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Analytical method development and validation for simultaneous estimation of cinitapride and pantoprazole in pharmaceutical dosage form

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ABSTRACT

A new, simple, precise, accurate, rapid as well as cost effective reverse phase HPLC method was developed for simultaneous estimation of cinitapride and pantoprazole in pharmaceutical dosage form. Chromatographic separation achieved isocratically on a C_{18} column by utilizing mobile phase Methanol: Water: Triethylamine (90: 10: 0.2v/v/v) at the flow rate of 1ml/min with UV detection at 277nm. The retention time of cinitpride and pantoprazole are 4.80min and 2.40min respectively. The method is accurate (99.2-102.9%), and linear within range 3-15µg/ml and 4-20µg/ml for ciitapride and pantoprazole respectively. The correlation coefficient was found to be r^2= 0.999 and 0.997 for CNP and PNP respectively. The LOD for cinitapride and pantoprazole 0.223µg/ml and 0.498µg/ml respectively and LOQ are 0.675µg/ml and 1.509µg/ml respectively. The proposed method is applicable for routine analysis of simultaneous estimation of cinitapride and pantoprazole in combine pharmaceutical dosage form.

Keywords: RP-HPLC, Cinitapride, pantoprazole, validation.

INTRODUCTION

Cinitapride(CNP), is a substituted benzamide gastroenteric prokinetic agent and antiemetic acting via complex but synergistic effect on serotonergic 5-HT2 receptor and dopaminergic D2 receptors in the neuronal synapses of the myenteric plexi.[1] It is not yet official in I.P., B.P. and U.S.P. But it is official in Martindale extra pharmacopoeia[2]. Chemically it is 4-amino-N-[1-(3-cyclohexen-1- ylmethyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide. It has an empirical formula $C_{21}H_{30}N_4O_4$ and molecular weight 402.4873g/mol. The use of cinitapride is efficient and safe in treatment of patient with disorder in the gastric emptiness related to gastroesophageal reflux and functional dyspepsia as well as in individuals that present irritable bowel syndrome with constipation and abdominal pain[3].

Pantoprazole(PNP), is widely used antiulcer drug, it is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H+,K+)- ATPase enzyme system at the secretary surface of the gastric parietal cell[3]. This effect is dose- related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus [4]. pantoprazole is official in IP, BP, and european pharmacopoeia. Chemically it is 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl) methyl] sulfinyl] 1H-benzimidazole. It has an empirical formula $C_{16}H_{15}F_2N_3O_4S$ and molecular weight 383.37g/mol. [5]

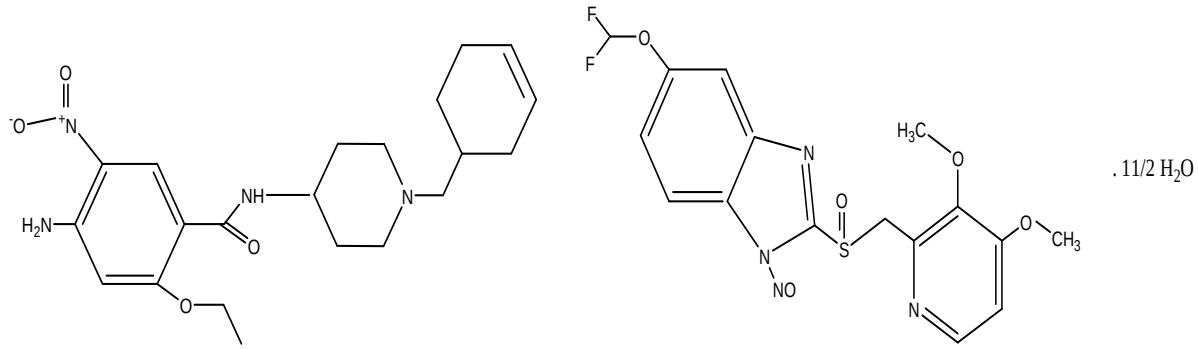

Fig. 1: structure of cinitapride and pantoprazole

The combination of cinitapride(3mg) and pantoprazole(40mg) is widely use to treat the patients suffering from non-ulcer dyspepsia or gastroesophageal reflux disease. It is also use for treating ulcers of duodenam, and the zollinger-ellison syndrome. [(5)]

