Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

QUANTIFICATION OF CINITAPRIDE AND PANTOPRAZOLE IN BULK AND ORAL DOSAGE FORM BY VISIBLE SPECTROPHOTOMETRIC METHOD

HEMALATHA PV., JERAD SURESH A. AND NIRAIMATHI V*

Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-600003, Tamilnadu, India.
Email: vnm_anr@yahoo.co.in

Received: 18 Feb 2012, Revised and Accepted: 28 Mar 2012

ABSTRACT

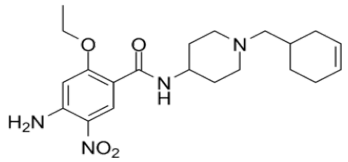
A new visible spectrophotometric method has been developed for the estimation of cinitapride and pantoprazole in bulk and capsule dosage form. The method makes use of diazotization followed by complexation for cinitapride (CNP) and redox cum complexation for pantoprazole (PNP). The complex of cinitapride showed λmax at 392.5nm and that of pantoprazole at 510nm. A good linearity with correlation coefficient within the limit was observed for the drugs at the concentration range of 10-60µg/ml for cinitapride and 1.2-5.4 µg/ml for pantoprazole. The reagents used were optimized. The developed methods were assessed for precision, accuracy, sensitivity. Thus a simple, easy to perform, economical, precise and accurate visible spectrophotometric methods have been developed for the estimation of cinitapride and pantoprazole in bulk and solid oral dosage form.

Keywords: Cinitapride (CNP), Pantoprazole (PNP), Diazotization, Redox, Accuracy, Complexation

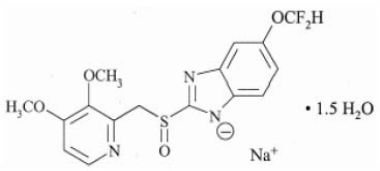
INTRODUCTION

Cinitapride is a substituted benzamide gastro enteric prokinetic agent acting via complex, but synergistic effects on seratonergic 5-HT_2 (inhibition) and 5-HT_4 (stimulation) receptor and dopaminergic D_2 (inhibition) receptors in the neuronal synapses of the myenteric plexus.[1,2] Cinitapride is indicated for the treatment of gastrointestinal disorders associated with motility disturbances such as gastro esophageal reflux disease, non-ulcer dyspepsia and delayed gastric emptying.[3] Pantoprazole[4] is a gastric proton pump inhibitor. Cinitapride is chemically 4-amino –N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide. Pantoprazole is chemically 6-(difluoromethoxy-2-[(3,4-dimethoxy pyridine-2-yl) methyl sulfynyl] -1H-benzimidazole. Only first derivative[5] and HPTLC[6] method has been reported so far for the combination. The aim of this project is to develop novel, precise and accurate colorimetric methods for the estimation of CNP and PNP in combined oral dosage form, which has not been reported till date. In this study CNP has been diazotized and then complexed with a keto-ester and PNP has been oxidized using bromine and then indirectly determined from the amount of unreacted bromine by the well known ferrous ammonium sulphate -1, 10-phenanthroline complex formation.

Structures[7]



Cinitapride
Mol wt: 402.49



Pantoprazole sodium sesquihydrate
Mol wt: 432.4

MATERIALS AND METHODS

Instrumentation

A Shimadzu model UV-1650 double beam UV-VIS spectrophotometer with a pair of 1cm matched quartz cells was used to measure absorbance.

Chemicals and reagents

The capsule dosage form was obtained from the local market. All the chemicals used in the preparation of the reagents were of analytical grade. Distilled water was used for the preparation of the reagents. All the solutions were freshly prepared just before the analysis. The modes of preparation of various reagents used are given below:

For Cinitapride

Sodium nitrite solution (2%): 2gms of sodium nitrite dissolved in water and made up to volume with water.

Hydrochloric acid (0.5 M): 4.3 mL of concentrated hydrochloric acid was made up to 100 mL in distilled water.

Ethyl aceto acetate (EAA) (2%) solution: 2 mL of ethyl aceto acetate was dissolved in distilled water and made up to volume with distilled water in a 100 mL volumetric flask.

Sodium hydroxide (1 mol L^{-1}): 4gms of sodium hydroxide was dissolved and made up to volume (100 mL) with water.

For Pantoprazole

Potassium bromate – bromide mixture

Dissolved 100mg of potassium bromate and 1g of potassium bromide in 10 mL of water and made up to volume in a 100 mL volumetric flask. Appropriate dilution of the stock solution was made to obtain a concentration of 40 µg/mL to be used in the determination.

