RESEARCH PAPER

Analytical method development and validation of pantoprazole and related substances by HPLC.

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ABSTRACT

Pantoprazole is a proton pump inhibitor (PPI) that inhibits the final stage of gastric acid production by creating a covalent link with two locations of the (H+, K+)-ATPase enzyme system on the secretory surface of the gastric parietal cell. The HPLC method for the determination of related substances in Pantoprazole has been developed and was specific, sensitive, precise, accurate, rapid and robust. The method allows quantification of potential related substances Pantoprazole, impurity A, impurity B, impurity C, impurity D & F &impurity E. Chromatographic separation was achieved on HPLC equipped with UV detector with column. Thermo Hypersil ODS (125 x 4.0) mm, 5µm and the gradient eluted with runtime, 30.0 min. The eluted compounds were monitored at 290 nm, the flow rate was 1.0 mL/min, and the column oven temperature was maintained at 40°C. The resolution between Pantoprazole & Impurity D & F and % RSD of area counts in standard solution was found to be 7.14 and 0.5 respectively, which was under the acceptance criteria. The high correlation coefficient (r2 > 0.999) values indicated clear correlations between the investigated compound concentrations and their peak areas within the test ranges. The developed method was validated according to ICH guidelines. The validation showed that developed method was valid and reliable for determination of pantoprazole and related substances.

Keywords: RP-HPLC, Analytical method development, Calibration curve, Validation

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INTRODUCTION

High performance liquid chromatography

A proton pump inhibitor called pantoprazole is used as a short-term treatment for oesophageal erosion and ulceration brought on by gastroesophageal reflux disease [1]. By forming a covalent bond with two sites of the (H+, K+) ATPase enzyme system on the secretory surface of the gastric parietal cell, the proton pump inhibitor (PPI) pantoprazole prevents the last phase of gastric acid generation [2]. Regardless of the stimulus, this dose-dependent effect suppresses both baseline and stimulated stomach acid output. This work set out to develop a novel, validated, straightforward, and repeatable RP-HPLC method for estimation in human plasma samples and dose forms. In biochemistry and analysis, high performance liquid chromatography, often known as high-pressure liquid chromatography, or HPLC, is a type of column chromatography that is frequently used to separate, identify, and quantify active compounds. A column that holds packing material (the stationary phase), a pump that pushes the mobile phase or phases through the column, and a detector that shows the molecules' retention times are the main components of HPLC [3–4].

MATERIALS AND INSTRUMENTS

Instruments: HPLC analyses for method development, forced degradation, and validation were performed on an Agilent technology equipped with a Rheodyne injector (20 µL) and a photodiode-array detector (PDA) utilizing empower software.

Chemicals: Methanol HPLC Grade, Water HPLC Grade, Acetonitrile HPLC Grade etc.

METHODOLOGY

Method Development Phase

Wavelength optimization& selection: The UV spectra of Pantoprazole and all contaminants were scanned from 190 to 400 nm. Pantoprazole's wavelength maxima were discovered to be 204 and 302 nm. All contaminants have a λ max of 290 nm, with the exception of impurity C at 305 nm. During the trials, it was discovered that baseline stability, mobile phase interference, and noise levels were better at 290 nm than at 303 nm. Furthermore, the appropriate detection and quantification limit for Pantoprazole and all impurities was discovered at 290 nm, and for impurity C at 305 nm; hence, 290 nm and 305 nm were chosen as detection wavelengths.

Mobile Phase optimization& selection: To achieve the required system suitability parameters for pantoprazole, preliminary trials were conducted using various columns (Thermo Hypersil ODS, Fortis C18, ACE C18 PFP, ACE C18 AR, YMC J Sphere), and various mobile phases made up of 10 mM potassium dihydrogen phosphate buffer (pH 6.5) and acetonitrile: methanol in varying proportions. Following a number of experiments, the best peak shape and resolution were found using gradient: linear elution using 10 mM

**Author for Correspondence: naveenbhadani12@gmail.com* potassium dihydrogen phosphate buffer (mobile phase-A) and ACN: Methanol (mobile phase-B). Retention times for Pantoprazole and the contaminants were found to be close to dead point. Gradient elution is therefore favoured over isocratic elution in this situation. 0.001N sodium hydroxide with ACN (50:50) is the recommended diluent. With good hydrophobicity, Thermo Hypersil ODS produced an isolated peak of related chemical A.

Diluent preparation: 0.001N Sodium Hydroxide with ACN in 50:50 was used for diluent.

Standard solution preparation: 5.628 mg of "Pantoprazole" were precisely weighed and placed into a 100 mL volumetric flask. Sonicated for two minutes after adding 100mL of diluent. 5 mL of the solution was then diluted to volume and combined in a 100 mL volumetric flask. They computed the ultimate concentrations.

Senstivity solution: 1 mL of the standard solution was then added to a ten mL volumetric flask, diluted to volume with diluent, and mixed. They computed the ultimate concentrations.

Sample/Test solution: 1 vial of "Pantoprazole" was transferred into a 10 mL volumetric flask. sonicated for two minutes after adding ten mL of diluent. One mL of the solution was then diluted to volume with diluent and combined in a ten mL volumetric flask. They computed the ultimate concentrations.

Chromatographic conditions: For chromatographic separation, the Thermo Hypersil ODS (125 x 4.0) mm, 5µm column was taken into consideration since it produces sharp, fully resolved peaks and satisfies all system suitability requirements. The auto sampler and column had an ambient temperature and a 1.0 mL/min flow rate. 20µl was chosen as the injection volume since it exhibits good UV absorbance within Beer's Lambert's range. The 30-minute run time was chosen.

VALIDATION PARAMETERS

The method developed here was validated as per ICH guidelines for its accuracy, linearity, precision, specificity, system suitability parameters, limit of detection and limit of quantification by using the following procedures. [5,6]

RESULTS

Selection of wavelength: The λ max for Pantoprazole, impurity A, B, D & F, E and impurity C was found to be 290 nm and 305 nm respectively.

Mobile phase selection: Gradient: Acetonitrile: Methanol (80:20) and 10 Mm potassium dihydrogen phosphate buffer (pH 6.5) were chosen for the linear elution of the mobile phase.

Acetonitrile gives sharp peaks and does not have adverse effect on tubing's and column chemistry even on long exposure.

RESULTS OF INITIAL TRIALS

Conditions 1: Mobile phase A: 10mM Potassium dihydrogen phosphate pH 6.5, Mobile phase B: 100% Acetonitrile, Column Temperature: 40° C, Injection Volume: 20µL, Gradient: Linear.

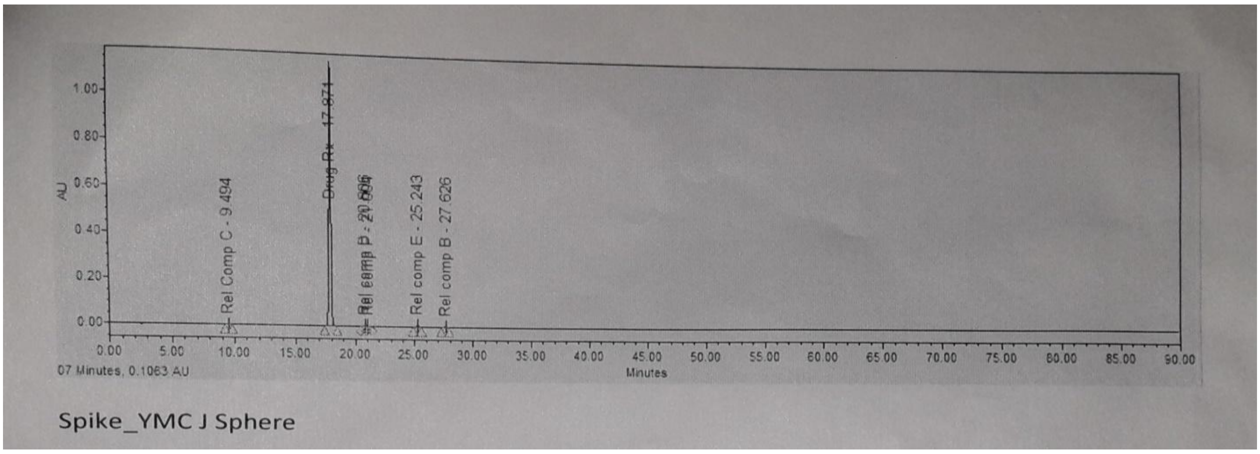
In 1st trials related compound, A peak merged with Pantoprazole peak (shown in table 2) so 2nd trials were performed using different columns and conditions.

