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FABRICATION AND DEVELOPMENT OF PANTOPRAZOLE LYOPHILIZED INJECTION FORMULATION BY USING FREEZE DRYING TECHNIQUES AND ITS ORGANIC IMPURITIES EVOLUTION AND METHOD OPTIMIZED BY UPLC

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ABSTRACT

A Novel formulation Pantoprazole lyophilized injection vial developed with critical quality attributes using Edetate disodium dehydrate (chelating agent) and Sodium hydroxide (pH adjusting agent-10.5-12) by using this formulation For the determination of known potential impurities present in Pantoprazole lyophilize, a simple, rapid Reverse phase – Ultra Performance liquid chromatography (RP-UPLC) method was developed and validated. injection finished drug product. Chromatographic separation attains using Buffer Preparation: Dissolved 1.32 g of Di Basic Ammonium Phosphate in to 1000 mL of Milli-Q water, Adjusted to pH 7.50 with Ortho Phosphoric acid and mixed well. Filter through 0.22 µ PVDF filter. Solvent Mixture Preparation: (Acetonitrile: Methanol) (70:30), Mobile Phase Preparation A: (Buffer: Solvent mixture) (85:15), Mobile Phase Preparation B: Solvent Mixture respectively. The components were efficiently separated in Waters Acquity BEH C18 50 mm x 2.1 mm 1.7µ particle size column. Flow gradient elution mode with initial flow rate of 0.4 mL.min-1 was used. The impurities were quantified at a working wavelength of 290 nm. Specificity, linearity, precision, ruggedness, accuracy, sensitivity (Limit of Detection & Limit of Quantitation), and robustness were validated in accordance with International Conference on Harmonization (ICH) standards. The present stability indicating method has a shorter run time, which is helpful for fast analysis of samples during quality control testing with reduced solvent consumption in a cost and time effective approach.

INTRODUCTION

Now a day's most of the peoples are suffering from the gastric problems. To overcome these issues so much of scientific work is being carried out and also couple of research articles are published. Recently acid reducing agents (ARAs) were/are Recommended to treat the gastrointestinal problem. The ARAs are divided into three classes in market i.e., antacid, histamine H2 receptor antagonists (H2RAs) and proton pump inhibitors (PPIs). Several review articles discuss these three classes medicated by co-administration [1-5].These review

articles have not documented the drug-drug interactions (DDIs) with the ARAs sufficiently and are inclined to target on the pH dependent DDIs and basic drugs. Accordingly, few new drugs have been approved and information is also available in the literature. There are any different formulations of oral dosage forms available in the market [6]. Common PPIs formulations such as omeprazole, esomeprazole, lansoprazole, dexlansoprazole, pantoprazole and rabeprazole are available in market without prescription [2, -5, 8-10]. The efficacy and acid suppression, comparison studies of the above formulations are reported long ago [11]. The main differences associated with pantoprazole are its irreversible and specific proton pump binding leading to greater bioavailability (1-1.9 hrs), % Protein binding capacity (98%), Time to peak plasma level (t_{max}: 2-3 hrs) [12] and its longer duration