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Method Development and Validation For The Quantification Of Pantoprazole By RP-HPLC And Spectrophotometry In Pharmaceutical Oral Dosage Form

Patil Akshay Kishore*, Dr.Rakesh Patel[2], Dr.Mohit Chaturvedi[3], Jeevan Patel[4], Gaurav Jain[5]

[1-4] School of Pharmacy, Dr. A.P.J Abdul Kalam University, Indore,
[5]Chameli Devi Institute of Pharmacy, Indore.

***Address of Correspondence: Patil Akshay Kishore**
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ABSTRACT

Simple, Precise, rapid and accurate methods were developed for the estimation of Pantoprazole in bulk and in dosage form. The methods are UV Spectroscopic method, Visible Spectroscopic method & RP-HPLC method. The λmax of of PNP was found to be 290nm in methanol. The bulk drug PNP obeyed Beer's law at 5-30µg / ml. The correlation coefficient was found to be 1 for both the drugs. The dosage form of the drugs was quantified by the following three methods. Method A involves standard absorbance method. The RSD proves there producibility of the method and thus the precision of the developed method. Method B involves determination of AUC for both standard and sample spectra obtained in Method A between two selected wavelength. The correlation coefficient was found to be 0.9990 for PNP. The recovery percentage was found to be 98 to102% which proves no interference by the sample matrix. Method C involves the derivatisation of the zero order spectra to second order spectra. The recovery spectra were also derivatized and used. All the methods have shown good linearity, precision and accuracy. The low % RSD values in recovery studies for all the above methods indicate that there is no interference due to excipients used in the formulations. Hence it is concluded that the developed UV – Visible and RP-HPLC methods were found to be simple, precise, accurate and rapid methods for the analysis of Pantoprazole in its pure form and in its pharmaceutical dosage formulation. Thus, all the above adopted methods can be effectively used for the routine analysis of Pantoprazole in pharmaceutical dosage form.

Keywords: Pantoprazole, UV Spectroscopic method, Visible Spectroscopic method, RP-HPLC method, Method Development.

INTRODUCTION

Analysis of drugs plays a major role in the development, manufacture and therapeutic use of drug. Quantitative analysis of raw materials and the final product is necessary to ensure that they meet certain specifications and to ascertain the amount of each component in the final product. Standard analytical procedures for newly designed and synthesized drugs are not available in any of the pharmacopoeias. So it becomes necessary to develop newer, accurate, specific, simple, easy to perform, reliable and economic analytical techniques for the estimation of the new drugs[1].

This thesis deals with pantoprazole (PNP). Pantoprazole has been recently included in Indian Pharmacopoeia – 2010. Extensive literature survey reveals that only first derivative and HPTLC method for the determination of pantoprazole (PNP) has been reported. There is no evidence for the estimation of PNP by UV-Visible spectrophotometry and RP-HPLC methods in bulk and in combined tablet dosage form. So, an attempt has been made is to develop a validated, simple, easy to perform, accurate, cost effective and rapid spectrophotometric methods for the estimation of PNP in bulk and combined oral dosage form[2].

MATERIALS AND METHOD

MATERIALS

Pantoprazole pure drug sample was generously gifted by Cipla Pvt. Ltd. Pune (India).The combined capsule dosage form was acquired from the local market. The capsule contained within it enteric coated tablet of pantoprazole sodium sesquihydrate equivalent to 40 mg of PNP. Methanol, and water used were analytical grade.

Instrumentation

1. Digital Balance
2. Shimadzu UV-Visible spectrophotometer, Model 1650 PC
3. A Shimadzu Prominence LC-20 AT with SP D-20 A UV detector- HPLC
4. pH meter

Experiementals

Ultra Violet Spectrophotometric Method

Selection Of Solvent

The solubility of PNP was studied as per Indian Pharmacopoeial standards. The solubility of the drugs was tested in both polar and non-polar solvents. Both the drugs were found to be freely soluble in methanol. So methanol was selected for further studies on PNP by UV spectroscopy.

Preparation Of Standard Stock Solution

Weighed accurately about 50 mg each of standard PNP in 50mL volumetric flasks. Dissolved in 10 ml of methanol and made up to volume with methanol to obtain stock solutions of concentration 1000 µg/ml of PNP.

Selection Of Absorption Maxima

The stock solutions of PNP were appropriately diluted to obtain a concentration of 10 µg/ ml. The dilutions were scanned in ultra violet region (200-400nm) against methanol blank. PNP showed maximum absorbance at 290 nm. Thus λ max for PNP were selected as 290 nm and used during further studies for the estimation of PNP by UV spectrophotometry.

Establishment Of Linearity

The stock solution (1000 µg/ mL) of PNP was serially diluted with methanol to obtain dilutions ranging 5-30 µg/ml. The final dilutions were scanned in ultraviolet region (200-400nm) against methanol blank. The λ max was found to be stable at 290 nm. The absorbance of the dilutions was measured at the selected λmax 290 nm.

Method A: Standard Absorbance Method

Twenty tablets of PNP was accurately weighed and crushed to fine powder.Tablet powder equivalent to 50 mg of PNP was weighed in a 100 ml volumetric flask, shaken vigorously with sufficient amount of methanol for half an hour and finally made up to volume with methanol. The resulting solution was filtered through Whatmann filter paper (No.41). First few ml of the filtrate was discarded and sufficient quantity of the filtrate was diluted with methanol to obtain a final concentration of 15µg/ml of PNP. The absorbance of the resulting solution was measured at 290 nm against methanol blank.