Table 1: Results of 1st trials

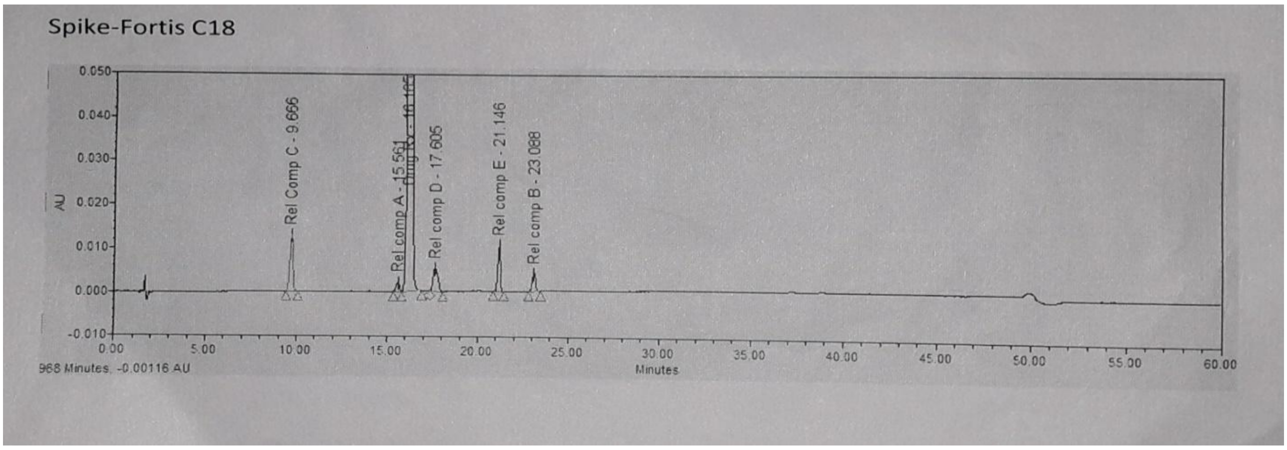
Column Name	Column Dimensions	Flow Rate (mL/min)	Observations
Thermo Hypersil ODS	(125 × 4.0) mm,5 µm	1.00	Related Compound A peak merged with Pantoprazole peak. Related Compound D&F coelution.
Fortis C18	(150 × 4.6) mm,3 µm	1.32	Related Compound A peak merged with Pantoprazole peak. Related Compound D&F coelution.
ACE C18 PFP	(250 × 4.6) mm,5 µm	1.32	Related Compound A peak eluted in tailing with Pantoprazole, But not fully resolved. Related Compound D&F separated.
ACE C18 AR	(250 × 4.6) mm,5 µm	1.32	Related Compound A peak merged with Pantoprazole peak. Related Compound C peak is not proper. Related Compound D&F not well resolved.
YMC J Sphere	(250 × 4.6) mm,4 µm	1.32	Related Compound A peak merged with Pantoprazole peak.

			Related Compound C peak is not proper. Related Compound D&F not well resolved.

