoprazole was 560 µg/mL and Cinitapride was 42 µg/mL in mixed standard solution. The mixed standard solution was further diluted with methanol to get concentration range of 240-560 µg/mL of Pantoprazole and 18-42 µg/mL of Cinitapride. Plotting a graph of peak area vs. concentration allowed the checking of linearity of detector response.

Procedure for assay

Assay was performed on marketed formulations containing Pantoprazole and Cinitapride.

Marketed capsules (20), of Cintodac (manufactured by Cadila Healthcare Limited, Ponda, Goa, India) were taken and weighed properly. Average weight of capsule was calculated. The pellets from each capsule were crushed properly; a powder equivalent to 40 mg Pantoprazole [≡ 3 mg Cinitapride] was transferred to 100 mL volumetric flask, approximate 60 mL methanol was added to it and shaken. The content in volumetric flask was sonicated properly volume was made up to mark with methanol and filtered with 0.22 µm nylon filter. A 20 µL of filtrate was injected into injector of liquid chromatographic system. Chromatogram was recorded and further calculations were carried out.

Table 1: Results of Analysis of Capsule Formulation

Parameters	Pantoprazole	Cinitapride
Label Claim (mg/capsule)	40	3
Amount of drug estimated* (mg) ± SD	38.39 ± 0.15	2.77 ± 0.05
% of label claim* (%) ± SD	95.95 ± 0.33	92.37 ± 1.45
% RSD	0.35	1.57

*six replicate injections.

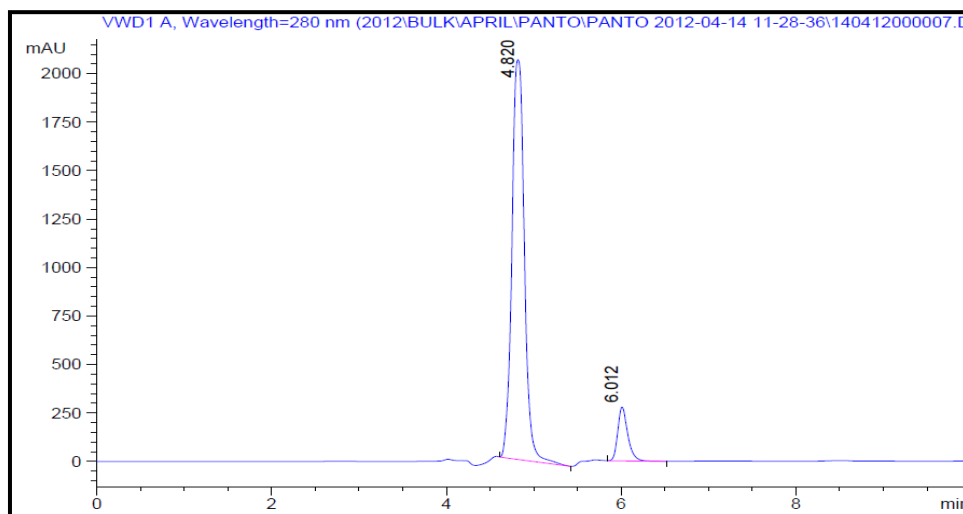


Fig. 1: Chromatogram of Marketed formulation of Pantoprazole tR 4.8 ± 0.05 min, Cinitapride tR 6.0 ± 0.05 min.

Method Validation¹²

Linearity: A 20 µL volume of Pantoprazole (240 µg/mL) and Cinitapride (18 µg/mL) was injected into HPLC system under the optimized chromatographic conditions for this study. The other injections have done with 5 different concentrations in the range of 240-560 µg/mL of Pantoprazole and 18-42 µg/mL of Cinitapride.

Precision: Intra-day and inter-day precision study of Pantoprazole and Cinitapride was carried out by estimating the corresponding responses nine times on the same day and on two different days.

Detection (LOD) and Quantification (LOQ) limit: The limit of detection (LOD) and limit of quantitation (LOQ) were determined using standard deviation of Y-intercept and average slope of six sets of calibration curves¹³. The limits of detection and quantification were calculated using the following formulae:

$$\text{LOD} = \frac{3.3 \text{ SD}}{S}$$

$$\text{LOQ} = \frac{10 \text{ SD}}{S}$$

Where, SD = Standard Deviation of Y-intercept,

S = Average of slope of the calibration curve.