Ferrous ammonium sulphate (350 µg/mL)

Dissolved 400 mg of FAS in 50 ml of distilled water, added 1 mL of dilute sulphuric acid and made up to 100 mL in a volumetric flask. Further dilutions were made to obtain a concentration of 350 µg/mL of FAS.

1, 10 - Phenanthroline

Dissolved 250 mg of 1, 10 – phenanthroline in distilled water with the aid of heat and made up to volume in 100 mL volumetric flask.

Hydrochloric acid ($5M^-$)

About 42.5 mL of concentrated hydrochloric acid was added to 20 mL of distilled water and made up to volume in 100mL volumetric flask with distilled water.

Hydrochloric acid (1M)

About 85 mL of concentrated hydrochloric acid was added to 100 mL of distilled carefully and made up to volume in 1000 mL volumetric flask with distilled water.

Ammonia solution (50 %)

About 50 mL of strong ammonia solution was added to 20 ml distilled water and made up to volume (100 mL) with water.

METHODS

Spectral characterization and linearity establishment

Cinitapride

A stock solution of CNP was prepared by dissolving accurately weighed quantity of standard CNP in distilled water and made up to volume with water to obtain a concentration of 500 µg/mL. From the stock solution 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml were transferred into six 50ml volumetric flasks. The solutions were diazotized by the addition of 2mL of 2% sodium nitrite solution and 2mL of 0.5 M hydrochloric acid at a temperature of about 0-5° C for 5 minutes. Then 2 ml of 2 % EAA was added to each reaction mixture followed by the addition of 1 mL of 1M sodium hydroxide, when a reddish orange coloured chromogen was formed. Finally the solutions were made up to volume with distilled water and shaken well. The absorbance of the reddish orange coloured chromogen was scanned between 350-800nm against distilled water blank. The chromogen gave maximum absorbance at 392.5 nm.

Pantoprazole

A stock solution of PNP was prepared by dissolving accurately weighed quantity of standard PNP in 1M hydrochloric acid and made up to volume with the same hydrochloric acid to obtain a final stock concentration of 1000 µg/mL. The stock solution was further diluted with distilled water to obtain a working standard solution of concentration 30 µg/mL. Transferred 1, 2, 3, 4 and 4.5 mL of the working standard solution of PNP (30µg/mL) into six 25 ml volumetric flask. To each flask was added 2 mL of potassium bromate – bromide mixture (35µg/mL) using a burette and 2mL of hydrochloric acid (5M), stoppered immediately, shaken well and kept aside for 5 minutes. To the reaction mixtures, 2 ml of FAS was added (350 µg/mL), shaken well and again kept aside for fifteen minutes until the reaction is completed. This was followed by the addition of 2 mL of 1, 10- phenanthroline when a blood red coloured chromogen was obtained. Finally the solutions were made up to volume with distilled water. The absorbance of the reddish coloured chromogen was scanned between 350-800nm against reagent blank. The chromogen gave maximum absorbance at 510 nm.

Analysis of the sample

The capsule dosage form contains CNP and PNP as separate extended release and enteric coated tablets respectively. So the drugs were analysed as separate entities by the above visible spectrophotometric methods respectively.

Cinitapride

Twenty tablets of CNP from the capsules were accurately weighed and ground to fine powder. Tablet powder equivalent to 25 mg of CNP was accurately weighed and shaken well with water for 20 minutes, made up to volume with water to obtain a concentration of 250 µg/mL. The solution was filtered through Whatmann filter paper No.41. First 10 ml of the filtrate was discarded, and then 6 ml of the filtrate was transferred to a 50 ml volumetric flask. The sample solution was diazotized, complexed with EAA and the absorbance was measured using the same procedure as that of standard CNP.

Pantoprazole

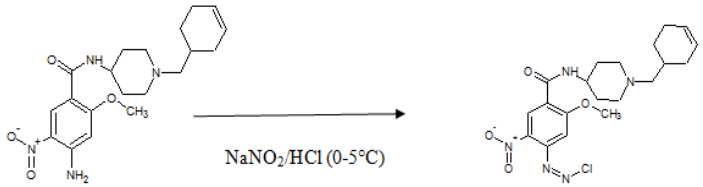
Twenty tablets of PNP from the capsules were accurately weighed and ground to fine powder. Tablet powder equivalent to 50mg of CNP was accurately weighed and shaken well with 1M hydrochloric acid for 20 minutes and made up to volume with 1M hydrochloric acid to obtain a concentration of 1000µg/mL. The solution was filtered through Whatmann filter paper No.41. First 10mL of the filtrate was discarded and the filtrate was appropriately diluted to obtain a concentration of 30 µg/mL with water. Transferred 3 mL of the first dilution (30µg/mL) to a 25mL volumetric flask and the same procedure for standard PNP was followed. The absorbance of the resulting solution was measured.