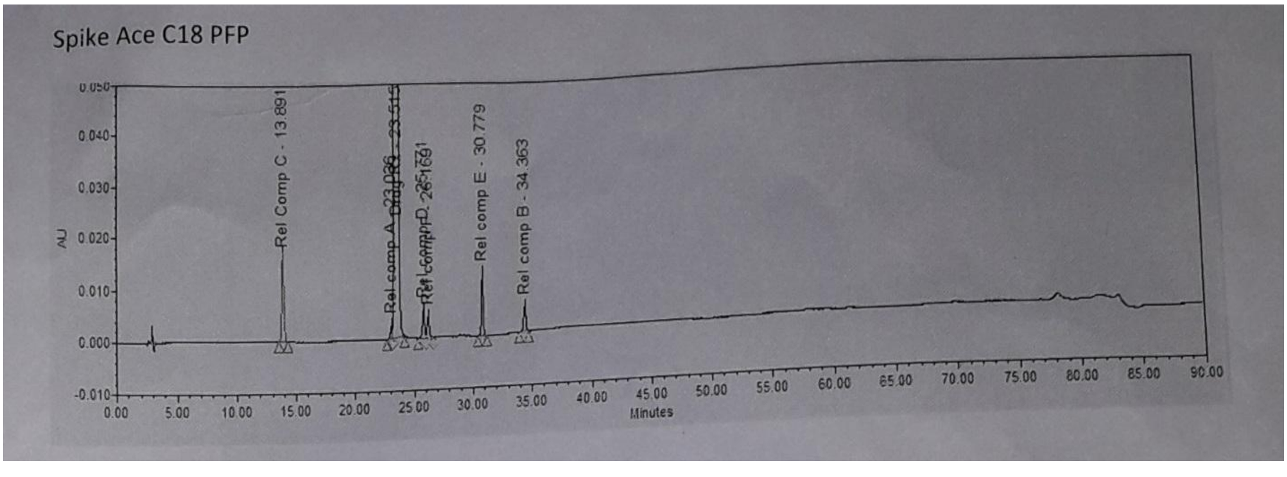
Chromatograms of trials



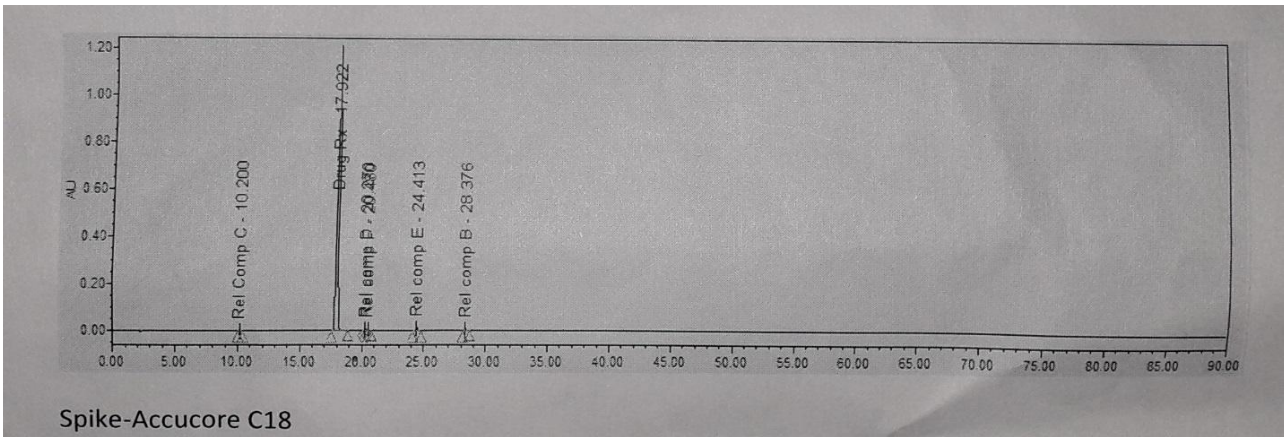
Graph 1: Column-Thermo Hypersil ODS



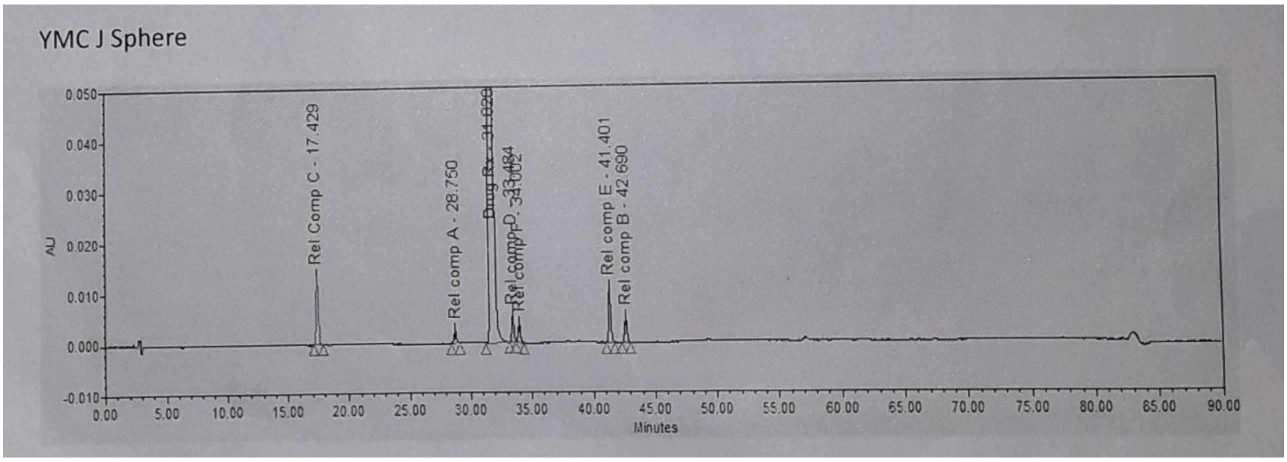
Graph 2: Column- Spike-Fortis C18



Graph 3: Column-Spike Ace C18 PFP



Graph 4: Column-ACE C18 AR



Graph 5: Column-YMC J Sphere

Conditions 2: Mobile phase A: 10Mm Potassium dihydrogen phosphate pH 6.5, Mobile phase B: Acetonitrile: Methanol (80:20), Column Temperature: 40° C, Injection Volume: 20µL, Gradient: Linear

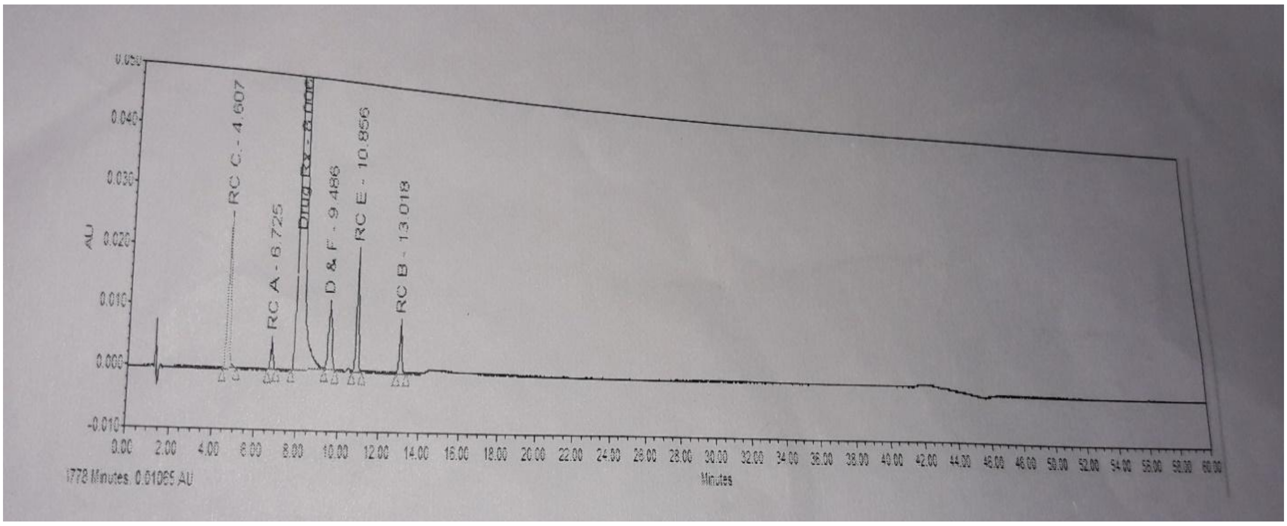
In 2nd trials Pantoprazole& related compound A was fully separated on column Thermo Hypersil ODS (shown in table 2) so 2nd trials were considered as final trials. Thermo Hypersil ODScolumn was selected for further research work.

Table 2: Results of 2nd trials

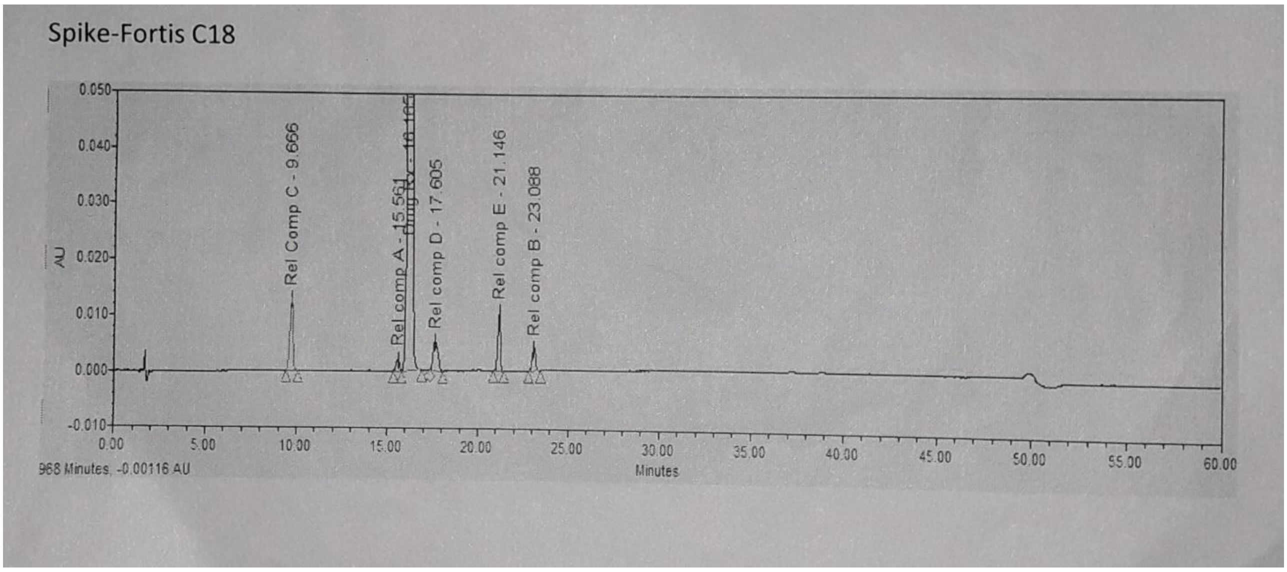
Column Name	**Column Dimensions**	**Flow Rate (mL/min)**	**Observations**
Thermo Hypersil ODS	(125 × 4.0) mm,5 µm	1.00	Pantoprazole &Related Compound A was fully separated. Related Compound D&F was fully resolved.
Fortis C18	(150 × 4.6) mm,3 µm	1.32	Related Compound A peak merged with Pantoprazole peak. Related Compound D&F not fully resolved.
ACE C18 PFP	(250 × 4.6) mm,5 µm	1.32	Related Compound A peak merged with Pantoprazole peak in fronting. Related Compound D&F resolved.

YMC J Sphere	(250 × 4.6) mm,4 µm	1.10	Related Compound D&F not fully resolved.

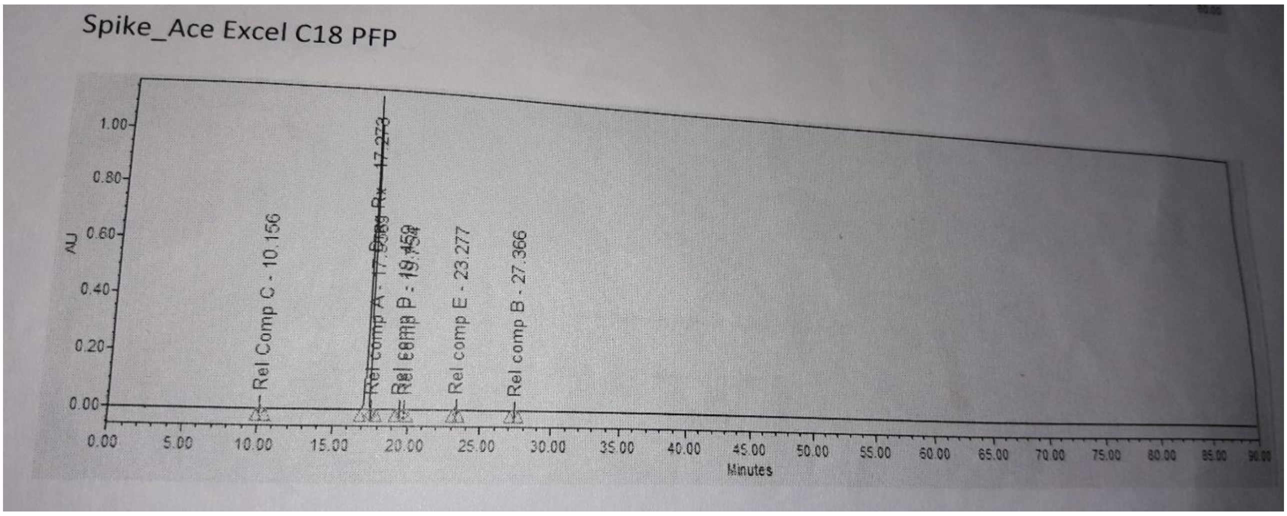
Chromatograms of trials.