The review of literature reveals that methods reported for estimation of cinitapride and pantoprazole either alone or with combination with other drugs. This includes determination of free levels of drug in human plasma by LC-MS, simple extractive colorimetric, RP-HPLC in human plasma as well as pharmaceutical dosage forms. The present study reports simple, precise accurate, and cost effective RP-HPLC method for simultaneous estimation of cinitapride and pantoprazole in pharmaceutical dosage form and the method is validated as per ICH guidelines. [(1, 6, 7, 8)]

MATERIALS AND METHODS

Reagents and chemicals:

- Cinitapride Hydrogn Tartrate Active pharmaceutical Ingredient (API) – Symed laboratories limited Hyderabad.
- Pantoprazole sodium sesquihydrate IP/USP – Cadila health care limited vadodara.
- Other reagents like methanol, water, triethylamine of HPLC grade – RFCL limited ankleswar, gujrat.

Instrumentation and chromatographic condition:

Analysis was performed by using analytical balance precisa XB220A, the HPLC used is of cyberlab LC-100 with UV detector. Column used in HPLC is, Kromasil 1005C_{18} 250mm×4.6mm ×5µm. Mobile phase consist of Methanol: Water: Triethylamine (90: 10: 0.2v/v/v) with the flow rate 1.0ml/min which degassed in a sonicator for 10min. the injection volume is 20µl and UV detection was at 277nm.

Preparation of stock solution:

Accurately weigh 137.2 mg of CNP hydrogen tartrate equivalent to 100 mg CNP and 112.8 mg of PNP sodium sesquihydrate equivalent to 100 mg PNP, transfer into the 10ml volumetric flask separately and dilute up to 10 using methanol. Pipette out 1ml from both and transfer in 10ml volumetric flask separately and make up to 10ml. From it Pipette out 3ml of cinitapride solution and 4ml of pantoprazole solution in same 10 ml volumetric flask and make up to 10ml with methanol(300ppm CNP/ 400ppm PNP). Pipette out 1ml from it and make up to 10ml using mobile phase. Serial dilutions of CNT and PNP were made from 3-15µg/ml and 4-20µg/ml respectively by taking 1, 2, 3, 4, 5ml from it and make upto 10ml with mobile phase.

Analysis of tablet formulation:

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 3mg CNP and 40mg PNP was weighed and dissolved in the 100ml of methanol (300ppmCNP/400ppm PNP). Pipette out 1ml from it in 10ml volumetric flask and add 2.7ml solution from 100ppm CNP solution and make up to 10ml with mobile phase.(30ppm CNP/40ppm PNP). The solution was further diluted to get sample chromatogram. A typical chromatogram obtained from a sample solution.

RESULTS AND DISCUSSION

Method development:

The HPLC method was developed and validated in terms of precision, accuracy and linearity according to ICH guidelines. The accuracy of the method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the Placebo. The mixtures were extracted and analyzed using the developed HPLC method. Linearity test solutions were prepared as described in

Formulation analysis. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for analytes were estimated by injecting a series of dilute solutions with known concentration. Values of LOD and LOQ were calculated by using σ (standard Deviation of response) and b (Slope of the calibration curve) and by using equations, LOD= (3.3 x σ)/ b and LOQ=(10xσ)/b. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. [9, 10]

Table. 1: Optimized chromatographic conditions

Parameter	Optimized Condition
Chromatograph	HPLC(Cyber Lab LC-100 With UV Detector)
Column	Kromasil 1005C18 25cm Long With I.D.-4.6mm, O.D- 8mm.
Mobile Phase	Methanol: Water: TEA (90: 10: 0.02%)
Flow Rate	1ml/Min
Detection	UV At 277nm
Injection Volume	20µl
Column Temperature	Ambient
Runtime	6min

Mobile phase optimization:
The symmetrical peak was obtained by measuring the response of eluent under the optimized condition after various experimental trials. The UV responses of both the drugs were found to be best at 277nm.

Mobile phase composition:
By injecting the 100ppm solution of both drugs in various mobile phase possibilities by modification of the ratio of organic phase and water and also the flow rate.