Method B: Area Under Curve

The AUC (area under curve) method involves the calculation of integrated value of absorbance selected wavelengths λ. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. The standard spectra obtained in the linearity characterization and the sample spectra for PNP obtained in method A were used. The AUC for PNP, the AUC between 248.4 and 314.0 nm for both standard and sample were determined. The calibration graph was plotted between AUC and concentration. The sample AUC was interpolated on the respective linearity chart and the concentration was determined.

Method C: Second Derivative

The zero order spectra obtained in the linearity characterization and method A for PNP were derivatized to get second order spectra. PNP showed a negative maxima at 290nm. The amplitude of the negative maxima were measured and plotted against concentration to determine the linearity.

Recovery Studies

The recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective preanalyzed sample. About20, 40 and 100% of standard drugs were added to the sample solutions and the absorbance was measured against methanol blank. The percentage recovered was calculated. The recovery studies were performed at three levels for all the three methods to confirm the accuracy of the above said methods.

Visible Spectro Photo Metric Method

The colorimetric methods were developed for the estimation of Pantoprazole. The visible spectrophotometric studies of PNP is based on the oxidation of PNP by excess of bromine and indirectly determining the amount of PNP using ferrous ammonium sulphate and 1,10 phenanthroline in Method A and ammonium thiocyanate in Method B.

Preparation of standard stock solution

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 1000µg/ml.

Methoda—using 1,10–phenanthroline

Spectral characterization and establishment of linearity

The stock solution was further diluted with distilled water to obtain a working standard solution of concentration 30 µg/ml. Transferred 1 – 4.5 ml of the working standard solution of PNP (30µg/mL) into six 25 mL volumetric flasks. To each flask was added 2 ml of potassium bromate – bromide mixture (35µg/mL) using a burette and 2 ml of hydrochloric acid (5M), stoppered immediately, shaken well and kept aside for 5 minutes. To the reaction mixtures, about 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 chromogenis obtained. Finally the solutions were made upto 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 510nm.

Method b—using ammoniumthiocyanate

Spectral characterization and linearity establishment

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 1000µg/ml. The stock solution was further diluted with distilled water to obtain a working standard solution of concentration 20 µg/ml. Transferred 0.5, 1, 1.5, 2, 2.5 and 3 ml of the working standard solution of PNP (20µg/ml) into six 25 ml volumetric flask. To each was added 2 ml of potassium bromate – bromide mixture (40µg/ml) using a burette and 2ml of hydrochloric acid (5M), stoppered immediately, shaken well and kept aside for 5 minutes. To the reaction mixtures, about 2 ml of FAS (400µg/ml)was added, shaken well and again kept aside for fifteen minutes until the reaction is completed. This was followed by the addition of 1ml of 3 mol L^{-1} ammoniumthiocyanate when a blood red coloured chromogen is 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 477 nm.

Reversephase-high performance liquid

Instrument

High Performance Liquid Chromatograph

1. Shimadzu prominence
2. UV-Visible Detector(SPD20A)
3. Auto sampler
4. Isocratic(LC-20AT)pump
5. RheodyneValveinjectorwith20µlfixedloop
6. ChromatographicColumn-PhenomenexGemini,C_{18}(5µ): 250x4.60mmi.d

Preparation of mobile phase

The mobile phase consisted of acetonitrile, methanol and phosphate buffer (pH-7) in the ratio of 40:20:40. Phosphate buffer was made from 0.2M potassium dihydrogen orthophosphate adjusted to pH-7 with triethylamine. The mobile phase was filtered through 0.2 µ membrane filter and used throughout the study.

Selection of wave length

A known concentration of standard 10 µg/ml solution of PNP was prepared in mobile phase containing acetonitrile, methanol and phosphate buffer (pH-7) in the ratio of 40:20:40 to obtain a concentration of 10 □g/ml. The solution was then scanned in the UV region in the UV spectrophotometer against the mobile phase asthe blank. The □$_{max}$ was observed to be 290nm.The□$_{max}$ was analysed using different ratios of mobile phase, different pH of the buffer. There was no significant change in the □$_{max}$. So 290 nm was selected as the detection wavelength for the estimation PNP by RP-HPLC.

Optimized chromatographic conditions

The following parameters were used for RP-HPLC analysis of PNP

Mode of operation	: Isocratic
Stationary phase	: C 18 Column (250mm×4.6mmi.d.,5µ)
Mobile phase	: Acetonitrile, Methanol and Phosphate buffer(pH-7)
Ratio	: 40:20:40%v/v
Detection wavelength	: 290 nm (PNP)
Flow rate	: 2.0ml/min
Temperature	: Ambient
Sample volume	: 20µl
Quantification method	: External Standard Calibration Method

Determination of PNP oral dosage form

Since the combined capsule dosage form contains PNP as separate tablets, these were studied as separate entities.

