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COMPARATIVE EVALUATION OF UV/VISIBLE AND HPLC ANALYTICAL METHODS WHEN APPLIED FOR ESTIMATION OF PENTOPRAZOLE SODIUM

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ABSTRACT

Simple and accurate spectrophotometric and HPLC method was developed for determination of Pantoprazole in lyophilized injection dosage form. The spectrophotometric method was developed by dissolving tablets in distilled water to make solution of 20ppm giving absorbance at 290nm. The experimental conditions were optimized and Beers law was obeyed over the applicable concentration range. The application of HPLC procedure depends on using a Thermo BDS Hypersil, C18 Column along with mobile phase consisting of Buffer pH 4 and acetonitrile (40: 60 v/v). Both techniques were applied successfully for analysis of Pantoprazole in different commercially available tablets and capsule. From the results obtained for both procedures percentage purity was found out.

KEYWORDS: Pantoprazole Na, Spectrophotometric, HPLC, Method development, Validation.

I. INTRODUCTION

Spectroscopy is the study of the interaction between matter and electromagnetic radiation. Historically, spectroscopy originated through the study of visible light dispersed according to its wavelength, by a prism. Later the concept was expanded greatly to include any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopy and spectrography are terms used to refer to the measurement of radiation intensity as a function of wavelength.[1,2]

Pantoprazole is the third proton pump inhibitor to be marketed in the UK; it is mainly used to treat too much acid secretions in the stomach, duodenal ulcer, benign gastric ulcer and gastro-

esophageal reflux diseases (GERD) and reflux oesophagitis. Pantoprazole has several advantages compared to its analogus (e.g., omeprazole and lansoprazole) such as specific binding site, greater stability in neutral pH environment and longer duration of action. It is more selective inhibitor of acid secretion than other proton pump inhibitors. In case of oral administration pantoprazole drug is destroyed in acid medium (stomach),due to necessity to pass intact through the stomach for reaching to duodenum for absorption, the pantoprazole is formulated as controlled release dosage forms.[3,4,5]

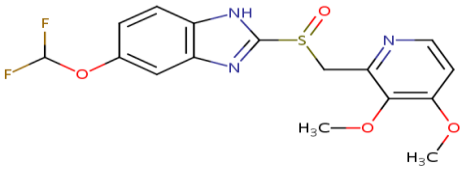

Figure 1: Pantoprazole.

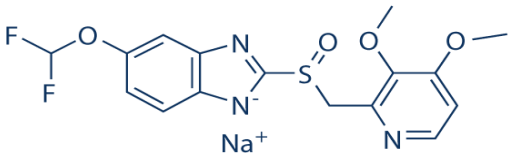

Figure 2: Pantoprazole Na.

II. MATERIAL AND METHODS

1. UV-Visible Spectrophotometer: Shimadzu UV-1800PC spectrophotometer using 1cm quartz cells.

2. HPLC: The HPLC analysis was carried with Waters 2695 with software version Empower 2- PDA detector and Shimadzu LC-2010C HT HPLC system with UV detector and auto sampler integrated with software LCsolution Version 1.25. The column used Thermo BDS Hypersil, C18 Column (150 mm x 4.6 mm, 5 µm particle size)

3. Diluent: Buffer:ACN 40:60

4. Mobile phase: Buffer:ACN 40:60

5. Preparation of standard solutions: For Spectrophotometric determination: Standard solution of Pantoprazole was prepared of 20ppm concentration. Using that standard solution a series of dilutions ranging from 10 ppm to 30 ppm was prepared. For HPLC determination: Weigh & transfer accurately about 20 mg of Pantoprazole Na working standard in to 100 ml volumetric flask. Add to it 70 ml of diluent and sonicate until it dissolve completely. Dilute up to the mark with diluent & mix well. Take 10 ml solution from above solution and transfer it to 20 ml volumetric flask and dilute it with diluent i.e., mobile phase up to the mark and sonicate it for 5 minutes.

6. Sample taken for analysis: For Spectrophotometric determination: Take equivalent to 40mg of Pantoprazole as sterile lyophilized powder i.e. PLIIV and dissolve it with distilled water in 10ml volumetric flask. Further dilute 1ml of above solution to 20ml in 20ml volumetric flask with diluent. Further dilute 1ml of above second stock solution to 10ml in 10ml volumetric flask with diluent. For HPLC determination: Take equivalent to 40mg of Pantoprazole as sterile lyophilized powder i.e. PLIIV and transfer one ampoule containing 10ml sodium chloride injection IP in it. Now transfer 0.5ml of above solution into 20ml of volumetric flask. Dilute with diluent up to the mark.