Type of organic modifier:
In the various ratio of mobile phase were tried but cinitapride shows peak tailing after adding the peak modifier triethylamine 0.02% which leads to peak symmetric and well defined sharp peak with good resolution. Hence methanol: water: triethylamine (90: 10: 0.2v/v/v) was optimized to get sharp and well resolved peak.

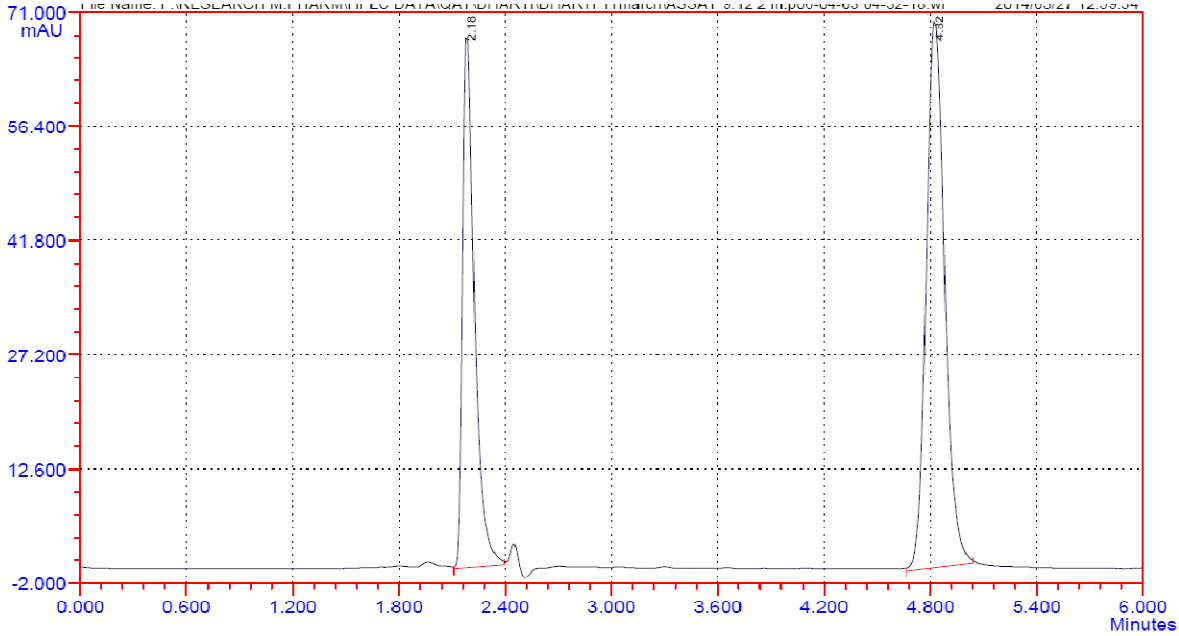

Fig. 2: chromatogram of standard CNP and PNP.

Validation of method:
Linearity and range:
For the construction of calibration curves, five calibration standard solutions were prepared over the concentration range. Linearity was determined for CNP and PNP in the range of 3-15µg/ml and 4-20µg/ml respectively. The correlation coefficient ('r^2') values were 0.999(n = 5) and 0.997(n=5)for CNP and PNP respectively. Typically, the regression equations for the calibration curve was found to be y = 3678x+2726 for CNP, y=2158x+1432 for PNP.