Preparation of standard solutions and Establishment of Linearity

Accurately weighed 100 mg of standard PNP in 50 ml volumetric flask, dissolved in mobile phase and made up to volume with the mobile phase to get a concentration of 2mg/ml of PNP. The solution was filtered through 0.2µ membrane filter and used further. Aliquot quantity of the standard solution (2mg/ml) of PNP was serially diluted with the mobile phase to get a concentration range of 140 µg/ml to 220µg/ml. The dilutions were injected in the 20µl loop one by one; the eluate were then detected at 290nm and the chromatograms were recorded. The peak area was plotted against concentration and the linearity was assessed us in regression analysis.

Analysis of formulation

Twenty tablets of PNP from the formulation were weighed accurately. Tablet powder equivalent to 100mg of PNP was accurately weighed, transferred to 50 ml volumetric flask. Dissolved in mobile phase with the aid of ultrasonication for 15 minutes and made up to volume with mobile phase to obtain a concentration of 2mg/ml. The solution was further diluted to obtain a concentration of 0.2mg/ml. The final dilution was then filtered through 0.2µ membrane filter and 20 µl was injected into the column. The eluate was detected at 290nm and the chromatogram was recorded.

Recovery studies

To 5ml of pre analysed sample stock solution (2mg/ml) 2.5 ml of standard stock solution (2mg/ml) was added and made up to 50 ml in a volumetric flask. The solution was then filtered and injected in to the column. The eluate was detected at 290 nm and the chromatogram was recorded.

Limit Of Detection And Limit Of Quantification

Calibration of standard of PNP was repeated for three times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of intercept.

System Suitability Studies

The system suitability studies carried out as specified in ICH guidelines and USP. The parameters like tailing factor, asymmetry factor, number of theoretical plate, capacity factor were calculated.

RESULT AND DISCUSSIONS

Ultra Violet Spectroscopic Method

Method A- Standard Comparison Method

The PNP was found to be very freely soluble in methanol. PNP showed absorption maxima at 290 nm. (fig-1).

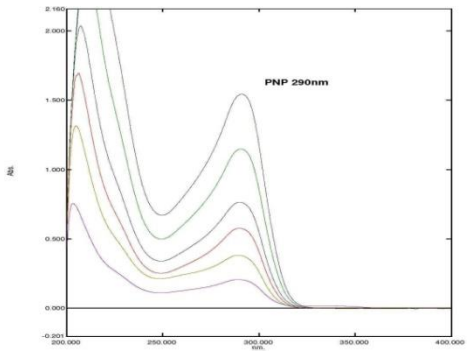


Fig 1: UV spectrum of PNP- λmax at 290 nm

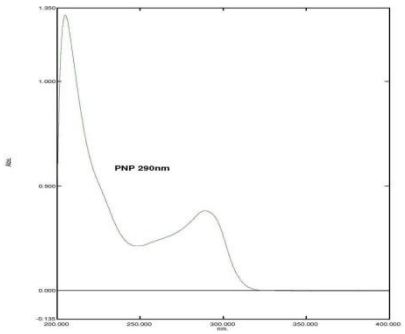


Fig 2: Overlain spectrum of PNP –λmax at 290 nm

The overlain spectrum of PNP is shown in fig-3.The absorbance corresponding to the concentration is tabulated in table-1. The five point calibration chart showed that thedrug obeyed Beer's law in the concentration range of 5–30 µg/mL. The linearity was assessed by the regression analysis as shown in the calibration chart. (fig-4) The correlation coefficient was found tobe1which was within the limit.

Table 1: Absorbance of PNP at 290 nm

S.No.	Concentration (µg/mL)	Absorbance at290nm*
1	5	0.192
2	10	0.384
3	15	0.579
4	20	0.769
5	30	1.149

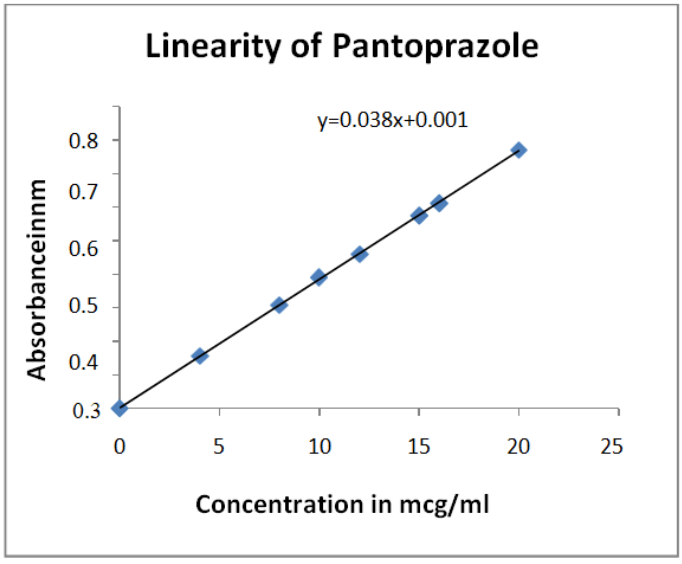


Fig 3: Calibration chart of PNP

Recovery studies

The recovery studies were performed on spiked samples at three levels (20%, 40%, 100%) using the standard addition technique. The results of the recovery analyses of PNP are tabulated in table-5. The results of the recovery studies confirm the accuracy of the developed method. Thus, it assures that the developed method shows no interference by the sample matrix.