of activity than other PPIs. In addition, pantoprazole is more stable than remaining PPIs in neutral to moderately acidic conditions and hence become less susceptible to get activated in slightly acidic human body compartments. So far a few clinically significant drug-drug interactions are reported with the pantoprazole [13-18]. In multiple *in-vivo* drug interaction studies, it is observed that the pharmacokinetics of pantoprazole and those of the co-administered agents has not extensively affected [12]. Full course (at most 40 days) of Pantoprazole is used to treat common gastrointestinal problems such as blotting of stomach by general medical practitioners. Oral administration of Pantoprazole during perioperative stage or chemotherapy may cause nausea, vomiting, or severe diarrhea for the patient and hence its bio availability is difficult. To overcome these issues intravenous formulation is used for the patient Gastroesophageal reflux disease(GRED) [19-22].It is unclear whether intravenous PPIs is more helpful than oral administration in preventing gastrointestinal (GI) bleeding in high bleeding risk patients with acute coronary syndromes (ACS).In the current market, Pantoprazole sodium finished product is commercially available with the trade name of PROTONIX IV Lyophilized powder from Wyeth Pharmaceuticals Inc, its available as 40mg/vial and Maximum daily dose is 240mg. Pantoprazole sodium active pharmaceutical ingredient is official in USP 40 and finished product was not yet official in any pharmacopeia. At present numerous pharmaceutical companies have developed formulations as well as so many analytical methods were published. In developed formulations we have observed the increment of organic impurities both at the stability conditions as well as at stress conditions [23]. Numerous methods are developed for the determination of pantoprazole in various instruments such as Specrophotometric, Chromgraphic, Thermogravametric , voltametry, human plasma [36-37] and However, there are no research papers are published on UPLC analytical methods are available in organic impurities present in Pantoprazole Sodium finished product. However, the organic impurities analytical method by HPLC with longer runtime, The developed UPLC analytical method by monitor the all impurities in very shorter run time, in view of the fact that UPLC offer the improved selectivity , sensitivity due to less chemicals and reagents consumption. Because of this reason the UPLC elected for the determination of impurities present in the Pantoprazole. The structure of impurities was given below. Fig:1. At Present pharmaceutical field require shorter run time analytical methods to deliver the projects within the timelines without criticalities. Hence it was anticipated to develop the organic impurities analytical methods by UPLC to meets the target. Forced degradation studies were performed to check the ability of the method by producing degradation profile similarly to observe the formal stability of the finished product under ICH stability conditions. These studies helpful to found the degradation pathways of the drug substance and drug product, similarly to resolve the stability related issues of the degradation products that are formed from the drug product and its placebo matrix.