III. RESULT AND DISCUSSION

1. Analysis by UV Spectrophotometer

Proper wavelength selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of Pantoprazole, the analytical condition were selected after testing the different parameters such as diluents, concentration, and other chromatographic conditions.

Our preliminary trials were by using different diluents consisting Acetonitrile, Methanol, Acetone etc. By using diluent distilled water best result was obtained and degassed in an ultrasonic bath.

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank (Figure 2). Pantoprazole shows λ_{max} at 290. The proposed analytical method is simple, accurate and reproducible.

2. Analysis by HPLC

Various mobile phase compositions and buffer systems were tried for achieving the optimum peak of PLIIV. Mobile phase composed of buffer pH 4 and acetonitrile (40:60 v/v) gave the optimum peak resolution hence mobile phase composition of buffer pH 4 and acetonitrile (40:60 v/v) at the flow rate of 1.0 ml/minute was finalized. Different buffer systems were also tried in order to get suitable peak shapes. Thermo BDS Hypersil, C18 Column (150 mm x 4.6 mm, 5 µm particle size) was selected. Detection was tried at various wavelengths but 278 nm was selected as the detection wavelength as Pantoprazole showed maximum absorption. The retention time was found to be 1.9 minutes for Pantoprazole Lyophilized Injection IV.

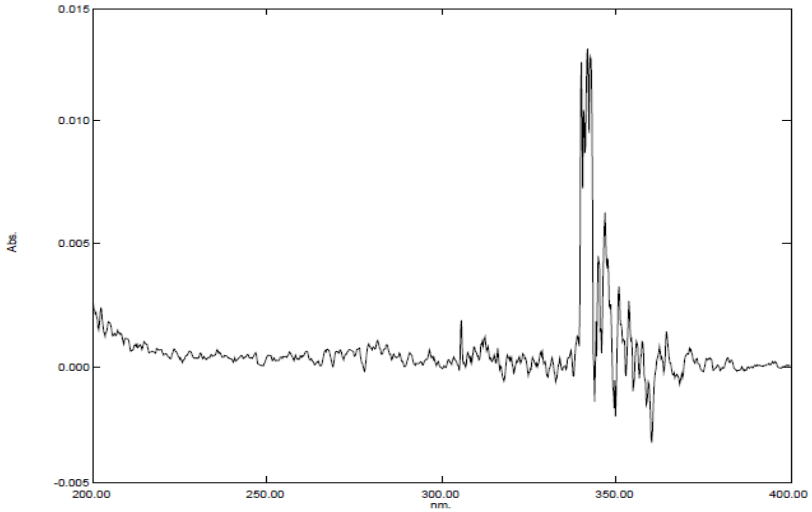

Figure 3: Scan of Blank i.e. Diluent on UV.

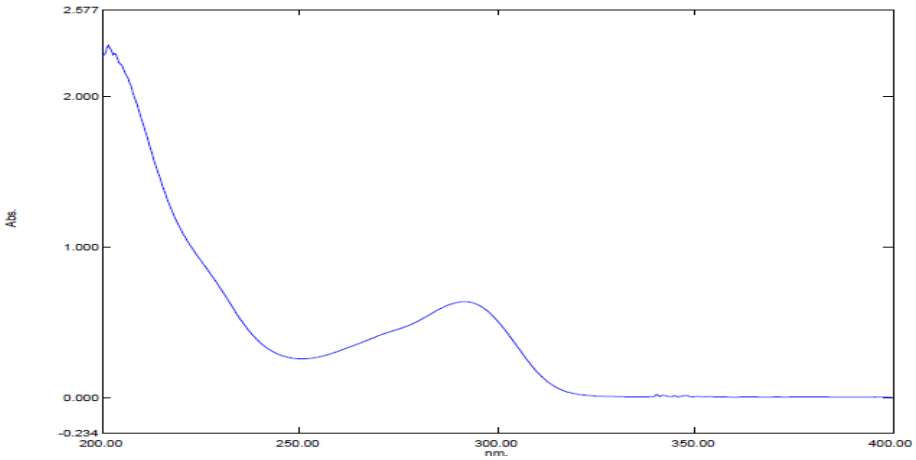

Figure 4: Scan of Pantoprazole standard on UV.