Table 2: Results of Recovery Studies of PNP

Expected % Recovery	Amount of drug added (mg)		Total Amount Assayed (mg)	Amount recovered (mg)	Assessed % Recovery	% Recovered ±S.D	RSD
	Sample	Std					
20%	40	8	48.38	8.05	20.14	100.69±.398	0.02
40%		16	56.53	16.3	40.87	101.34±0.87	0.02
100%		80	79.6	39.27	98.17	100.47 ±0.73	0.01

Method b– area under curve

The AUC (area under curve) method involves the calculation of integrated value of absorbance, between the two selected wavelengths λ1 and λ2.The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. The AUC between two selected wavelengths is digitally calculated and given by the in-built software in the UV spectrophotometer. The spectra obtained in method A were used.

Pantoprazole

The AUCs of the standard spectra of PNP were determined between 248.4 (λ_1) and 314.0 nm as (λ_2) and showing fig-6 and the AUC corresponding to concentration is given in table-7.

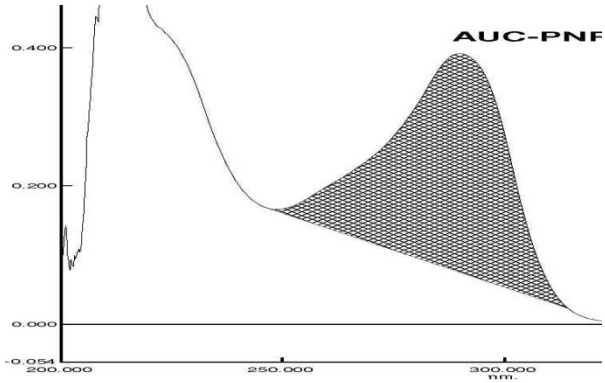


Fig 4: AUC of PNP

Table 3: Area under Curves of PNP

S.No.	Concentration (µg/mL)	Area under curve*
1	5	4.707
2	10	9.046
3	15	14.551
4	20	18.984
5	30	28.539

Recovery studies (pnp)

The AUC of the spectra obtained for the recovery studies (spiked samples at three levels 20%, 40%, 100%) of the samples (PNP) were interpolated on the linearity chart of the respective drugs and the concentrations were determined. The recovery percentage was calculated using the formula

$$\text{Recovery \%} = \frac{\text{Original amount— Standard Amount}}{\text{Standard Amount}} \times 100$$

Table 4: Results of Recovery Studies of PNP using AUC

Expected %recovery	Amount of drug added mg		Total Amount assayed (mg)*	Amount recovered (mg)	Assessed % Recovery	%Recovered ±S.D	%RSD
	Sample	Standard					
20%	40 mg	8	48.03	8.15	20.38	101.10±0.313	0.0153
40%		16	56.00	16.13	40.31	100.80	0.0155
100%		80	79.09	39.21	98.02	98.02±0.717	0.0073

Method c–second derivative

Derivative spectrophotometry involves the conversion of normal spectrum to its first, second or higher derivative spectrum. The second derivative (D^2) spectrumis the plot of the curvature of the D^0 spectrum or a plot of $d^2A/ d\lambda^2$ against λ. The characteristic of the second order spectra is that it represents a negative maximum just at the original λ max of the analyte. The amplitude of the negative maxima downwards is directly proportional to the concentration of the analyte.

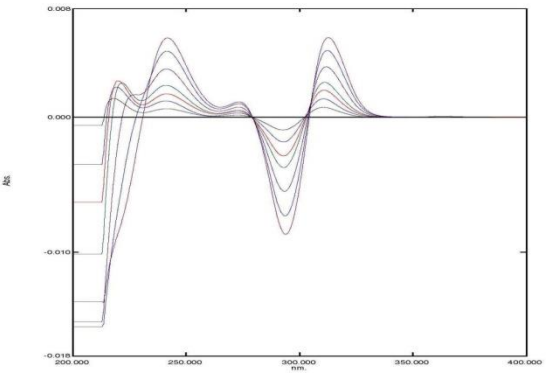


Fig 5: Over lain II Derivative spectrum of PNP

Recovery studies (PNP)

The recovery studies were performed for PNP. Recovery studies were performed based on standard addition technique. The zero order spectra obtained for recovery studies (20%, 40%, and 100%) in method A were derivatized. The amplitudes of the negative maxima were interpolated on the respective second derivative linearity charts and the concentrations were determined. Using the concentration, the recovery percentage was determined and tabulated in table-10 for PNP. The low RSD value indicates that recovery was appreciable and confirms that the interference due to sample matrix is negligible. Thus the method is accurate for PNP.