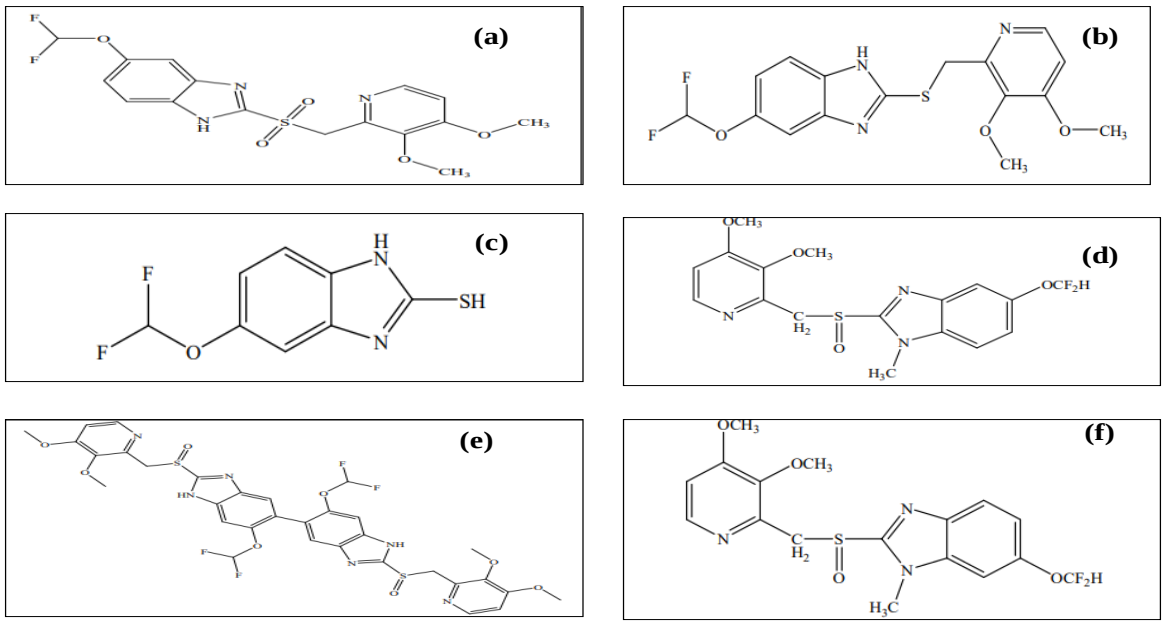


Fig :1 (a) 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl) methyl]sulfonyl]-1H-benzimidazole(Related Compound-A)**, (b)** 5-(Difluoromethoxy)-2-[[(3,4- dimethoxy-2-pyridyl)methyl] thio]-1H-benzimidazole(Related Compound-B)**, (c)** 5-(Difluoromethoxy)-1H-benzimidazole-2-thiol (Related Compound-C)**,(d)** 5-(Difluoromethoxy)-2-[(RS)- [(3,4-dimethoxy pyridin-2-yl) methyl]sulfinyl]-1-methyl-1H-Benzimidazole (Related Compound-D)**,(e)** Mixture of sterio isomers of 6,6'- bis (difluoromethoxy) -2,2'- bis[[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1H,1'H-5,5'-bibenzimidazolyl(Related Compound-E)**,(f)** 6-(Difluoromethoxy)-2-[(RS)- (3,4-dimethoxy pyridin-2-yl) methyl]sulfinyl]-1-methyl-1Hbenzimidazole (Related Compound-F)

The developed method was validated as per the USP and ICH requirements [38-44]. This paper describes the first time development of novel formulation and evaluation of organic impurities by UPLC.

MATERIALS AND METHODS

Materials

Acetonitrile, Di-Ammonium Hydrogen Phosphate ,Ortho Phosphoric Acid , Ammonia Solution, Methanol, Purified water, Edetate disodium dehydrate, Sodium hydroxide,All other analytical grade chemicals were purchased from S.D Fine chemicals Mumbai, India. Panoprazole Sodium USP Active Pharmaceutical regent and its organic impurities gift samples.

Optimization of formulation development and Lyophilisation cycle:

The formulation development of pantoprazole sodium Lyophilized vial for Injection and a preparation method thereof [45]. The Lyophilized powder injection is prepared from require quantity pantoprazole sodium API and Edetate disodium dehydrate is dissolved into water clear and bright solution; Replenish water for injection to full dose, mix homogeneously with glass rod and then adjusted the pH value is in between 10.5-12.0 by using sodium hydroxidesolution. The Pantoprazole Sodium bulk solution was preliminary filtered through 0.22µm EDF filter (PALL life sciences Pvt Ltd) into 20 CC vials and closed with half sealed stoppers under aseptic conditions. The Pantoprazole Sodium injection solution was subjected for lyophilization using lyophilizer (Lyodel model: LYO1550). The lyophilization cycles comprise three steps i.e freezing, primary drying (sublimation to remove unbound water) and secondary drying (desorption to

remove the bond water). During drying period, the visually inspected and selected products were dried by frozen technique at -40°C. The primary drying cycle was taken for 3 hrs whereas the secondary drying taken for 7 hrs. All through drying period, the vacuum was maintaining constantly at 50 m Torr whereas the temperature and required time was simultaneously changed respectively. After lyophilization process, sealed caps of the vials, stored at 2-8°C, protected from light [Ref].

Risk assessment of formulation variables:

The main objective of this study to check the impact on CQA of finished drug product. We have to check the following parameters.