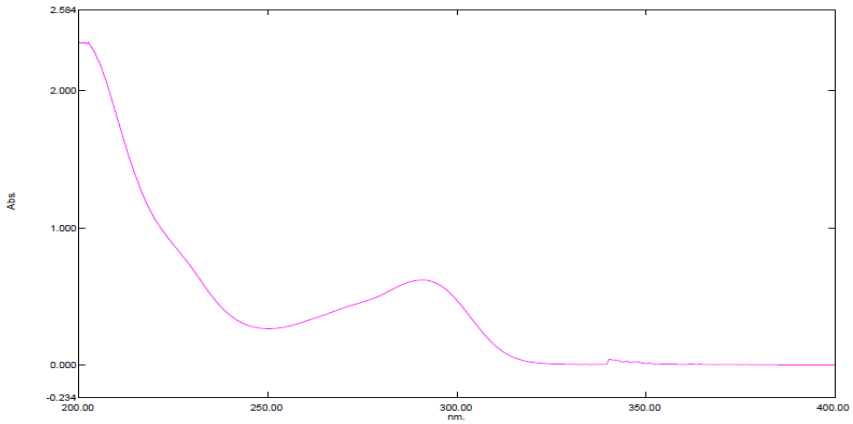

Figure 5: Scan of Pantoprazole Sample on UV.

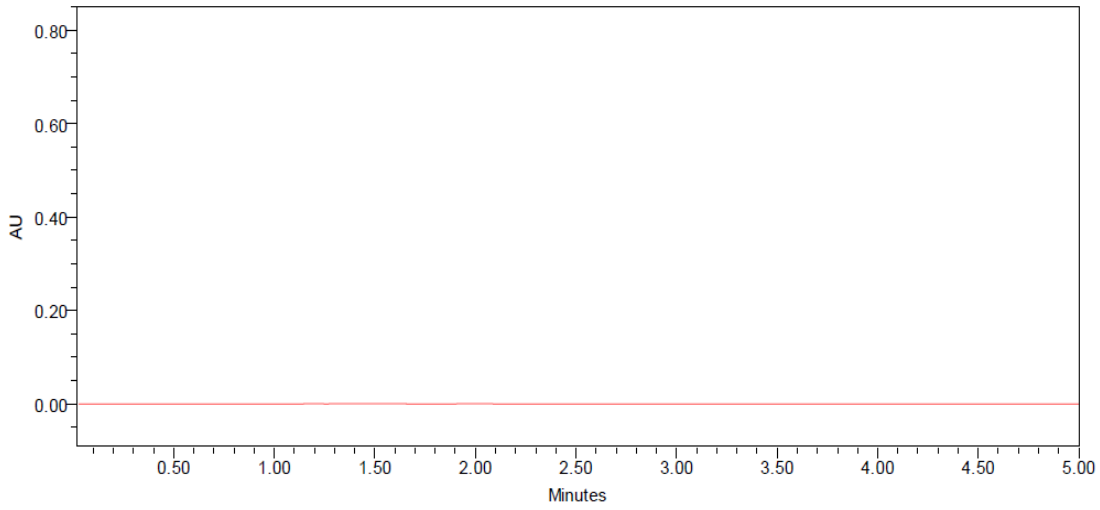

Figure 6: Chromatogram of blank solution on HPLC.