Table 5: Results of Recovery Studies of PNP-II Derivative method

Expected% Recovery	Amount ofdrug addedinmg		Totalamountassayed(mg)*	Amountrecovered(mg)	Assessed % Recovery	% Recovered ±S.D	RSD
	Sample	Standard					
20%	40 mg	8	47.94	8.09	20.21	101.07±0.313	0.0155
40%		16	56.00	16.15	40.37	100.93±0.626	0.0155
100%		80	80.50	40.70	101.74	101.74 ±.939	0.0092

Table 6: Optical parameters of PNP-UV Spectrophotometry

S.No.	Optical Parameter	Pantoprazole Method		
		A	B	C
1.	Wavelengthλmax	290nm	290nm	290nm

2.	Molarabsorptivity	14746.97	----	----
3.	Beer'slawlimitµg/ml	5-30	5-30	5-30
4.	Regressionequation	y =0.038x+0.001	y =0.955x-0.095	y=0.215x+0.035
5.	Slope	0.03815	0.955	0.215
6.	Intercept	0.001	-0.095	0.035
7.	Correlationcoefficient	1.000	0.9990	0.999
8.	Sandell'ssensitivity	0.0260	----	----
9.	LOD	0.4144	----	----
10.	LOQ	1.2560	----	---
11.	RSD	0.8022	0.9548	0.4782

Visible spectrophotometric method

The visible or colorimetric determination of pantoprazole is based on the oxidation of PNP to its sulphone derivative by by *insitu* generated bromine followed by determination of unreacted bromine by two different reaction schemes. In method A, the residual bromine is treated with excess of iron (II), and the resulting iron (III) is complexed with thiocyanate and measured at 470 nm. Method B involves treating the unreacted bromine with a measured excess of iron (II) and remaining iron (II) is complexed with 1,10-phenanthroline at a raised pH, and measured at 510 nm. In both methods, the amount of bromine reacted corresponds to the amount of PNP.

Method A- using1,10-phenanthroline

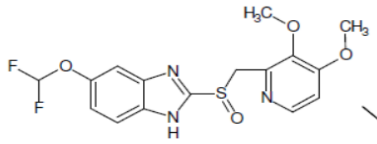
The colorimetric estimation of PNP is based on the oxidation of PNP by the*insitu* liberation of bromine during the reaction between excess bromate-bromide andhydrochloric acid.Pantoprazole is oxidized to form the sulphone analogue. The unreacted bromine left after oxidation of PNP is determined indirectly 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 (ferroin) which is a very well-known complexation reaction used for the estimation of iron in the ferrous state. The complex shows λ max at 510 nm. When a known excess amount of bromated -bromide mixture is allowed to react with increasing amount of PNP, there occurs decrease in the amount of bromine for oxidation of FAS to ferricammonium sulphate. Thus, an increase in the concentration of the FAS, available for complexation with 1, 10-phenanthroline is observed from the increase in the absorbance, as shown by the slope in the calibration chart (fig-12). Since the complex is formed and is stable only in alkaline media, ammonia solution is used.

REACTIONMECHANISM

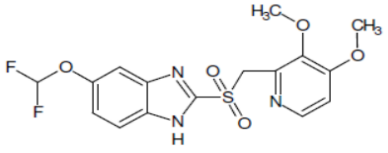
I. Oxidation

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

(potassium bromate-bromide) *bromine*

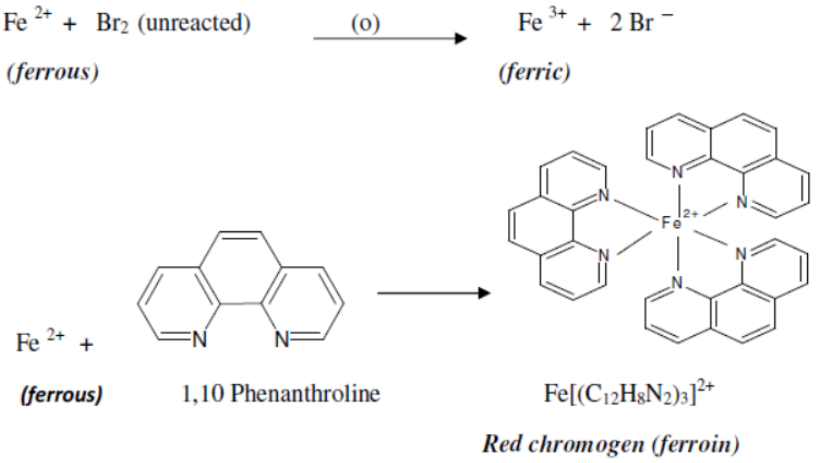


Pantoprazole Br_2 *(o)*



Oxidised form of panatoprazole

II. **Complexation** (Vogels., 2007)



The estimation of PNP was also performed within the Beer'srange. The amount of the reagents, the condition of the reagents and the order of the addition of reagents were maintained throughout the analysis of the sample. The overlain spectrum is shown in fig-12.

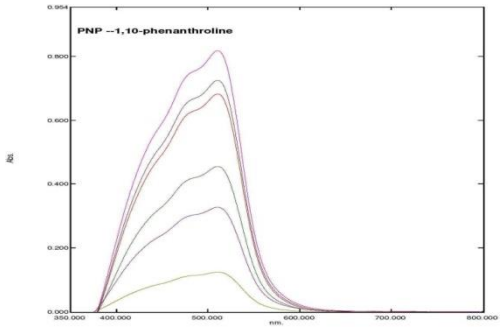


Fig 6: Overlain spectra PNP using 1,10-phenanthroline

Table 7: Absorbance of PNP using 1,10-phenathroline

S.No.	Concentration (in µg/mL)	Absorbance*
1	1.2	0.160
2	2.4	0.318
3	3.6	0.466
4	4.8	0.622
5	5.4	0.703