Effect of order of addition on product quality attributes

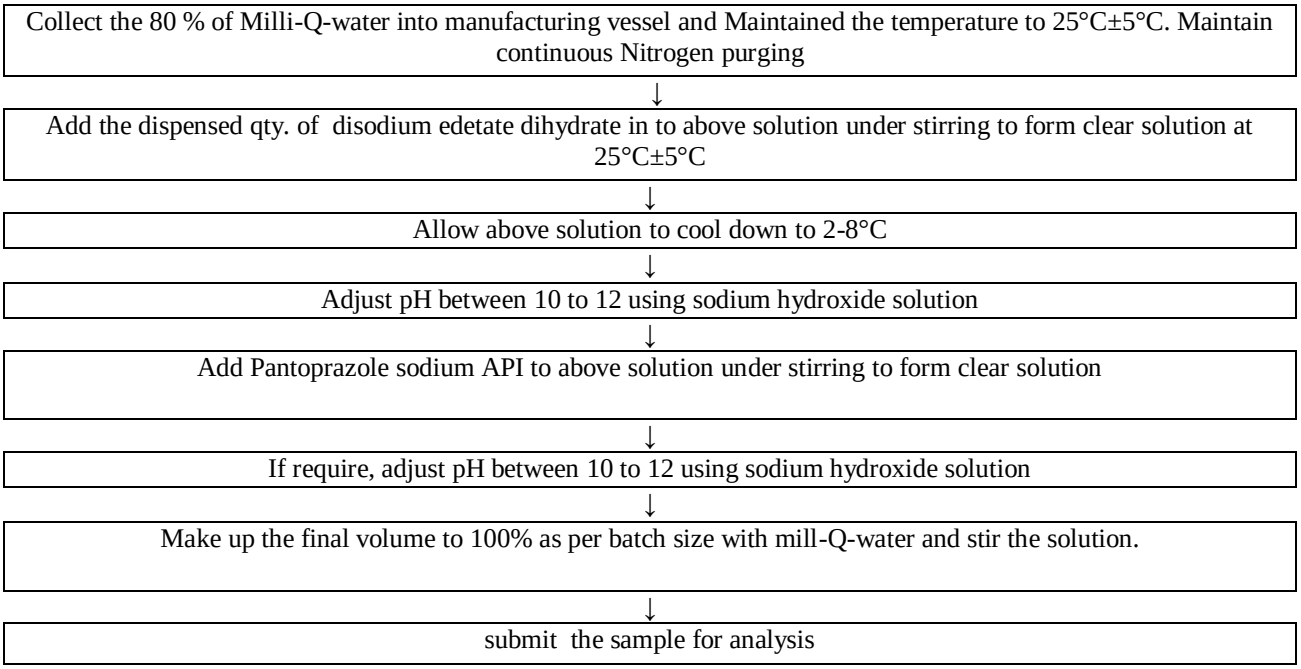
Taken Bulk solution of Pantoprazole sodium for Injection at concentration of 20 mg/mL was manufactured in two different order of addition. Manufacturing process flow is mentioned in figure no. 1 and 2 **Effect of manufacturing temperature on Product Stability**

Taken Bulk solution of Pantoprazole sodium for Injection was prepared at 20 mg/mL concentration at two different temperatures i.e. 25°C ±5°C & 5°C±3°C. The data was reported in below table 5 and 6.

Table: 1 Initial risk assessment of the formulation variables

Drug Product CQAs	Formulation variables			
	Order of addition	Manufacturing temperature	Light intensity during manufacturing	Effect of Dissolved Oxygen
Description	Low	Medium	Medium	Medium
Assay of Pantoprazole	High	High	High	Medium
Related substance	High	High	High	Medium
pH	Low	Medium	Low	Low

Process-I



Process-II

Collect the 80 % of Milli-Q-water into manufacturing vessel and Maintained the temperature to 25°C±5°C. Maintain continuous Nitrogen purging

↓

Add the dispensed qty. of disodium edetate dihydrate in to above solution under stirring to form clear solution at 25°C±5°C

↓

Allow above solution to cool down to 2-8°C

↓

Add Pantoprazole sodium API to above solution under stirring to form clear solution

↓

Adjust pH between 10 to 12 using sodium hydroxide solution

↓

Make up the final volume to 100% as per batch size with mill-Q-water and stir the solution.

↓

submit the sample for analysis

Effect of intensity of light on Product quality attributes

Taken Bulk solution of Pantoprazole sodium for injection was prepared at 20 mg/ mL. Prepared bulk solution was exposed to different light conditions as mentioned in below table 5 and 6

Effect of dissolved oxygen on product quality attributes

Taken Bulk solution of Pantoprazole sodium for injection was prepared at concentration of 20 mg/mL with nitrogen purging (DO less than 1 ppm) and without nitrogen purging. Above samples were Samples were analysed for Initial and critical quality attributes like description, assay of Pantoprazole, related substances. Batch Results were reported in below Table.5 and 6.

PREPARATION OF STANDARD AND SAMPLE SOLUTIONS FOR ORGANIC IMPURITIES AND ASSAY

Preparation of Diluted standard solution:

Initial Standard stock solution of PAN (400 µg/mL)was prepared by dissolving in diluent. From stock solution was further diluted to obtain a concentration of (0.8 µg/mL). All impurities were prepared by initially soluble in required amount of acetonitrile, followed by using diluent at desired concentration levels for validation purpose.