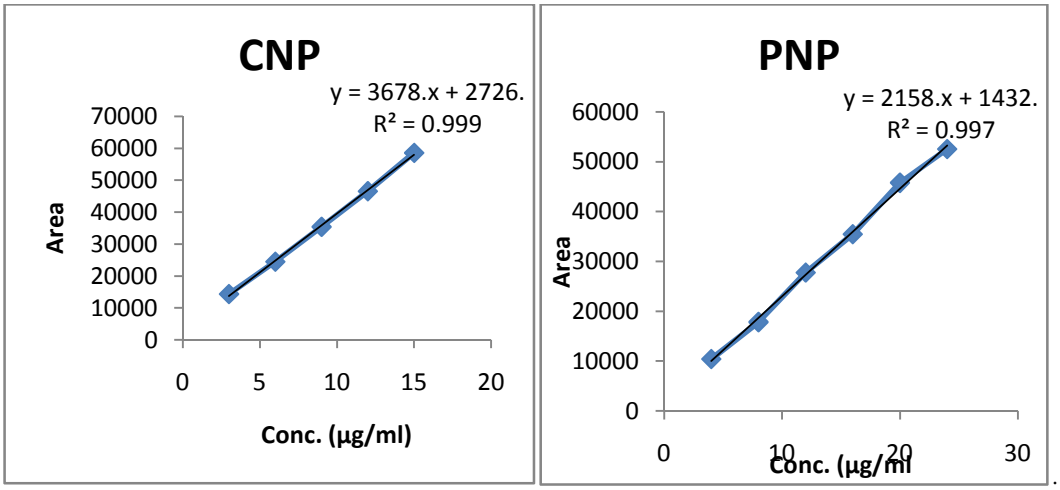

Fig. 3: calibration curve of CNP and PNP

Precision and accuracy:

The precision study of method is done by performing five replicate analyses of the same working solution. Intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The results indicated the good precision of the developed method. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for CNP 100.26 and for PNP 101.11.

Table 2: Regression analysis data and summary of validation parameter for proposed method:

Sr.no.	Validation parameter	HPLC method	
		CNP	PNP
1	Linearity range	3-15µg/ml	4-20µg/ml
	Linearity equation	Y=3678x+2726	Y=2158x+1432
2	Slop	3678	2158
	Intercept	2726	1432
3	Correlation co-efficient	0.999	0.997
4	Precision (%RSD)		
	Repeatability(n=6)	1.0584	0.6937
	Intraday(n=3)	1.6822	1.6086
	Interday(n=3)	0.5378	0.1788

Table. 3: Results of formulation analysis and accuracy studies

Drug	Formulation study		Recovery study		
	%Assay found	%RSD	Level	%recovery	%RSD
			50%	99.2272	0.2901
CNP	98.66	0.249	100%	99.9011	0.3345
			150%	101.4133	0.1798
			50%	102.142	0.6364
PNP	102.87	0.411	100%	99.4932	0.5718
			150%	102.9743	0.5362

Limit of detection (LOD) and Limit of quantitation (LOQ):

Table. 4: The LOD and LOQ values

	CNP	PNP
LOD(µg/ml)	0.223	0.4982
LOQ(µg/ml)	0.6758	1.5098

Specificity:

An investigation of specificity should be conducted during validation of identification tests, the determination of impurities and the assay. [(11)]