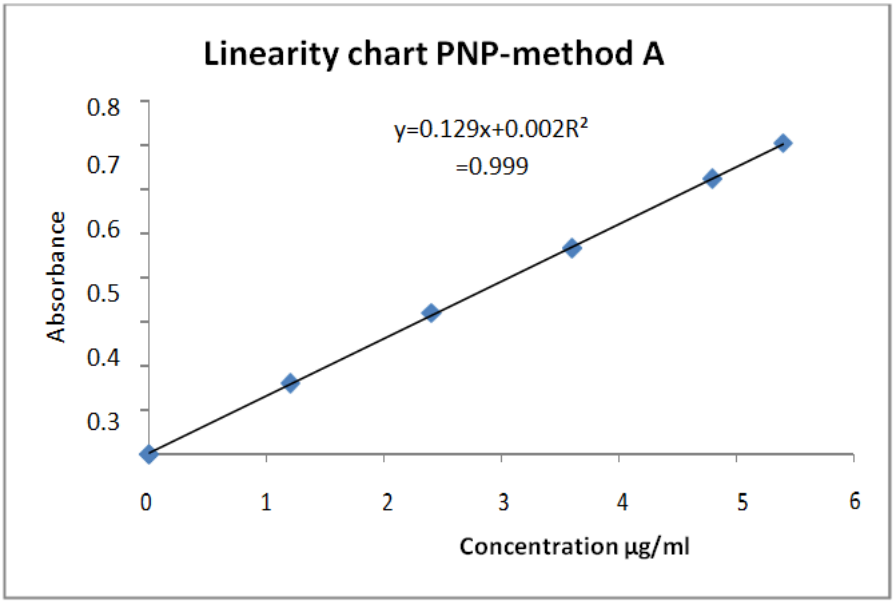
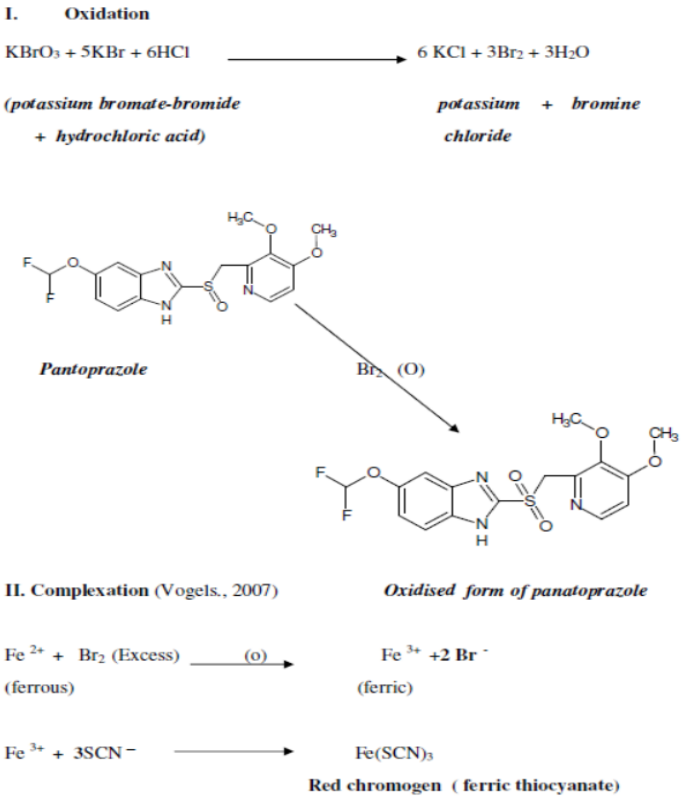


Fig 7: Calibration chart of PNP using 1, 10- phenanthroline

Method B–using ammonium thiocyanate (atc)

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 FAS to ferric ammonium sulphate. This in turn is complexed with ammonium thiocyanate to form a blood red complex of ferric thiocyanate which is a very well-known reaction. The complex shows λmax at 477nm.

REACTION MECHANISM



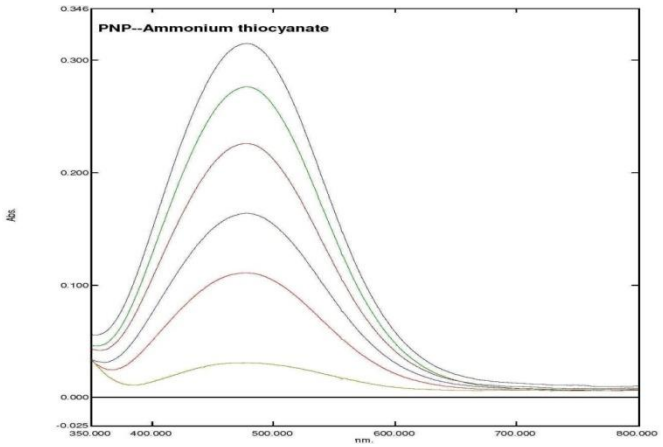


Fig 8: Overlain spectra PNP–ATCat477nm

Table 8: Absorbance of PNP–ATC

S.No.	Concentration(in µg/mL)	Absorbance*
1	0.4	0.315
2	0.8	0.276
3	1.2	0.216
4	1.6	0.164
5	2.0	0.111
6	2.4	0.061

Recovery studies (PNP-Method A&B)

Accuracy of the developed method was determined by the usual recovery studies. The recovery studies were performed by standard addition technique. It involves the addition of standard drug to the preanalysed sample. The resultant sample was then subjected to colorimetric reaction as discussed in method A & B separately. The results are shown in the table-15 & 16 for method A & B respectively. The results of recovery studies were found to lie between 98-102 %, for method- A and for method–B, 99-102%, which was within the limit. The results of the recovery studies indicate that the method developed shows no interference by the sample matrix excipients in the formulation.