RESULTS AND DISCUSSION

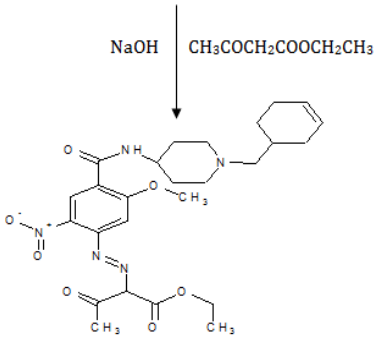
Cinitapride

The colorimetric method is based on the diazotization[8] of the primary aromatic amino group in CNP. The diazotized CNP is then coupled with EAA in alkaline media. The diazotisation of drugs containing free amino group is a well known reaction. In this method the primary aromatic amino group in CNP is diazotized using sodium nitrite solution and hydrochloric acid. Diazonium compounds are unstable and would decompose at elevated temperature[9], hence temperature was maintained below 5°C until the addition of sodium hydroxide. The amino group is converted into diazonium salt which on treatment with EAA forms a complex. The complex is formed only in an alkaline medium, provided by the addition of sodium hydroxide solution. The complex is reddish orange in colour for which the maximum absorbance was observed at 399nm.

Mechanism of Reaction[10]



Cinitapride ***Diazonium salt of Cinitapride***



Diazo Coupling of Cinitapride - EAA

Optimisation

Diazotization and coupling reaction condition

The conditions for the diazotization are well established to be at 0-5°C. An elevated temperature leads to incomplete diazotization and decomposition of diazotized compound which could be assessed by the non linear response of the diazotized mixture in UV spectrophotometry at 410nm.The volume and strength of the sodium nitrite and hydrochloric acid used was optimized. It was found that an increase or decrease from 1-2mL and 1-4% of sodium nitrite brought about no change in response or very less absorbance. By trial and error it was established that 2 mL each of 2% sodium nitrite solution, 0.5M hydrochloric acid and 2% EAA were found to give good linear relationship between absorbance and concentration. Any change in the volume or strength of the above reagents showed deviation from the linearity. Similarly the strength and volume of sodium hydroxide were also optimized. It was observed that 1mL of 1M sodium hydroxide was optimal to ensure complete complexation and good colour intensity. Finally the concentration of the analyte was also studied. Lower the concentration (below 10µgm) lower was the colour intensity as a result, the absorbance was very less and did not show significant difference. Higher concentration (above 60µg/mL) showed deviation from the linearity. Thus 10-60µg/mL of CNP obeyed Beer's law[11] and was selected for the study. The correlation coefficient was found to be 0.999 as shown in fig 1.

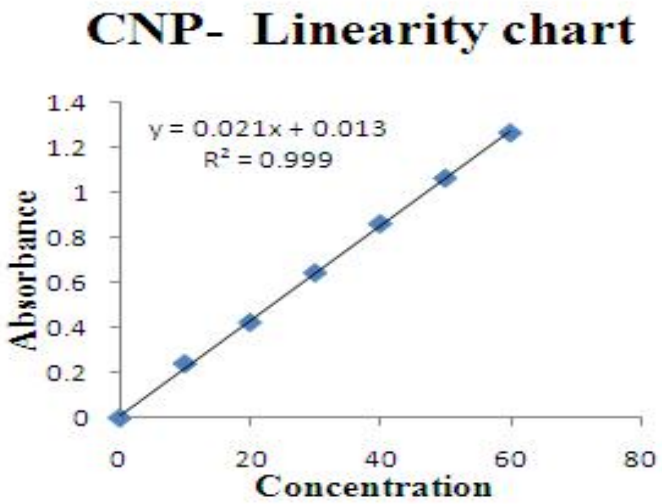


Fig. 1: Calibration chart of cinitapride.

Pantoprazole

The colorimetric estimation of PNP is based on the oxidation of PNP by the *insitu* liberation of bromine during the reaction between excess bromate-bromide and hydrochloric acid. The unreacted bromine left after oxidation of PNP is determined indirectly by the oxidation of large excess FAS to ferric ammonium sulphate. The unreacted FAS then treated with 1, 10-phenanthroline form a blood red complex of FAS - 1, 10-phenanthroline which is a very well known complexation reaction used for the estimation of iron in the ferrous state. The complex shows λmax at 510 nm. When a known excess amount of bromate-bromide mixture is allowed to react with increasing amount of PNP, there occurs decrease in the amount of bromine for oxidation of FAS to ferric ammonium sulphate.