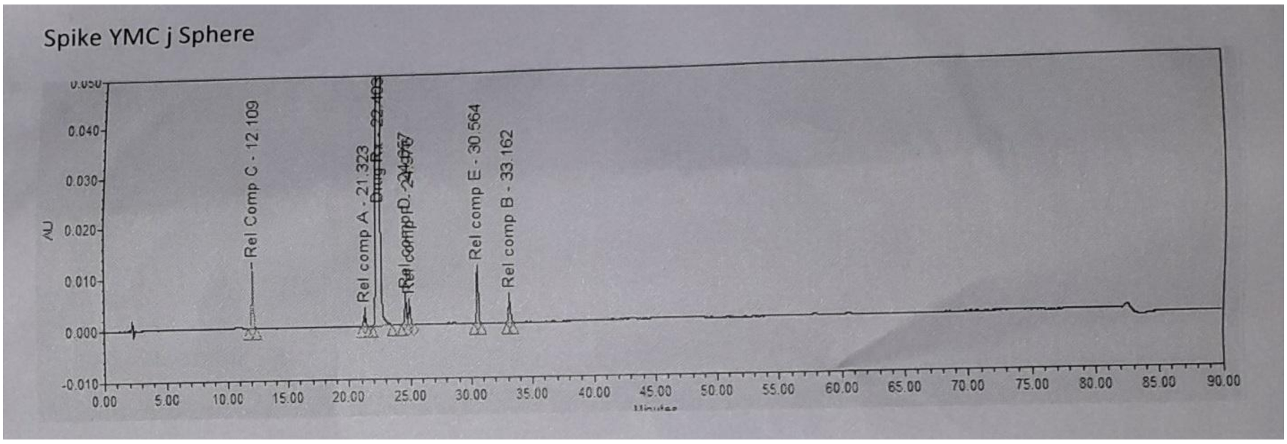
Graph 6: Column-Thermo Hypersil ODS



Graph 7: Column-Spike-Fortis C18



Graph 8: Column-Spike_Ace Excel C8 PFP



Graph 9: Column-Spike YMC j Sphere

Mobile phase preparation: 10mM Potassium dihydrogen phosphate buffer pH of 6.5 (mobile phase-A) & 50 volumes of acetonitrile and 20 volumes of methanol was used as mobile phase-B. Finally filtered through 0.45µm PVDF membrane filter and degassed it.

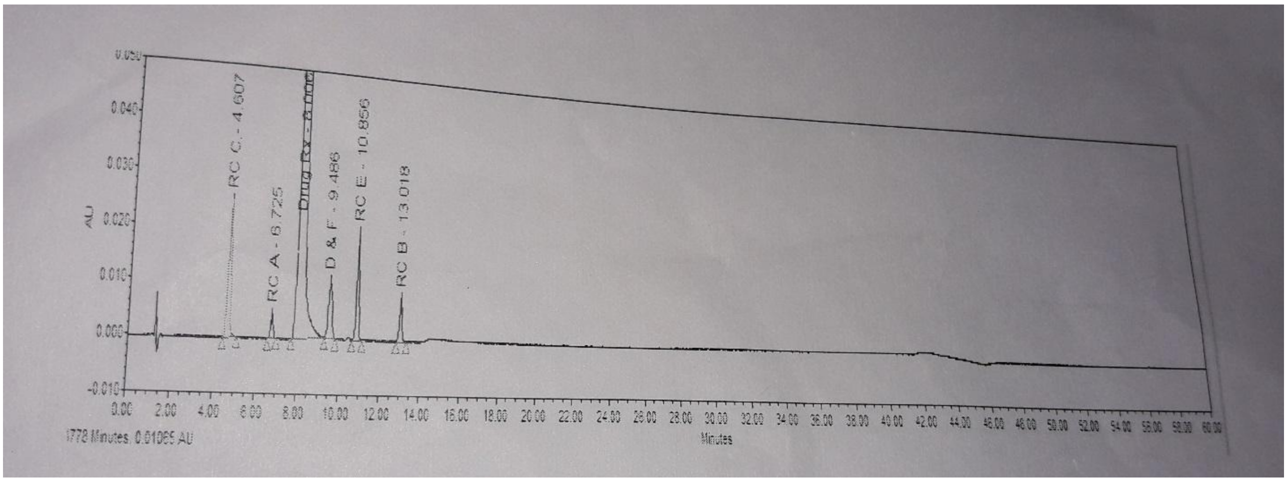
Diluent preparation: 0.001N Sodium Hydroxide with ACN (50:50) was used for diluent.

Standard solution preparation: 5.628 mg of "Pantoprazole" were precisely weighed and placed into a 100 mL volumetric flask. Sonicated for two minutes after adding 100mL of diluent. 5 mL of the solution were then added to a 100 mL volumetric flask, diluted to volume with diluent, and stirred. 2.8 µg/mL was the fin al concentration.

Sensitivity solution: 1mL of the standard solution was then added to a 10 mL volumetric flask, diluted to volume with diluent, and mixed. 0.28 µg/mL was the final concentration.

Sample/test solution: 1 vial of 'Pantoprazole' was transferred into a 10 mL volumetric flask. Add 10mL of diluent and sonicate for 2 minutes. Further, 1mL of the solution was added to a 10 mL volumetric flask and diluted to volume with diluent before being combined. The ultimate concentrations were 400 µg/mL

Chromatographic Conditions:
Equipment : Liquid Chromatography equipped with UV detector
Column : Thermo Hypersil ODS (125 x 4.0) mm, 5µm
Column temperature : 40°C
Flow rate : 1.0 mL/minute
Injection volume : 20µl
Run time : 30 minutes
Detection Wavelength : 290 nm for impurity C at 305 nm



Graph 6: Column-Thermo Hypersil ODS

Graph 6 depicted the totally separated peak of Pantoprazole and the highly resolved related molecule D&F under optimum chromatographic conditions. Pantoprazole, impurity A, impurity B, impurity C, impurity D & F, and impurity E had retention times of 8.00, 6.725, 13.018, 4.607, 9.486 and 10.855 minutes, respectively [7,8].

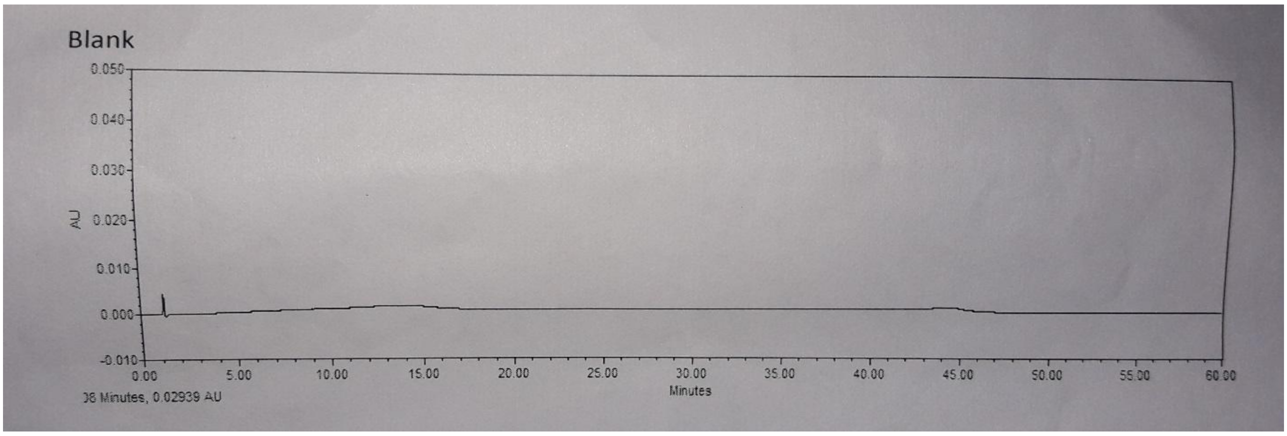
Validation parameters results:

System suitability: According to the USP, system appropriateness tests are an essential component of chromatographic procedures. In the case of HPLC, system appropriateness is determined by assessing relevant characteristics such as plate number, resolution, asymmetry, and capacity factor.