Table 9: Results of recovery studies PNP– 1,10-phenanthroline

Expected %recovery	Amount ofdrug addedinmg		Totalamountassayed(mg)*	Amountrecovered(mg)	Assessed % Recovery	% Recovered ±S.D	RSD
	Sample	Standard					
20%	40 mg	8	47.52	8.07	20.17	100.84 ±0.152	0.0075
40%		16	59.09	19.6	49.09	98.18±1.14	0.0234
100%		80	80.25	40.8	101.99	101.99±0.79	0.0077

Table 10: Optical parameters of PNP by colorimetry

S. No	OpticalParameter	Pantoprazole	
		MethodA (1,10-PHT)	MethodB (ATC)
1.	Wavelengthλmax	510nm	477 nm
2.	Molarabsorptivity	5022.02	95581.60
3.	Beer'slawlimit (µg/mL)	1.2-5.4	0.4-2.4
4.	Regressionequation	y=0.129X + 0.002	y=-0.1297X +0.372
5.	Slope	0.129	-0.1297

6.	Intercept	0.002	0.372
7.	Correlation coefficient	0.999	0.998
8.	Sandell's Sensitivity	0.008	0.13
9.	LOD	0.234	18.9
10.	LOQ	0.707	57.4

RP-HPLC method

A new effort has been made to develop simple, precise, cost-effective and a ccurate methods for the estimation of Pantoprazole in tablet dosage form.

Detector wavelength

A solution of 10µg/ml of PNP standard drug in mobile phase was scanned in the UV region to determine the detector wavelength. It was observed that PNP showed λ max at 290 nm. Hence this was selected as the detecting wavelength for theestimation of PNP by HPLC.

Reverse phase-HPLC

The analytes are more polar in nature RP-HPLC method was selected.

Optimization of the chromatographic condition

The chromatographic condition was optimized using the standard drugs PNP. The optimization procedure involves the optimization of mobile phase in relation to its compatibility to the system suitability parameters as per ICH guidelines.

By trial and error method the mobile phase containing acetonitrile, methanol and phosphate buffer (pH-7) in the ratio of 40:20:40 %v/v was selected for the study.The mobile phase of pH-7 and flow rate of 2 ml/minute was selected for the study of PNP. Thus it was observed that the optimized chromatographic condition was common for PNP which is the main advantage of the developed method.

System suitability parameters

The system suitability parameters for the drug PNP in theoptimized chromatographic condition were calaculated and present in the table-18.All the values were compared with the standard values given by ICH guidelines and found to be compatible. The number of theoretical plates for PNP was found to be 5091 respectively which confirms the good efficiency of the column for the drugs and nature of mobile phase.

Table 11: System suitability parameters for PNP

S. No.	Parameter	Pantoprazole
1.	Theoretical Plates	5091
2.	Tailing factor	1.32
3.	Capacity factor	0
4.	Temperature of the column	ambient
5.	Retention time	3.5minutes
6.	Correlation coefficient (r^2)	1
7.	Correlation coefficient (r^2)	1
8.	Limit of Detection (LOD) (µg/ml)	1.9
8.	Limit of Quantitation (LOQ) (µg/ml)	5.8

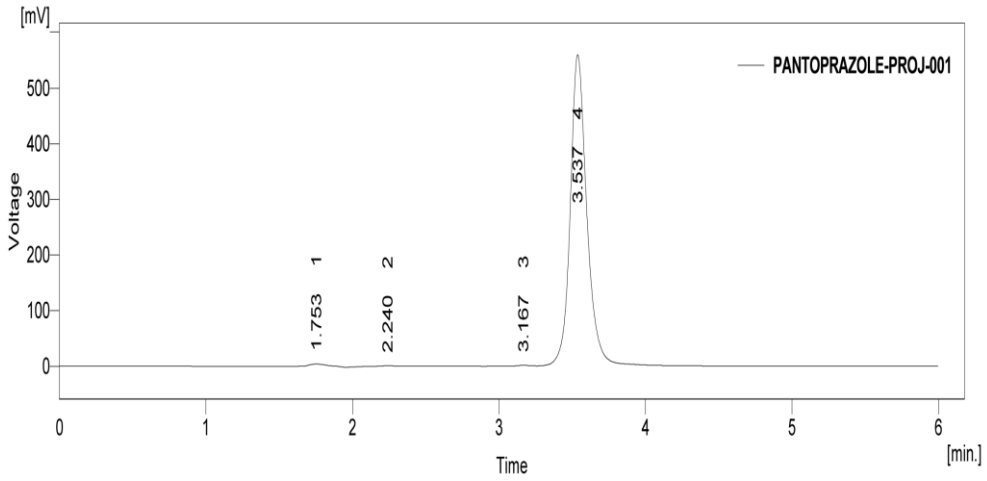


Fig 9: Chromatogram of PNP (Rt-3.5minutes)

Establishment Of Linearity

Linearity of the drug's concentration with respect to the instrumental response (peak area) was established for PNP. The peak areas with respect to concentration are presented in the table 20. A calibration chart was plotted using concentration along X –axis and peak area along Y-axis. The linearity of response under the optimized chromatographic condition was analysed using regression analysis. PNP obeyed Beer's lawat 140-220µg/ml. The correlation coefficient was found to be equal to 1 for PNP which is shown in the respective linearity chart.