Thus, an increase in the concentration of the FAS, available for complexation with 1,10-phenanthroline which is observed from the increase in the absorbance, as shown by the slope in the calibration chart (fig 2). Since the complex is formed and is stable only in alkaline media, ammonia solution is used.

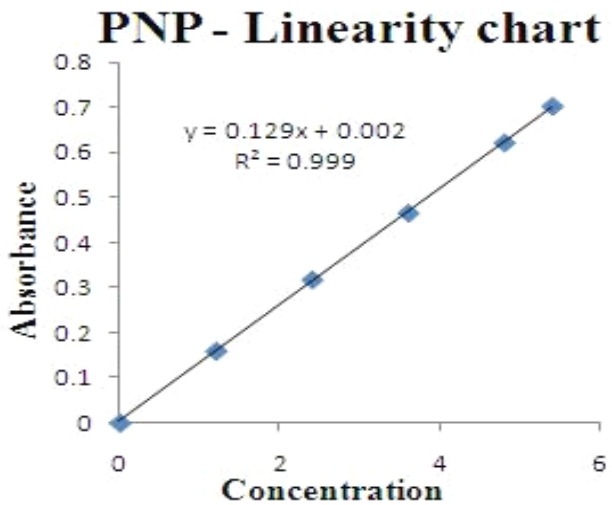


Fig. 2: Calibration chart of pantoprazole.

Optimization

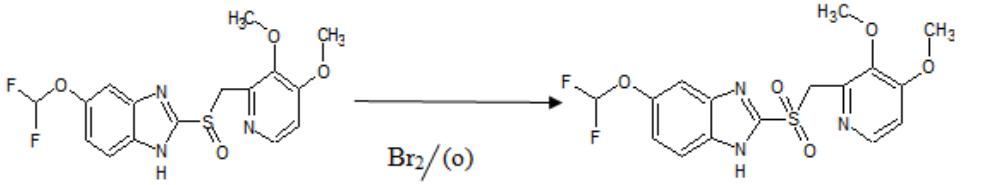
The strength and volume of hydrochloric acid and bromate-bromide mixture was optimised. Any change in the volume of the reagent resulted in either insufficient liberation or large excess of bromine which did not give any good response. Thus 1ml of 5M hydrochloric acid and 2ml of bromate –bromide mixture and 2ml of 350 µg/mL of FAS was selected for the complete reduction of the unreacted bromine. Moreover, 2mL of 1, 10- phenanthroline was required for the complete complexation of the excess unreacted FAS.

Mechanism of Reaction [12]

I. Oxidation

$KBrO_3 + 5KBr + 6HCl \longrightarrow 6\,KCl + 3Br_{2(g)}\downarrow + 3H_2O$

(potassium bromate-bromide) ***bromine***



Pantoprazole ***Oxidised product of Pantoprazole***

II. Complexation

$Fe^{2+} + Br_2$ (unreacted) $\xrightarrow{(o)}$ $Fe^{3+} + 2\,Br$

(ferrous) ***(ferric)***

Fe^{2+} + 1,10 Phenanthroline $Fe[(C_{12}H_8N_2)_3]^{2+}$

(ferrous) ***Red chromogen (ferroin)***

The complex is formed only in an alkaline medium which is provided by the addition of ammonia solution. About 2 ml of ammonia solution was sufficient to neutralize and slighltly raise the pH of the solution at which the complexation is complete. Any more increase in volume of the ammonia solution, drastically increases the pH of the solution resulting in the instability of the complex. The chromogen was red in colour which showed a maximum absorbance at 510 nm. Higher concentration of the analyte required very high concentration and large excess of the bromate-bromide mixture. The above developed method obeyed Beer's law at the concentration of 1.2-5.4 µg/mL of PNP. The correlation coefficient was within the limit 0.999. The optical parameters of both CNP and PNP was represented in table 1.

Accuracy and Precision (CNP&PNP)

Precision of the developed methods for the drugs have been ascertained by the reproducibility of the results, when repeated six times gave a percentage purity of 98.94 ±0.000014 for CNP and 98.64 ± 0.00021 for PNP. The drugs obeyed Beer's law at the concentration range of 10-60 and 1.2-5.4 µg/mL for CNP & PNP respectively. The method precision was assessed from the results of six replicate analysis. The assay percentage was appreciable and shown in table 2.