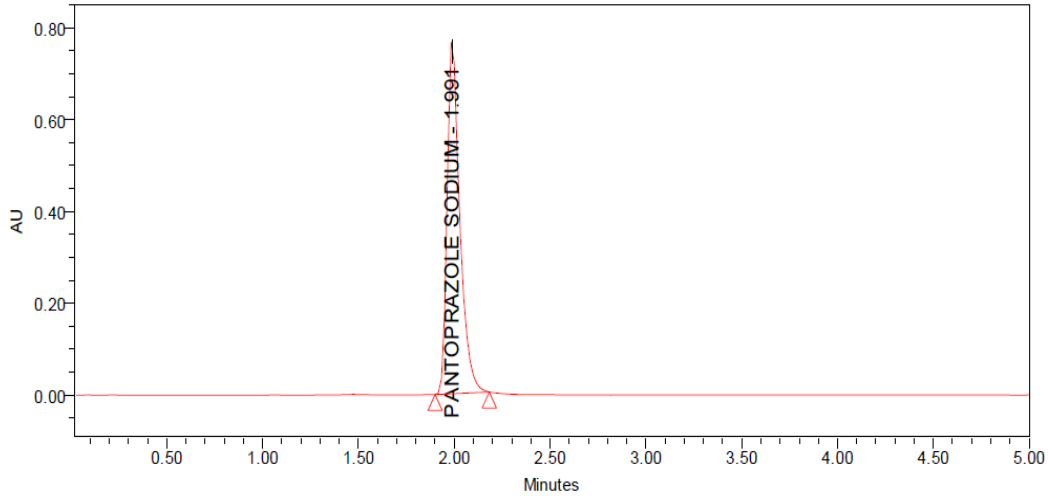

Figure 7: Chromatogram of standard solution on HPLC.

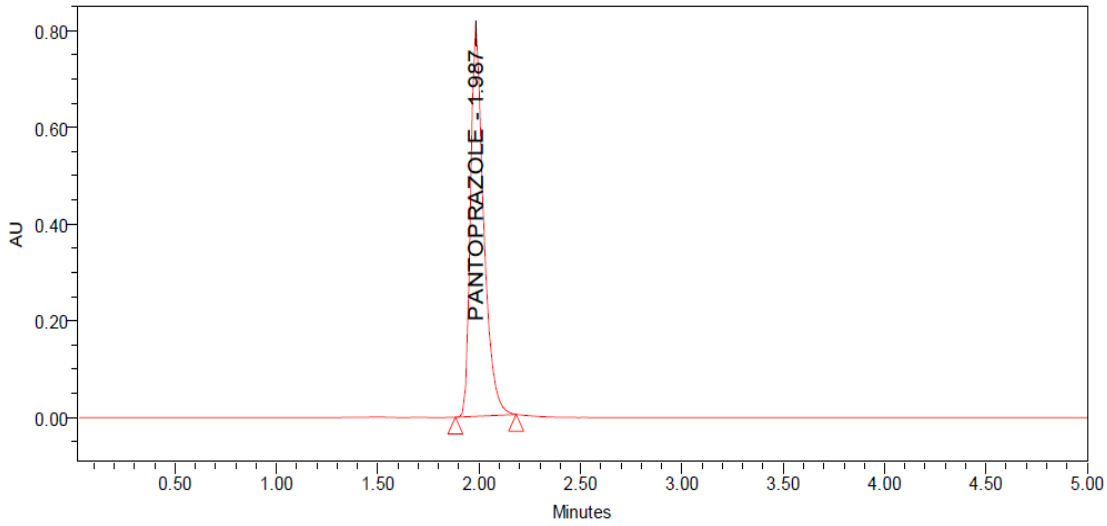

Figure 8: Chromatogram of sample solution on HPLC.

Analytical Method Validation of proposed method

The validation of proposed method was based on ICH guidelines and USP-39.

Linearity on UV

The calibration curve was plotted in the range of 10 to 30 µgmL^{-1} to determine the correlation coefficient and y-intercept. The sample solution was serially diluted from 10 to 30 µgmL^{-1} in distilled water and scanned at 290 nm wavelength to get the absorbance of respective dilution.

Linearity on HPLC

Five concentrations such as 50.1, 75.2, 100.2, 125.3 & 150.3 µg/ml for Pantoprazole Na were prepared and the linearity graph was plotted using concentration verses peak area as shown in Figure 10. Graph of Residuals against concentration was also plotted as per shown in Figure 11. A linear relationship was obtained between peak areas and quantity analyzed in the range of 50% to 150% (50.1-150.3 µg/ml).

Precision on UV

Intraday precision was determined by estimating the response of six samples of 20 µgmL-1 on the same day.

Precision on HPLC

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by carrying out six independent assays of Pantoprazole Lyophilized injection IV at 100 µg/ml concentration. The mean area and % relative standard deviation (RSD) was calculated. % RSD should be ≤ 2 %.

Accuracy on UV

The accuracy of the method was determined by calculating the percentage recoveries. So, these 10, 20, and 30 µgmL-1 dilutions were analyzed thrice to assess the percentage recoveries.