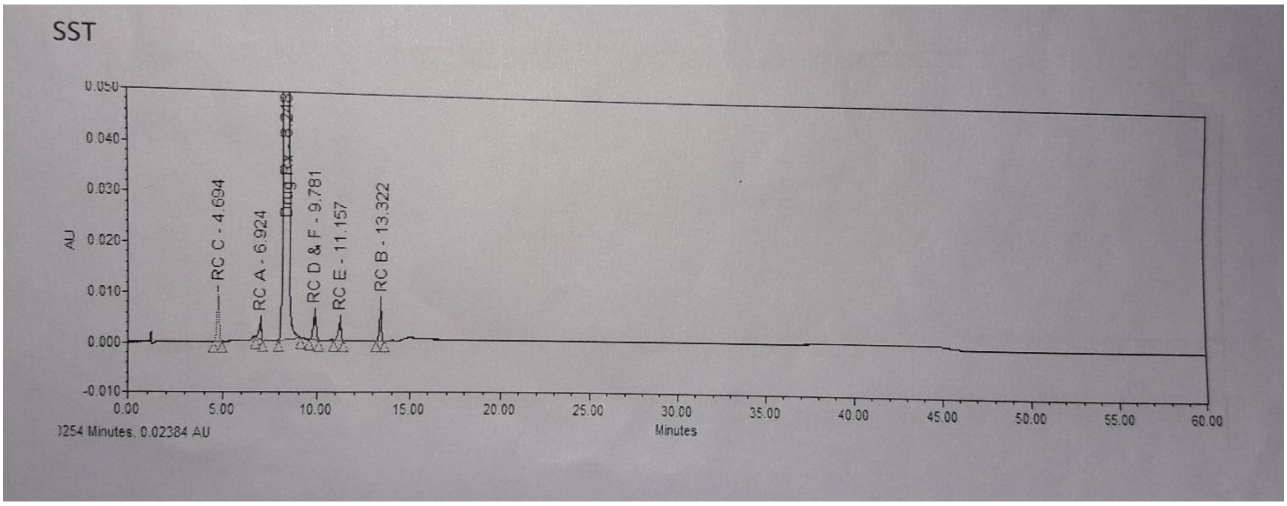
Resolution is a set of parameters that describe the separation capacity of the entire chromatographic system in relation to the mixture's individual components. The relative standard deviation indicates how precise the average of outcomes is. The resolution between Pantoprazole and Impurity D & F and the percentage RSD of area counts in standard solution were found to be 7.14 and 0.5, respectively, which met the acceptance standards indicated in table 3 [9]

Table 3: System suitability study data

System suitability parameters	Observed value	Acceptance Criteria
Resolution between Pantoprazole & Impurity D & F	7.14	NLT 1.5
% RSD of area counts in standard solution (n=6)	0.5	NMT 5.0



Graph 10: Chromatogram of Blank



Graph 11: Chromatogram of standard solution for System suitability

System precision: System precision was calculated by injecting the standard solution six times and % RSD was calculated. The % RSD was found to be less than 5.0 % indicating the precision of the system. The data is represented in table 4 [10].

Table 4: System precision study data

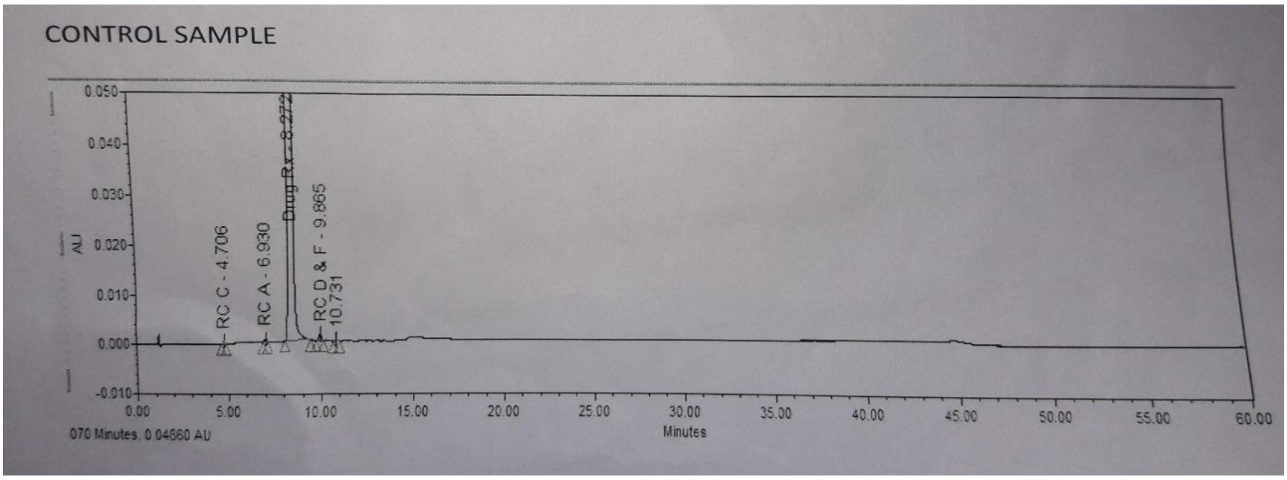
Standard Area counts at 290 nm	
Injection	**Pantoprazole**
1	107207
2	106375
3	106174
4	106521
5	106642
6	107403
Mean	**106720**
SD	**483**
RSD	**0.5**

Method precision: Method accuracy indicates the reproducibility of the method. It was determined by different preparations of test solution and quantify impurities against standard solution.

Control sample: Two impurities were not detected in control sample therefore method precision demonstrated by spike sample. Results are shown in table 5.[11]

Table 5: Results of control sample

Main peak RT (min)	**8.26**									
Degradants	**RT**	**RRT**	**RF**	**Area counts**			**% w/w**			**% RSD**
Impurity C	4.91	0.57	0.30	3770	3638	3589	0.007	0.006	0.006	2.6
Impurity A	6.91	0.84	1.00	4245	4356	4448	0.025	0.025	0.026	2.3
Impurity D & F	9.85	1.19	1.00	13659	13879	13845	0.080	0.081	0.081	0.9
Impurity E	ND	ND	1.00	0	0	0	0.000	0.000	0.000	NA
Impurity B	ND	ND	1.00	0	0	0	0.000	0.000	0.000	NA
Unspecfied degradant_1	4.26	0.52	1.00	2207	1936	2213	0.013	0.011	0.13	7.5

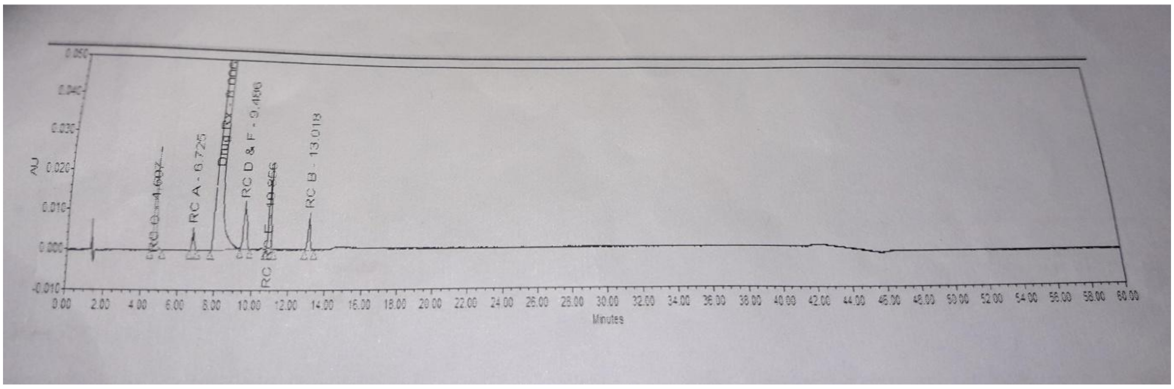


Graph 13: Chromatogram of Control sample

Spiked sample: It was prepared by adding known quantity of the analytes. %RSD of total impurities and other known & unknown impurities found within acceptance criteria i.e. %RSD should be less than 10.0% that suggests method was reproducible under the developed chromatographic conditions. Results are shown in table 6.