Recovery studies: For both drugs recovery studies were carried out by applying the method to drug sample to which known amount of Pantoprazole and Cinitapride corresponding to 80, 100 and 120 % of label claim had been added (Standard addition method). At each level three determinations were carried out.

Robustness: To evaluate robustness of the developed method, few parameters were varied. These parameters included variation in

flow rate, percentage of methanol in mobile phase and wavelength of detection.

Specificity: The specificity of the method was ascertained by analyzing standard drug and sample solutions of capsule formulation. The retention time of Pantoprazole and Cinitapride was confirmed by comparing the retention time with that of the standard drug to indicate that there is no interference of the excipients in the capsule formulation.

RESULTS AND DISCUSSION

Linearity: Pantoprazole and Cinitapride showed good correlation coefficient in concentration range of 240-560 µg/mL ($r^2 = 0.999$) and 18-42 µg/mL ($r^2 = 0.999$), respectively.

Table 2: Linear Regression Data for Calibration Curves

Parameter	Pantoprazole	Cinitapride
Linearity range*	240-560 µg/mL	18-42 µg/mL
r^2	0.999	0.999
Regression Equation	$Y = 51.95x + 71.93$	$Y = 56.36x + 70.58$
Slope	51.95	56.36
Intercept	71.93	70.58

*n = 6

Precision: Intra-day and inter-day precision study of Pantoprazole and Cinitapride was carried out by estimating the corresponding responses nine times on the same day and on two different days for the concentration of 400 µg/mL and 30 µg/mL for Pantoprazole and Cinitapride, respectively and expressed as % RSD. The results for intra-day and inter-day precision of both drugs are shown in table 3.

Table 3: Results of Precision Study*

Parameters	Intra-day precision		Inter-day precision	
	Mean Area ± S.D.	% R.S.D.	Mean Area ± S.D.	% R.S.D.
Pantoprazole	3310.02 ± 49.41	1.49	2955.36 ± 53.96	1.82
Cinitapride	4022.60 ± 62.74	1.56	3961.77 ± 69.13	1.74

*n = 9

Detection (LOD) and Quantification (LOQ) limit: The LOD values were found to be 0.52 µg/mL and 0.27 µg/mL for Pantoprazole and Cinitapride, respectively. LOQ values were found to be 1.6 µg/mL and 0.82 µg/mL for Pantoprazole and Cinitapride, respectively.

Recovery studies: The proposed method when used for subsequent estimation of Pantoprazole and Cinitapride from pharmaceutical dosage form after spiking with additional standard drug solution the results obtained was shown in table 4.

Robustness: Each factor selected was changed at three levels (-1, 0, 1). One factor was changed at one time to estimate the effect. The parameters included variation in flow rate, percentage of methanol in the mobile phase and wavelength of detection. Insignificant variability in retention time was observed.

System Suitability Parameters: System suitability tests were done to verify the resolution and reproducibility of the chromatographic system. System suitability parameters for the optimized method were found to be in acceptable limit.

Table 4: Results of Recovery Studies*

Level	% of Pantoprazole recovered	% of Cinitapride recovered
80%	99.55 %	98.92 %
100%	99.50 %	99.59 %
120%	99.54 %	98.64 %
% R.S.D.	0.03	0.49

*three replicate injections.

Table 5: Results of Robustness Data

Level	Mobile Phase Mean* ± SD		Flow rate Mean* ± SD		Wavelength of detection Mean* ± SD	
	Pantoprazole	Cinitapride	Pantoprazole	Cinitapride	Pantoprazole	Cinitapride
-1	4.83 ± 0.015	6.02 ± 0.005	4.91 ± 0.043	6.09 ± 0.03	4.95 ± 0.025	6.07 ± 0.041
0	4.82 ± 0.020	6.0 ± 0.003	4.82 ± 0.031	6.00 ± 0.005	4.82 ± 0.045	6.00 ± 0.042
+1	4.81 ± 0.010	5.98 ± 0.005	4.78 ± 0.041	5.92 ± 0.017	4.77 ± 0.015	5.95 ± 0.021

*n=3

Table 3: System Suitability Parameters

Parameters	Pantoprazole	Cinitapride
No. of Theoretical Plates	5005	14304
Tailing factor	0.85	0.69
Resolution	5.05	

CONCLUSION

A simple, accurate, precise, and robust HPLC method was developed for the simultaneous analysis of Pantoprazole and Cinitapride and validated as per ICH guidelines. The method could be employed for simultaneous estimation of the both drugs in drug analysis laboratories.