Accuracy on HPLC

The accuracy of the method was determined by recovery experiments known concentrations of working standard was added to the fixed concentration of the pre-analyzed Pantoprazole Lyophilized injection IV sample. Percent recovery was calculated by comparing the area with

pre-analyzed sample. Three different solutions of Pantoprazole were prepared in triplicate at level of 50%, 100% and 150% of its predefined concentration (50, 100, 150 µg/mL) and the percentage mean and individual recovery was calculated. Data from the linearity was considered for accuracy.

Robustness on UV

Robustness of the stated method was evaluated by deliberate variation in the solvent. Deliberately changed tap water and HPLC water instant of distilled water. The results were reported in terms of %Assay.

Robustness on HPLC

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was observed that the variations like sonication time & change in wavelength etc.

Specificity on UV

Specificity of the proposed method was determined by analyzing the standard dilution and sample.

Specificity on HPLC

Spectral purities of Pantoprazole Lyophilized injection IV peak were evaluated for the interference of the Lyophilized Injection IV excipients, degradation components or due to the presence of impurities as per the methodology.

RESULT AND DISCUSSION

Analytical method validation of developed method using UV Spectrophotometer.

Linearity on UV

The linearity of developed method was evaluated at seven different concentrations from 10 to 30 μgmL^{-1}. Correlation coefficient, intercept and slope value were also determined for statistical investigation. The observed absorbance of each test sample was plotted against the corresponding concentration and a controlled linear line was obtained Figure 10 So, it is concluded that the proposed method is linear in the specified range of concentrations.

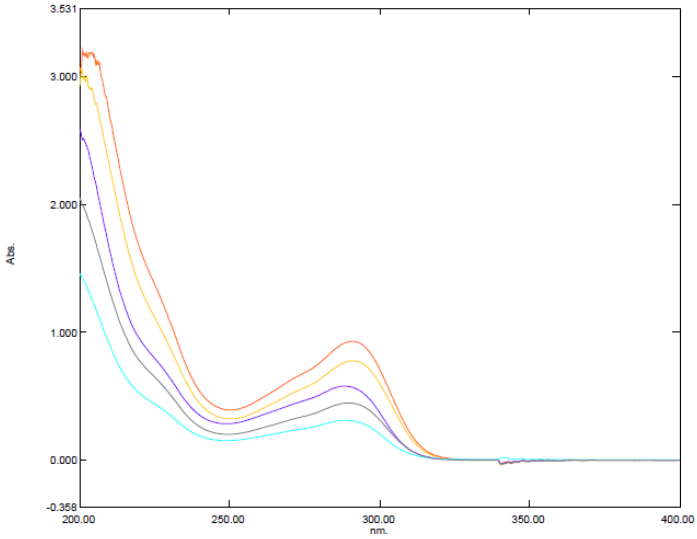

Figure 9: Scan of Linearity Range on UV.

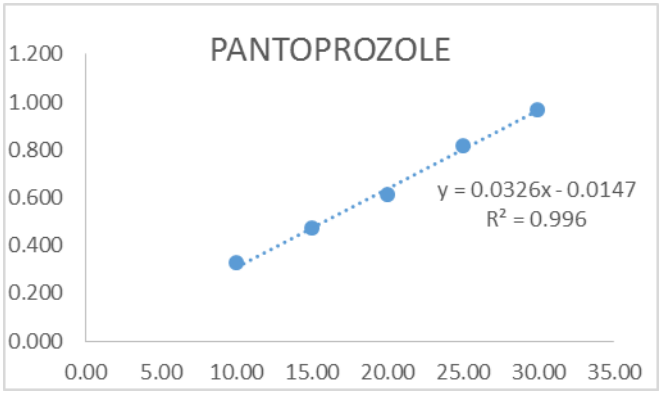

Figure 10: Scan of Linearity Plot on UV.

Table 1: Linearity Concentration Levels of Pantoprazole on UV.

% level	Volume of stock solution	Diluted to (ml)	Final concentration in ppm
50%	1 ml	10	10
75%	1.5 ml	10	15
100%	2 ml	10	20
125%	2.5 ml	10	25
150%	3 ml	10	30

Table 2: Observation table for linearity of Pantoprazole on UV.