Table 6: Results of spiked sample

Main peak RT (min)	**8.26**									
Degradants	**RT**	**RRT**	**RF**	**Area counts**			**% w/w**			**% RSD**
Impurity C	4.70	0.57	0.30	131082	132497	131276	0.230	0.223	0.231	0.6
Impurity A	6.91	0.84	1.00	68933	69007	68644	0.404	0.404	0.404	0.3
Impurity D & F	9.85	1.19	1.00	261843	263634	260551	1.533	0.534	1.525	0.6
Impurity E	11.18	1.35	1.00	34190	34053	33828	0.200	0.199	0.198	0.5
Impurity B	13.35	1.62	1.00	55397	55606	54906	0.324	0.325	0.321	0.5



Graph 14: Chromatogram of Spiked sample

Linearity & Range: In order to establish a linear relationship between the detector response peak area of each individual standard and the amount or concentration of analyte present in the sample, a set of calibration standards was established.

Pantoprazole: The drug shows linear response in the range of 0.16-7.80ug/mL at selected wavelength (shown in table 7 and figure 1). Correlation coefficient was found to be 0.99994. According to ICH Q2 (R1), Correlation coefficient should be equal to one, but, usually, values higher than 0.990 are considered significant.

Table 7: Calibration curve data of Pantoprazole

Linearity level	**Pantoprazole (290nm)**	
	ug/mL	**Area count (µV*sec)**
Linearity-1	0.16	5553
Linearity-2	0.21	6838
Linearity-3	0.32	10685
Linearity-4	0.39	13110
Linearity-5	0.78	27545
Linearity-6	1.56	49597
Linearity-7	3.90	123563
Linearity-8	7.80	249281
	Slope	**31809**
	Intercept	**653**
	CC	**0.99994**
	RF	**1.00**

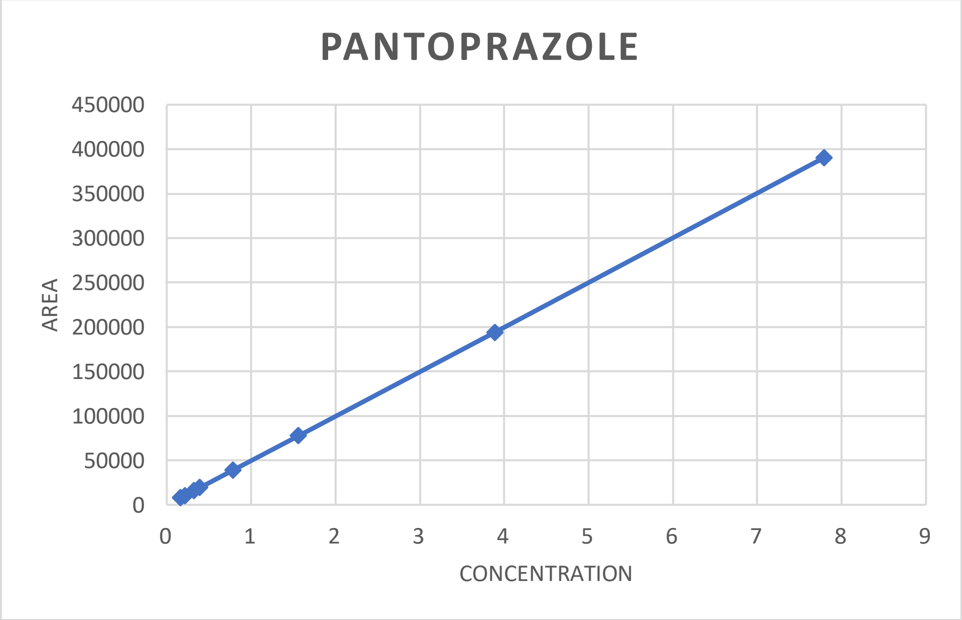


Figure 1: Calibration curve of Pantoprazole

Impurity A: The impurity A shows linear response in the range of 0.14-6.83ug/mL at selected wavelength (shown in table 8 and figure 2). Correlation coefficient was found to be 0.99999. According to ICH Q2 (R1), correlation coefficient should be equal to one, but, usually, values higher than 0.990 are considered significant.

Table 8: Calibration curve data of Impurity A

Linearity level	**Impurity A(290nm)**	
	ug/mL	**Area count (µV*sec)**
Linearity-1	0.14	5378
Linearity-2	0.18	6966
Linearity-3	0.28	10823
Linearity-4	0.34	13459
Linearity-5	0.68	27158
Linearity-6	1.37	53714

Linearity-7	3.42	135203
Linearity-8	6.83	272159
	Slope	**39834**
	Intercept	**-318**
	CC	**0.99999**
	RF	**0.80**

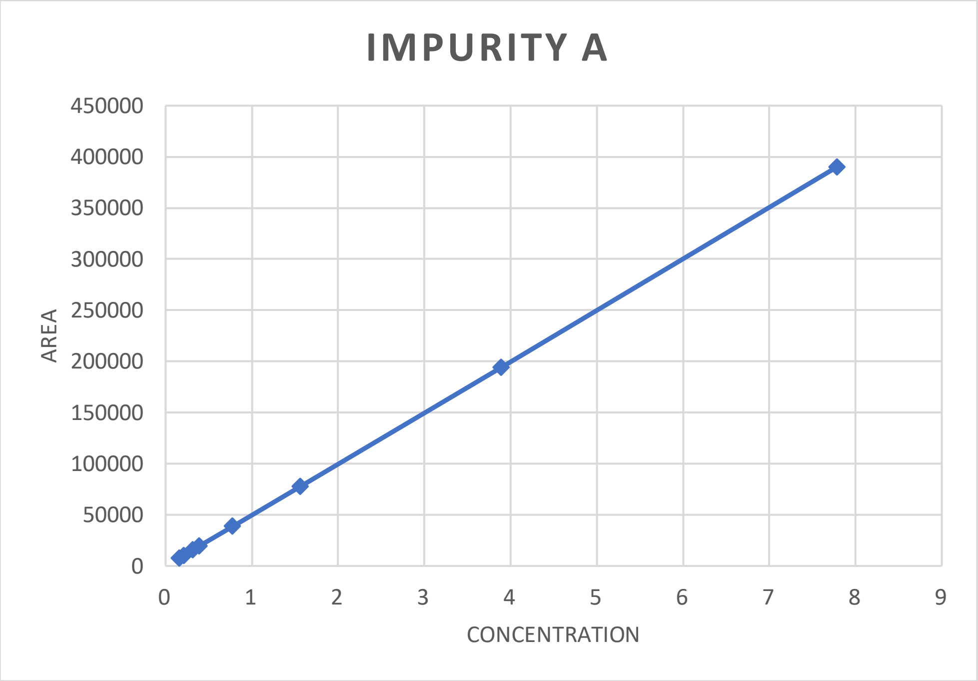


Figure 2: Calibration curve of Impurity A

Impurity B: The impurity B shows linear response in the range of 0.16-7.79ug/mL at selected wavelength (shown in table 9 and figure 3). Correlation coefficient was found to be 1.00000. According to ICH Q2 (R1), correlation coefficient should be equal to one, but, usually, values higher than 0.990 are considered significant.