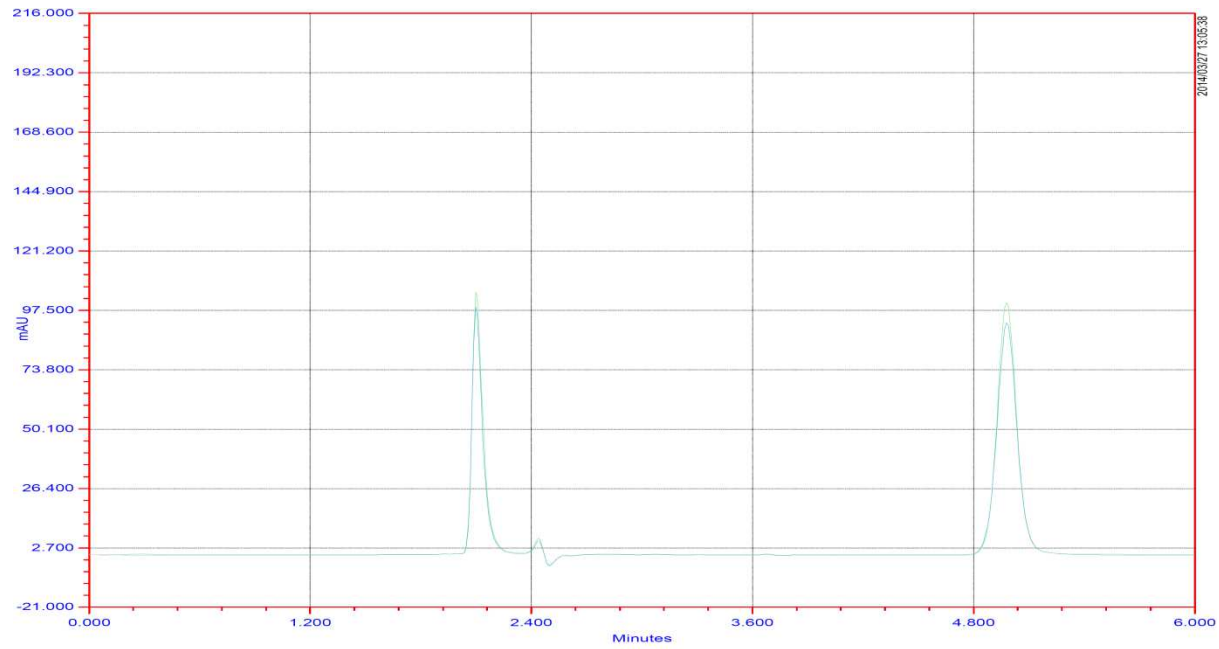

Fig. 4 Specificity Chromatogram consists of A) Mobile Phase, B) Formulation,C) standards of CNP (30 µg/ml) and PNP (40 µg/ml)

Robustness:

The robustness is reliability of an analysis with respect to deliberate variation in the method parameter. Method was determine by change in method like mobile phase ratio, change in flow rate show there was no mark change in the chromatographic parameters, which demonstrates that the method developed is robust[(12)].

Table. 5: robustness study

Chromatographic parameter	%Recovery	
	CNP	PNP
Flow rate		
0.9ml	99.5449	102.9047
1.0ml	99.22	102.142
1.1ml	98.4241	101.8695
Mobile phase composition (Methanol:water:TEA)		
88:12:0.02%	98.2144	102.9015
90:10:0.02%	99.22	102.142
92:8:002%	98.4242	103.9605

CONCLUSION

A new RP-HPLC method has been developed for simultaneous estimation of CNP and PNP in marketed formulation. The method gave good resolution for both the drugs with a short analysis run time within 6 min. The developed method was validated. It was found to be novel, simple accurate precise, sensitive and cost effective. Hence the proposed RP-HPLC method is suitable for routine assay of cinitapride and pantoprazole in pharmaceutical dosage form in quality control laboratories.

REFERENCES

[1] Hemalatha PV, Jerad Suresh A., Niraimathi V., *Int. J. of pharmacy &P.ceu. Res***. 2012.**
[2]Martindale (PhP) pharmaceutical press **2007,** 1551.1.
[3] Patel G.H., Prajapati S.T., Patel C. N. *Int. J. Pharm***.2012,** 4253-4260.
[4] Zalak N. Mewda, Naresh Kalsariya, Renu Chauhan, Shailesh Shah, Dinesh Shah *Int. J. Pharm. Sci. Rev. Res*. **2012**, (17):102-105.
[5] *USP 25–NF 20* (United States Pharmacopeial Convention, Rockville, MD, **2002**): 2256.
[6]Honggang lou, Hong Yuan, Zourong Ruan, Donghang Xu, Quan Zhou chromatographia **2008.**
[7]Ch.V.Suresh, G. Vidya Sagar *Int. J. Res. Ayurveda & pha*. **2011**,2 (6):1825-1826.
[8] Varsha Jagatiya, Jitendra Patel. *Int. J.Chem Tech.Res.* **2012**.(4):1396-1401.

[9] ICH-Q2B Validation of Analytical Procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland**,1996.**
[10]Jay Breaux Kevin Jones, and Pierreboulas,Understanding and implemenlting efficient analytical method development and validation by pharmaceutical technology analytical chemistry and testing **2003.**
[11] B. Thangabalan, A. Elphine prabahar, R. kalaichelvi, P. Vijayarajkumar *E-J. Of chem***.2009,6 (S1)**: S21-S24.
[12]G. Nagarajan, P. Nagesh, B.V. Ramana, C. Triveni, *Int. Res. J. Of pha*. **2013,** 4(2):131-135.
[13] A.Madhukar, K.Jagadeeshwar, K.Naresh, Armstrong vinod raj, B. Jayapaul, S. Naazneen *Der pharma chemica***, 2011,** 3 (6):494-499.
[14] Aniruddha R. Chabukswar, Swati C. Jagdale, Bhanudas S. Kuchekar, Pradeep D Lokhande1, Santosh N. Shinde, Kunal D. Ingale, Anuja K. Kolsure, *Der Pharma Chemica*, **2010**, 2(4): 148-156