For Lyophilized vial:

Taken 5 vials (40mg/vial) and remove flip off seals. Added about 10 mL diluent in each vial and dissolved the contents. Carefully transferred the liquid contents of each vial into a 100 mL of volumetric flask. Further wash and transferred each vial with sufficient amount of diluent into the same volumetric flask. Made up to the volume with diluent and mixed. Further diluted 5 mL to 25 mL with diluent and mixed well. Filtered through 0.22 µ PVDF filter. (400 µg /mL)

For Bulk solution: Transfer 4.0 mL of bulk solution (20mg/mL) into 200 mL volumetric flask. Added about 5-10mL mixture of Acetonitrile: Water (1:1) and made up to the mark with diluent and mixed well.

Placebo Preparation (For finish product): Taken 5 vials of placebo and remove flip off seals. Added about 10 mL diluent in each vial and dissolved the contents. Carefully transferred the liquid contents of each vial into a 100 mL of volumetric flask. Further wash and transferred each vial with sufficient amount of diluent into the same volumetric flask. Made up to the volume with diluent and mixed. Further diluted 5 mL to 25 mL with diluent and mixed well. Filter through 0.22 µ PVDF filter.

DETERMINATION OF % DRUG CONTENT:

For Lyophilized vial: From the organic impurities sample stock solution further diluted 3 mL to 100 mL with diluent and mixed well. Filter through 0.22 µ PVDF filter.

For Bulk solution: Transferred 5.0 mL of bulk solution (20mg/mL) into 200 mL volumetric flask. Added about 5-10 mL mixture of Acetonitrile: Water (1:1) and made up to the mark with diluent and mixed well. Further diluted 3 mL of this solution into 25 mL with diluent and mixed well.

A part from the above trails, we have chosen the best formulation i.e which formulation meets the acceptance criteria.

Comparison of developed formulation and RLD: In-house developed formulation and RLD formulation data was comprise for the intended usage.

The Comparison data was tabulated in section:7

INSTRUMENTATION

The experimental work was conceded out on Waters-Acquity UPLC system is consists of with high pressure binary gradient pump, column oven compartment, Photo diode array detector, Auto injector, Computer with Empower-3 software for data acquisition. The main drug components along with their impurities were separated using Waters Acquity BEH C18 50 mm x 2.1 mm 1.7µ column.

Summary of UPLC method Optimization for Organic impurities and Assay

Pantoprazole sodium for injection is not official in USP. Whereas, Pantoprazole sodium and Pantoprazole sodium DR tablets are official in USP. Pantoprazole sodium is a proton pump inhibitor

As per USP monograph of Pantoprazole sodium DR tablets, there were numerous challenges faced during development of stability indicating assay and related substance method for the Injection product. All the methods which are available (Pharmacopeia and non-pharmacopeia) till date having number of limitations such as reproducibility of related substances results, shifting of impurities peaks, co-eluting impurities peak and also long run time. Hence, to address all these issues a single UPLC method Developed, which is also cost effective and less time consuming

As per USP monograph of Pantoprazole sodium USP the HPLC column is 3.9-mm,15-cm column that contains 4-mm packing L1. Since the method transferred to UPLC, Waters Acquity BEH C18 (50 × 2.1) mm, 1.7µm has been selected.

The compendial USP method is by HPLC. The method for the Related substances of Pantoprazole in Pantoprazole sodium for injection was initiated on UPLC to reduce the run timeAfter performed different trails, the mobile phase and dilune was given as follows.

Buffer Preparation: Dissolved 1.32 g of Di Basic Ammonium Phosphate in to 1000 mL of Milli-Q water, Adjusted to pH 7.50 with Ortho Phosphoric acid and mixed well. Filtered through 0.22 µ PVDF filter.

SOLVENT MIXTURE PREPARATION*:* Filtered Acetonitrile and Methanol separately through 0.22 µ PVDF filter and mixed 70 volumes of Acetonitrile and 30 volumes of Methanol. *Mobile Phase A Preparation*: Taken 85 volumes of Buffer Preparation and 15 volumes of solvent mixture preparation, mixed them well and sonicated for 5 to 10 minutes. *Mobile Phase Preparation B*: Solvent Mixture Preparation. In the existing gradient programme was separate all the organic impurities were well separated from the main peak ,all impurities shown symmetric peak shape and resolution between each impurity is more than 1.5 and satisfactory. To ensure the column back pressure fix the column oven temperature is 40°C. While optimizing the diluents which one was given best main drug recovery on the API and sample i.e finalized for the sample extraction &impurity solubility. The preparation of diluent is transferred 25 mL of Ammonium hydroxide (Ammonia 30% in water) solution in to 500 mL volumetric flask and made up the volume with Milli-Q water and mixed well. Filtered through 0.22 µ PVDF filter.