Table 9: Calibration curve data of Impurity B

Linearity level	**Impurity B(290nm)**	
	ug/Ml	**Area count (µV*sec)**
Linearity-1	0.16	7629
Linearity-2	0.21	10258
Linearity-3	0.32	15907
Linearity-4	0.39	19457
Linearity-5	0.78	38789
Linearity-6	1.56	77548
Linearity-7	3.89	193981
Linearity-8	7.79	390334
	Slope	**50109**
	Intercept	**-343**
	CC	**1.00000**
	RF	**0.63**

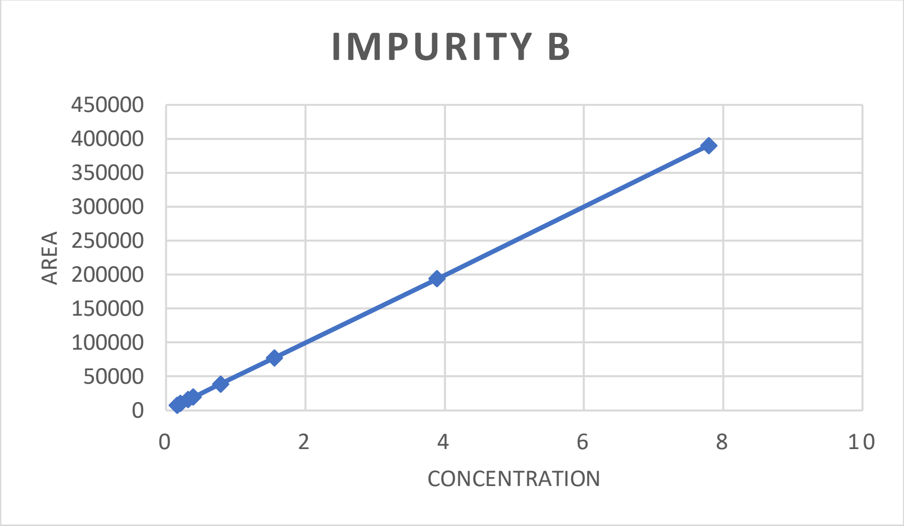


Figure 3: Calibration curve of Impurity B

Impurity C: The impurity C shows linear response in the range of 0.16-7.84ug/mL at selected wavelength (shown in table 10 and figure 4). Correlation coefficient was found to be 0.99997. According to ICH Q2 (R1), correlation coefficient should be equal to one, but, usually, values higher than 0.990 are considered significant.

Table 10: Calibration curve data of Impurity C

Linearity level	**Impurity C (305 nm)**	
	ug/mL	**Area count (µV*sec)**
Linearity-1	0.16	16416
Linearity-2	0.21	23063
Linearity-3	0.33	36334
Linearity-4	0.39	44881
Linearity-5	0.78	91548
Linearity-6	1.57	186907
Linearity-7	3.92	482892
Linearity-8	7.84	979341
	Slope	**125324**
	Intercept	**-5444**
	CC	**0.99997**
	Calculated RF	**0.25**

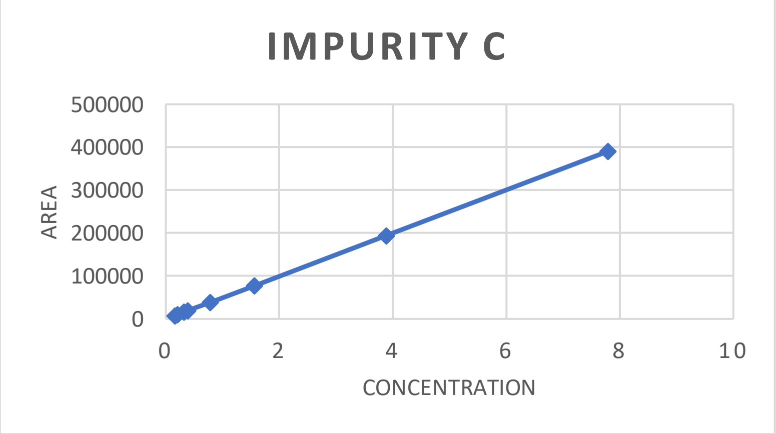


Figure 4: Calibration curve of Impurity C

Impurity D & F: The impurity D & F shows linear response in the range of 0.16-8.22ug/mL at selected wavelength (shown in table 11 and figure 5). Correlation coefficient was found to be 1.00000. According to ICH Q2 (R1), correlation coefficient should be equal to one, but, usually, values higher than 0.990 are considered significant.

Table 11: Calibration curve data of Impurity D & F

Linearity level	Impurity D & F(290nm)	
	ug/mL	**Area count (µV*sec)**
Linearity-1	0.16	5996
Linearity-2	0.22	7944
Linearity-3	0.34	12348
Linearity-4	0.41	15136
Linearity-5	0.82	30402
Linearity-6	1.64	60960
Linearity-7	4.11	152079
Linearity-8	8.22	305699
	Slope	**37181**
	Intercept	**-169**
	CC	**1.00000**
	RF	**0.86**

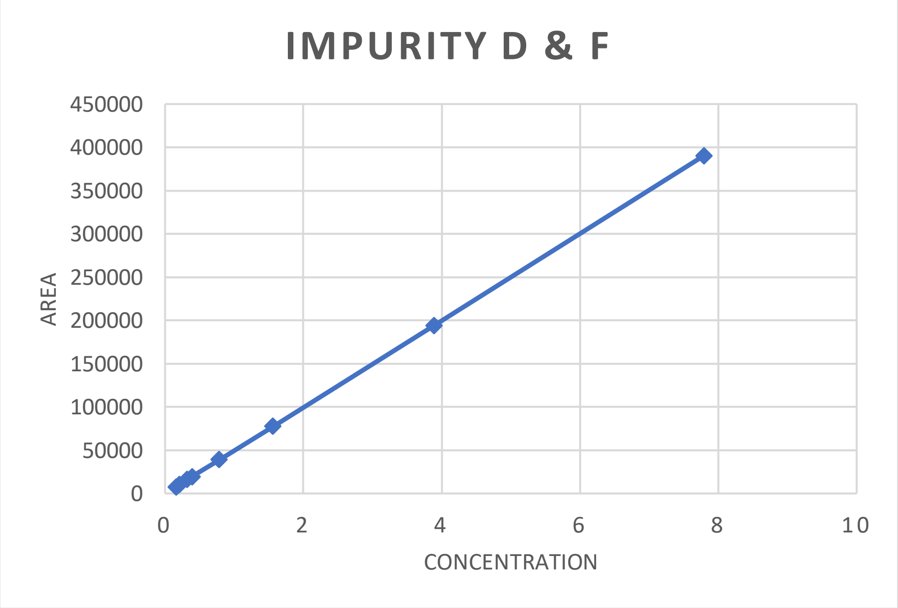


Figure 5: Calibration curve of Impurity D & F

Impurity E: The impurity E shows linear response in the range of 0.15-7.47ug/mL at selected wavelength (shown in table 12 and figure 6). Correlation coefficient was found to be 0.99999. According to ICHQ2 (R1), correlation coefficient should be equal to one, but, usually, values higher than 0.990 are considered significant. [12]

Table 12: Calibration curve data of Impurity E

Linearity level	Impurity E(290nm)	
	ug/mL	**Area count (µV*sec)**
Linearity-1	0.15	4163
Linearity-2	0.20	5610
Linearity-3	0.31	8505
Linearity-4	0.37	10450
Linearity-5	0.75	20994
Linearity-6	1.49	42645
Linearity-7	3.74	106559
Linearity-8	7.47	215378
	Slope	**28828**
	Intercept	**-391**
	CC	**0.99999**
	RF	**1.10**