ACKNOWLEDGEMENT

The authors are thankful to Fedun Pharma, Palghar (E), Thane, India and Symbio Labs Ltd., Hyderabad, India for providing gift sample of Pantoprazole and Cinitapride, respectively and President of this society and Principal of this college for providing required facilities to carry out this research work.

REFERENCES

- Sweetman SC, The Martindale Complete Drug Reference, 34th ed., Pharmaceutical Press.; 2007. p. 1259,1283
- Budavari S., The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, 14th ed., Published by Merck Research Laboratory, NJ; 2006. p. 2294, 7014
- Suslu I., Altinoz S., Yildiz E. Determination of pantoprazole in tablet dosage forms by two different spectrophotometric methods. *Fab J. Pharm. Sci.* 2003; 28: 85-92.
- Basavaiah K., Anil U.R., Tharpa K. Spectrophotometric determination of pantoprazole sodium in pharmaceuticals using n-bromosuccinimide, methyl orange and indigo carmine as reagents. *Iran. J. Chem. Chem. Eng.* 2009; 28(1): 31-36.
- Noubarani M., Keyhanfar F., Motevalian M., Mahmoudian M. Improved HPLC method for determination of four ppi's, omeprazole, pantoprazole, lansoprazole and rabeprazole in human plasma. *J. Pharm Pharmaceut Sci.* 2010; 13(1): 1-10.
- Sivakumar T., Manavalan R., Valliappan K. Development and validation of a RP-HPLC method for simultaneous determination of domperidone and pantoprazole in pharmaceutical dosage forms. *Acta Chromatogr.* 2007; 18: 130-142.
- Zeinab Abdelaziz el-Sherif, Afaf Osman Mohamed, Mohamed Galal el-Bardicy, Mohamed Fayez el-Tarras. Reversed-phase High Performance Liquid Chromatographic method for the determination of lansoprazole, omeprazole and pantoprazole sodium sesquihydrate in presence of their acid-induced degradation products. *Chem. Pharm. Bull.* 2006; 54(6): 814-818.
- Thangabalan P., Kumar V. Spectrophotometric analysis of cinitapride in tablet dosage form using 2.0 M Sodium Benzoate solution as hydrotropic solubilizing agent. *J. of Ana. Chem.* 2011; 1(2): 47-50.
- Roy S. M. N., Mangaonkar K. V., Desai A. Y., Yetal S. M. RP-HPLC method for the determination of cinitapride in the presence of its degradation products in bulk drug. *E-Journal of Chemistry.* 2010; 7(1): 311-319.
- Humaira S., Dey A., Appala R. S., Sanaullah S., Development and validation of a rapid RP HPLC method for the determination of cinitapride hydrogen tartarate in solid oral dosage forms. *E-Journal of Chemistry.* 2011; 8(3): 1424-1429.
- Validation of Analytical Procedure: Text and Methodology. The International Conference on Harmonization, Q2 (R1), IFPMA, Geneva: 2005, p. 1-13.
- Pharne A. B., Santhakumari B., Ghemud A. S., Jain H. K., Kulkarni M. J., Bioanalytical Method Development and Validation of Vildagliptin A Novel Dipeptidyl Peptidase IV Inhibitor by RP-HPLC Method. *Int J Pharm Pharm Sci.* 2012; 4(3): 119-123.
- Sojitra C., Rajput S., Development and validation of RP-HPLC methods for simultaneous estimation of naproxen and esomeprazole magnesium trihydrate in combined pharmaceutical formulation. *Int J Pharm Pharm Sci.* 2012; 4(3): 533-537.