Table 1: Optical Parameters of CNP and PNP - Visible Spectrophotometry

S. No	Optical Parameter	Cinitapride	Pantoprazole
1.	Wavelength λmax	392.5	510nm
2.	Molar absorptivity mol L^{-1}	8837.83	5022.02
3.	Beer's law limit (µg/ml)	10 - 60	1.2-5.4
4.	Regression equation	Y=0.021X + 0.013	Y=0.129X + 0.002
5.	Slope	0.021	0.129
6.	Intercept	0.013	0.002
7.	Correlation coefficient	0.999	0.999
8.	Sandell's sensitivity	0.046	0.008
9.	LOD	4.40	0.234
10.	LOQ	13.33	0.707

Table 2: Result of Tablet Assay

Drug	Label claim	Amount determined*	Percentage Purity (%)	S.D	R.S.D
CNP	0.003 gm	0.02968 gm	98.94	± 0.000014	0.4752
PNP	0.040 gm	0.0395gm	98.64	± 0.000215	0.5442

**mean value of six determinations*

Accuracy of the developed method for the samples were determined by recovery studies. It was performed on spiked samples at three levels. To a known amount of the analyzed sample the standard drug was added and the total amount was analysed by the above proposed colorimetric method. The results of the recovery studies (table 3) show that there is no interference by the additives in the formulation and the proposed methods could be applied for the tablet formulation of both the drugs irrespective of the nature of the excipients. All the essential parameters like assay percentage, correlation coefficient, LOD, LOQ, the results of precision and accuracy were within the limits and acceptable.

Table 3: Recovery Studies

Drug	Amount of drug added		Recovery by the proposed methods*		
	Sample	Standard µg/ml	% recovery	S.D	R.S.D
CNP	30µg/mL	8.1	102.09	± 0.59	2.14
		15	98.18	± 0.54	1.10
		30	98.26	± 0.33	0.33
PNP	2.4µg/mL	0.6	98.7	± 0.87	4.43
		1.2	99.04	± 1.52	3.07
		2.4	101.99	± 0.74	0.73

CONCLUSION

New visible spectrophotometric methods have been developed for the estimation of CNP and PNP. The methods were found to be simple, selective, sensitive, accurate and reproducible. Thus the above proposed methods could be applied for the quality control of CNP and PNP in pharmaceutical formulation in industry.

ACKNOWLEDGEMENT

The authors are thankful M/S Zydus Cadila, Ankleshwar, Gujarat (India) and Knis Laboratories, Chennai, TN (India) for providing Cinitapride and pantoprazole as gift samples respectively. The authors are also thankful to the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College for rendering full support to carry out the study.

REFERENCES

1. Roberts DJ. The pharmacological basis of the therapeutic activity of clebopride and related substituted benzamides. Curr Ther Res . 1982; 31**:** S1–S44.
2. Fernandez AG and Roberts DJ Cinitapride hydrogen tartrate. Drugs Fut. 1991; 16**:** 885–892.
3. www.cipladoc.com
4. Graham L. Patrick. An Introduction to Medicinal Chemistry. 4th ed. Oxford University Press; 2010; 674.
5. Paresh U. Patel, Shivang K. Prabtani. First Derivative Spectrophotometric Method for Simultaneous Estimation of Pantoprazole and Cinitapride in Combined Dosage Form. Int J of Pharm Research**,** 2011; 3 (2): 53-54.
6. Patel GH, Prajapati ST, Patel CN. HPTLC method development and validation for simultaneous determination of cinitapride and pantoprazole in capsule dosage form. Research J of Pharm and Tech. 2011; 4 (9): 1428-1431.
7. www.drugbank.com
8. Shahar Yar M. Pharmaceutical Analysis: Methods of Analysis and Assay: Miscellaneous Methods of analysis. Available from: URL: nsdl.niscair.res.in/revised +miscellaneous+method+of+ analysis.pdf/2007
9. www.chemguide.co.uk. 2007: Jim Clark. Making of diazonium salts from phenyl amine.
10. Chand Pasha, Narayana B. A simple spectrophotometric method for the determination of mosapride by diazotization method. Yanbu Journal of Engineering and Science 2010; 1(143H): 68-73.
11. Willard H, Lynne Merritt, John A Dean,Frank A Seattle. Instrumental methods of Analysis. 6th ed. New Delhi: 1986; p 67-68
12. Adnan A Badwan, Lina N. Nebulas, AI Olnflri MM, Nidal H. Daraghmeh, Mahmoud K. Shour, Abroad M. Abdoh I, Jaber AMY Analytical profiles of drug substances and excipients 2002; 29 p.213
13. Vogels Qualitative Inorganic Analysis. 7 th ed. New Delhi: Dorling Kindersely India (pvt) Ltd: 2007. p. 124,128.