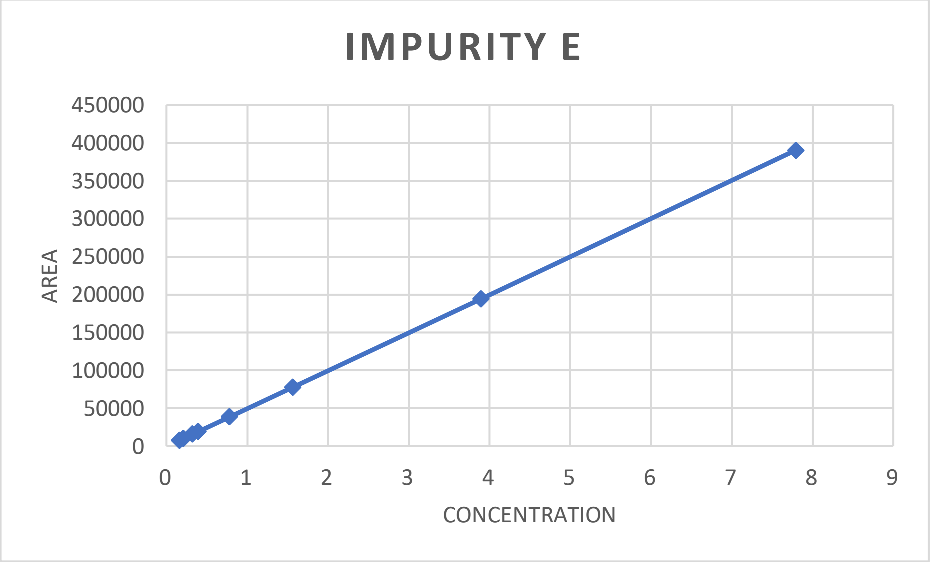


Figure 6: Calibration curve of Impurity E

6.2.4Accuracy:

Analytical recovery tests were conducted utilizing impurity preparations to assess the accuracy of the suggested procedures. The percentage recovery of contaminants by spiking them to the drug material was used to measure accuracy. Three replicate samples at three concentration levels were made in order to conduct accuracy studies. Data on recovery rate and mean recovery rate were obtained. ICH Q2 (R1) states that if the mean percentage recovery falls between 80 and 120 percent, accuracy is considered satisfactory and acceptable. Every result was determined to meet the acceptance criteria (table 13).

Table 13: Determination data of accuracy

Component Name	% Recovery			Average Recovery
	Sample-1	Sample-2	Sample-3	(%)
Impurity C	91.4	92.4	91.5	91.5
Impurity A	92.3	92.4	91.9	92.2
Impurity D & F	88.9	89.6	88.5	89.0
Impurity E	88.0	87.7	87.1	87.6
Impurity B	110.6	111.8	111.8	111.4

Peak purity analysis proved that the procedure was specific. With purity angle values below the purity criterion, the results showed that the Pantoprazole peak was spectrally pure. It was determined that the suggested analysis was interference-free with an acceptable peak purity. Neither the peak of pantoprazole nor the impurities co-elute with one another. There was no interference from the blank solution.

The control sample, spiked sample, and individual impurity solution all had retention times that were identical. The capacity of the method to clearly evaluate the analyte in the presence of additional components that may be expected to be present, such as contaminants, degradation products, and matrix components, indicates that the method was specific according to the ICH criteria for specificity. Specificity results were tabulated in table 15-20

Table 14: Specificity data of control sample

Component Name	RRT as per USP	Individual Identification Solution	Control Sample			
		RT (min)	RT (min)	RRT	Purity Angle	Purity Threshold
Impurity C	0.60	4.671	NA	NA	NA	NA
Impurity A	0.90	6.914	6.93	0.84	4.243	6.897
Pantoprazole	1.00	NA	8.270	1.00	1.257	10.444
Impurity D & F	1.20	9.864	9.864	1.19	4.598	2.418
Impurity E	1.30	11.126	NA	NA	NA	NA
Impurity B	1.50	13.294	NA	NA	NA	NA

Table 15: Specificity data of spiked sample

Component Name	RRT as per USP	Individual Identification Solution	Spiked Sample			
		RT (min)	RT (min)	RRT	Purity Angle	Purity Threshold
Impurity C	0.60	4.671	4.699	0.57	0.195	0.392
Impurity A	0.90	6.914	6.930	0.84	0.35	0.624
Pantoprazole	1.00	NA	8.284	1.00	0.903	10.989
Impurity D & F	1.20	9.864	9.808	1.18	3.767	0.316
Impurity E	1.30	11.126	11.183	1.35	0.334	0.663
Impurity B	1.50	13.294	13.350	1.61	0.335	0.688

Table 16: Specificity data of sample spiked with Impurity D

Component Name	RRT as per USP	Individual Identification Solution	Sample Spiked with Impurity D			
		RT (min)	RT (min)	RRT	Purity Angle	Purity Threshold
Impurity C	0.60	4.671	NA			
Impurity A	0.90	6.914	NA			
Pantoprazole	1.00	NA	NA			
Impurity D & F	1.20	9.864	9.940	1.20	0.479	0.769
Impurity E	1.30	11.126	NA			
Impurity B	1.50	13.294	NA			

Table 17: Specificity data of sample spiked with Impurity F

Component Name	RRT as per USP	Individual Identification Solution	Sample Spiked with Impurity F			
		RT (min)	RT (min)	RRT	Purity Angle	Purity Threshold
Impurity C	0.60	4.671	NA			
Impurity A	0.90	6.914	NA			
Pantoprazole	1.00	NA	NA			
Impurity D & F	1.20	9.864	9.819	1.18	0.871	1.145
Impurity E	1.30	11.126	NA			

Impurity B	1.50	13.294	NA			

Table 18: Specificity data of Pantoprazole standard solution

Component Name	**RRT as per USP**	**Individual Identification Solution**	**Pantoprazole Standard Solution**			
		RT (min)	**RT (min)**	**RRT**	**Purity Angle**	**Purity Threshold**
Impurity C	0.60	4.671	NA			
Impurity A	0.90	6.914	NA			
Pantoprazole	1.00	NA	8.308	1.000	0.176	0.445
Impurity D & F	1.20	9.864	NA			
Impurity E	1.30	11.126	NA			
Impurity B	1.50	13.294	NA			

Table 19: Final results of specificity

Component Name	**Remarks**
Impurity C	Peaks specific & peak purity passed.
Impurity A	Peaks specific & peak purity passed.
Pantoprazole	Peaks specific & peak purity passed.
Impurity D & F	Impurity D & F peak purity failed in sample and impurity spiked sample solutions. This is due to their known co-elutions. Their peak purity was demonstrated by spiking them separately in sample solution at specification levels. Peak purity of both D&F passed in sample with individual spiking.
Impurity E	Peaks specific & peak purity passed.
Impurity B	Peaks specific & peak purity passed.

6.2.6Limit of Detection and Limit of Quantitation: The lowest analyte concentrations that can be determined using analytical techniques are LOD and LOQ. The following formulas were used to get the LOD and LOQ:

$LOQ = 10\sigma/S$ and $LOD = 3.3\ \sigma/S$

where S is the calibration curve's slope and σ is the standard deviation of the lowest standard concentration. Table 21 presents the findings. [13]

Table 20: LOD & LOQ of quantification of Pantoprazole, Impurity A and Impurity B, Impurity C, Impurity D & F and Impurity E

Drug & impurities	**LOD (ug/mL)**	**LOQ (ug/mL)**
Pantoprazole	0.104	0.156
Impurity A	0.037	0.091
Impurity B	0.104	0.200
Impurity C	0.105	0.157
Impurity D & F	0.110	0.164
Impurity E	0.100	0.132

DISCUSSION AND CONCLUSION

The HPLC approach, which was designed for the purpose of determining related chemicals in pantoprazole, was robust, fast, exact, sensitive, and specific. Pantoprazole, impurities A, B, C, D&F, and E, as well as possible associated compounds, can all be quantified using this method. Chromatographic separation was accomplished using an HPLC with a UV detector and a Thermo Hypersil ODS column (125 x 4.0) mm and 5µm. The gradient eluted after 30.0 minutes of runtime. The column oven temperature was kept at 40°C, the eluted chemicals were measured at 290 nm, and the flow rate was 1.0 mL/min. The percentage RSD of area counts in standard solution and the resolution between Pantoprazole and Impurity D & F were found to be 0.5 and 7.14, respectively, falling within the acceptable range. Clear correlations between the examined compound concentrations and their peak areas within the test ranges were demonstrated by the high correlation coefficient (r2 > 0.999) values. When recovery studies were conducted using the spike approach, the accuracy was found to be between 80.0% and 120.0%.

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