Limit Of Detection And Limit Of Quantification

The LOD and LOQ were determined from the average of the three determinations of the slope and standard errors of estimate of the respective linearity charts. The LOD was found to be 1.9 µg/ml for PNP; LOQ as 5.8 µg/ml for PNP respectively.

Table 12: Peak area of PNP

S.No.	Concentration(µg/mL)	Peak area
1.	140	3964.82
2.	160	4442.76
3.	180	4966.63
4.	200	5542.18
5.	220	6082.93

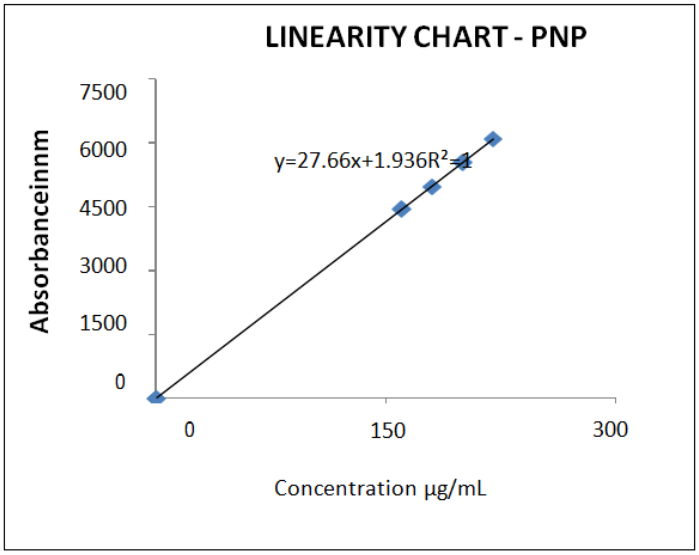


Fig 10: Calibration chart of PNP-HPLC

Validation of the proposed RP–HPLC method

Accuracy

The accuracy of the developed method was determined by performing recovery studies using the standard addition technique. A known amount of the standard drugs were added to the respective samples and the chromatogram was recorded for the same. The recovery studies were performed on 50% spiked samples and injected in duplicate. The analysis was performed thrice. The results of the recovery are shown in table-21. The percentage recovery was found lie between 98.89 and 100.00 % and 99.50 and 101% for PNP. The results of the recovery analysis suggest that the developed method has no interference by the sample matrix.

Table 13: Results of recovery studies of PNP

Expected %Recovery	Amount added(mg)		Totalamo untassaye dinmg*	AmountRec overedinmg	Assessed % Recovery	% Recovery	SD	RSD
	Sample	Standard						
			60.40	20.4	51	100.66		
50 %	40	20	60.69	20.7	51.73	101.15	0.6937	0.0069
			59.70	19.7	49.24	99.50		

Specificity

The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well –shaped peaks indicate the specificity of the method. Better resolution was found for the drug peak with no interference proved that the method was found to be selective to the drug.

Precision

The precision of the developed method was assessed by performing the analysis thrice which is tabulated in table-22.

Table 14: Results of Purity studies of PNP

S.No.	Label claim	Amount present (mg) ±SD*	RSD	%Purity ±SD	RSD
1.		39.5 ±0.0001	0.0031	98.8 ±0.313	0.0030
2.		40.0 ±.00009	0.0023	100.02 ±0.234	0.0023
3.	40mg	40.0 ±.00019	0.0046	100.02 ±0.469	0.0047

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CONCLUSION

Simple, Precise, rapid and accurate methods were developed for the estimation of Pantoprazole in bulk and in dosage form. The methods are UV Spectroscopic method, Visible Spectroscopic method & RP-HPLC method. The λmax of of PNP was found to be 290nm in methanol. The bulk drug PNP obeyed Beer's law at 5-30µg / ml. The correlation coefficient was found to be 1 for both the drugs. The dosage form of the drugs was quantified by the following three methods. Method A involves standard absorbance method. The RSD proves there producibility of the method and thus the precision of the developed method. Method B involves determination of AUC for both standard and sample spectra obtained in Method A between two selected wavelength. The correlation coefficient was found to be 0.9990 for PNP. The recovery percentage was found to be 98 to102% which proves no interference by the sample matrix. Method C involves the derivatisation of the zero order spectra to second order spectra. The recovery spectra were also derivatized and used. All the methods have shown good linearity, precision and accuracy. The low % RSD values in recovery studies for all the above methods indicate that there is no interference due to excipients used in the formulations. Hence it is concluded that the developed UV – Visible and RP-HPLC methods were found to be simple, precise, accurate and rapid methods for the analysis of Pantoprazole in its pure form and in its pharmaceutical dosage formulation. Thus, all the above adopted methods can be effectively used for the routine analysis of Pantoprazole in pharmaceutical dosage form.

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