Parameter for Linearity	Values	Acceptance Criteria
Correlation coefficient R	0.99801	$\geq$ 0.99
%Y – axis intercept	-2.39	$\leq \pm$ 5 %
Slope of regression line	0.03	To be reported
Y Intercept	-0.01	To be reported

The method was considered to be linear in the range on 10-30 µg/ml for Pantoprazole as Correlation coefficient & %Y-axis intercept should be within the limit.

Linearity on HPLC

Five concentrations such as 50.1, 75.2, 100.2, 125.3 & 150.3 µg/ml for Pantoprazole Na were prepared and the linearity graph was plotted using concentration verses peak area as shown in Figure 11. Graph of Residuals against concentration was also plotted as per shown in Figure 12. A linear relationship was obtained between peak areas and quantity analyzed in the range of 50% to 150% (50.1-150.3 µg/ml).

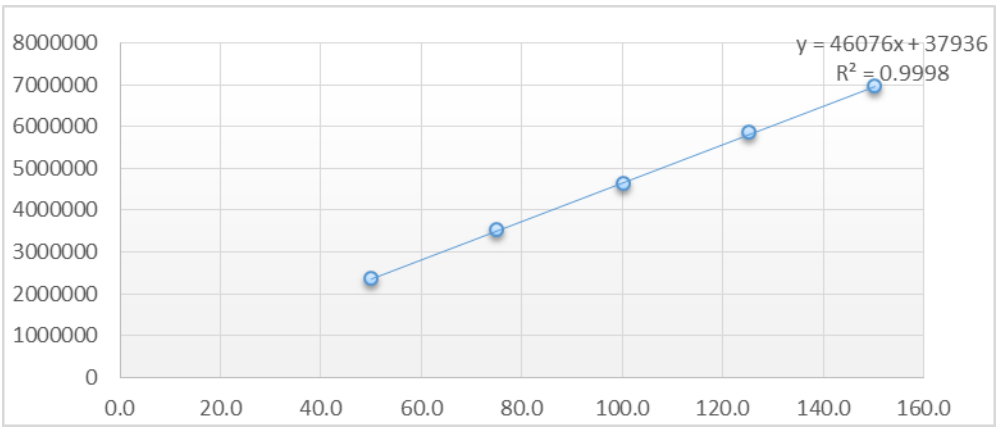

Figure 11: Linearity plot for Pantoprazole Na on HPLC.

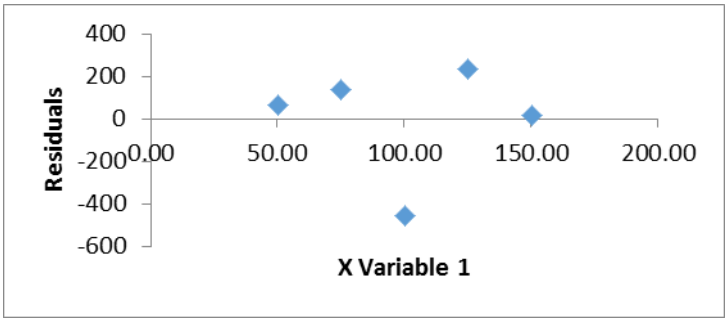

Figure 12: Plot of Residuals against concentration for Pantoprazole Na on HPLC.

Table 3: Linearity Concentration Levels of Pantoprazole.

% level	Volume of stock solution	Diluted to (ml)	Final concentration in ppm
50%	5 ml	20	50.1
75%	7.5 ml	20	75.2
100%	10 ml	20	100.2
125%	12.5 ml	20	125.3
150%	15 ml	20	150.3

Table 4: Observation table for linearity of Pantoprazole Na.

Parameter for Linearity	Values	Acceptance Criteria
Correlation coefficient R	0.9990	≥ 0.999
%Y – axis intercept	0.82	≤ ± 5 %
Slope of regression line	46075.78	To be reported

The method was considered to be linear in the range on 50.1 – 150.3 µg/ml for Pantoprazole Na as Correlation coefficient & %Y-axis intercept should be within the limit.

Accuracy on UV

The percentage recovery of Pantoprazole was tabulated in table 5. The method was considered to be accurate as the % individual recovery was within the acceptance criteria of 97-103 % and the % mean recovery was within the acceptance criteria of 98 – 102 %.

Table 5: Recovery at Different Concentration Levels.

Accuracy level	% recovery of Pantoprazole
50%	99.86
	100.20
	100.86
100%	99.37
	99.20
	99.20
150%	100.86
	101.08
	100.64
Means recovery	100.14
Minimum recovery	99.20
Maximum recovery	100.86

Accuracy on HPLC

The percentage recovery of Pantoprazole Na was tabulated in table 6. The method was considered to be accurate as the % individual recovery was within the acceptance criteria of 97-103 % and the % mean recovery was within the acceptance criteria of 98 – 102 %.

Table 6: Recovery at Different Concentration Levels.

Accuracy level	% recovery of Pantoprazole Na
50%	98.0
	97.6
	99.7
100%	101.6
	101.6
	101.5
150%	98.3
	98.1
	98.7
Means recovery	99.47
Minimum recovery	98.4
Maximum recovery	101.6

Precision on UV

The exactness of the method as defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations was well within the acceptance limit of ≤ 2 %. Refer table 7.

Table 7: Method Precision on UV.

Sample No.	% Assay of Pantoprazole
Sample 01	99.86
Sample 02	99.86
Sample 03	100.03
99Sample 04	99.37
Sample 05	99.86
Sample 06	100.20
Mean	99.86
STD Dev	0.28
% RSD	0.28

Precision on HPLC

The exactness of the method as defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations was well within the acceptance limit of ≤ 2 %. Refer table 8.

Table 8: Method Precision on HPLC.

Sample No.	% Assay of PLIIV
Sample 01	99.9
Sample 02	98.6
Sample 03	100.4
Sample 04	100.7
Sample 05	101.3
Sample 06	99.9
Mean	100.1
STD Dev	7706.66
% RSD	0.22

Intermediate Precision on UV

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. Refer table 6 for % Assay of Pantoprazole.

Table 9: Intermediate Precision on UV.

Sample No.	% Assay of Pantoprazole
Sample 01	98.67
Sample 02	98.51
Sample 03	98.84
Sample 04	99.00
Sample 05	98.51
Sample 05	98.34
Mean	98.643
STD Dev	0.24
% RSD	0.25

Intermediate Precision on HPLC

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. Refer table 7 for % Assay of PLIIV and table 10 for comparison of two independent repeatability.

Table 10: Intermediate Precision on HPLC.

Sample No.	% Assay of PLIIV
Sample 01	101.1
Sample 02	100.0
Sample 03	101.8
Sample 04	100.9
Sample 05	101.6

Sample 05	101.5
Mean	101.1
STD Dev	4349
% RSD	0.09

Robustness on UV

The robustness of the developed method in this experimental work was evaluated by changing the solvent from distilling water to tap water and HPLC water. The results represented that no critical change was recorded as mention in Table 11.

Table 11: Robustness of Developed Method.

Sr. No.	Concentration	Solvent	Absorbance	% Recovery
1	20	Tap Water	0.593	99.16
			0.593	99.16
			0.590	98.66
2	20	HPLC Water	0.597	99.83
			0.598	100
			0.599	100.17

Robustness on HPLC

Method was found to be robust as system suitability criteria was achieved for all the robustness parameters tested. Deliberate change in parameter does not have any significant effect on the method performance, which demonstrated that the developed HPLC method was robust. The results were shown in Table (12).

Table 12: Robustness Result for Pantoprazole.

Parameter	System suitability	% Assay
	% RSD	
As per method		
--	0.22	99.4
pH		
3.8	0.77	101.5
4.2	0.48	100.6
Flow rate		
0.8 mL/Minutes	0.27	100.7
1.2 mL/minutes	0.59	100.6

IV. CONCLUSION

Despite the number of methods described by the other researchers for analysis of Pantoprazole the proposed UV-Visible Spectrophotometric method and HPLC method for

determination of Pantoprazole in pharmaceutical samples is simple and rapid than other sophisticated instruments. All the samples were analyzed within the range. These methods are very appropriate for routine analysis of active drugs in the laboratories. The procedures are easy to execute and require less sample handling than methods described in the literature. This spectroscopic method is selective, simple and rapid which can be very beneficial for the routine analysis of Pantoprazole Lyophilized Injection IV in pharmaceutical injection dosage form and solid dosage form. The following table 13 gives the summary of result.

Table 13: Summary of Results.

Parameters	On UV	On HPLC
% Recovery	100.14	99.47
Precision	99.86	100.1
Intermediate Precision	98.6	101.1

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