The concentration was set at 400g/mL to improve impurity responses. Impurity solutions were prepared at 0.2%Level, in accordance with the ICH recommended limits for organic impurities in drug products. and API and impurities were injected to determine retention times and relative retention times.

Pantoprazole is having absorbance maxima at 200 nm and 290nm. As per the USP monograph of Pantoprazole sodium DR tablets and Pantoprazole sodium API, the wavelength used in Related substances method is 290 nm and 285 nm. Considering low solvent interference and majority of the absorbance of the impurities are at 290 nm, selected 290 nm.Spectral data from a Photo Diode Array detector (PDA) show that the majority of Pantoprazole sodium impurities have wavelength maxima around 290 nm (Figure 2). As a result, the same wavelength of 290 nm has been chosen for impurity quantification. 1.5 µL injection volume was chosen for sufficient area counts, and precise area counts for impurities and main drugs were discovered.

Table: 2 Gradient Program:

Flow rate mL/min	Time (Min)	% Mobile phase A	% Mobile phase B
0.4	0.01	86	14
0.4	1.70	86	14
0.4	6.10	42	58
0.4	6.30	86	14
0.4	8.00	86	14

Table: 3 Elution of Impurities

S.NO	Impurity Name	RT (Mins)	RRT
1	Related Compound A	2.040	0.63
2	Related Compound B	4.950	1.52
3	Related Compound C	1.173	0.36
4	Related Compound D	3.893	1.21
5	Related Compound E	4.697	1.54
6	Related Compound F	3.963	1.23
7	Pantoprazole	3.227	1.00

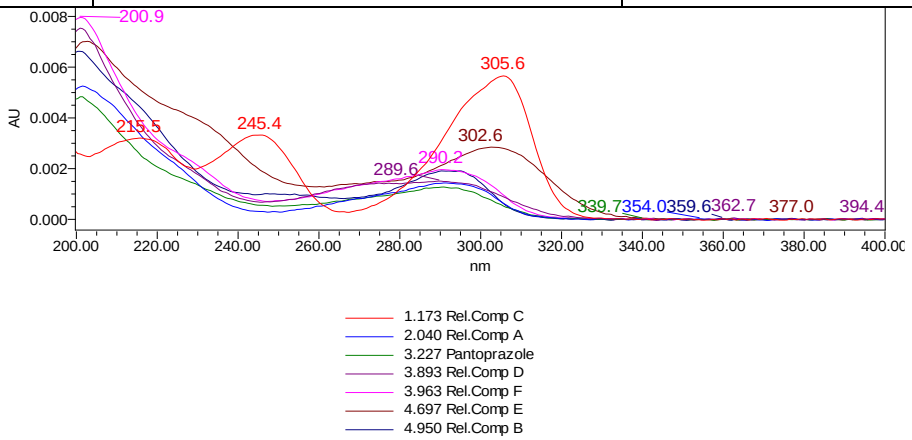


Fig 2: Spectra of pantoprazole and impurities

Table: 4 Chromatographic System suitability results

Component Name	Tailing factor	% RSD for six replicate injections
Pantoprazole	1.2	1.85

Table: 5 Data comparison of the critical quality attributes

method validation of finalized analytical

S. No.	Test	Without nitrogen purging	With nitrogen purging
1	Description	Complies	Complies
2	pH	11.10	11.07
3	Assay	102.2	99.8
4	Related substance		
	Related compound A (%)	0.000	0.088
	Related compound B (%)	0.011	0.006
	Related compound C (%)	0.004	0.003
	Related compound D&F (%)	0.0160	0.020
	Related compound E	0.000	0.025
	Any unspecified degradation products (%)	0.084 (RRT 0.63)	0.060 (RRT 1.13)
	Total degradation product (%)	0.244	0.295
5	Colour of solution (APHA units)	158.3	162.5

SYSTEM SUITABILITY FOR RELATED SUBSTANCES:

The system is suitable if,

i. From six replicate standard injections: % RSD for Pantoprazole peak in standard solution is not more than 5.0%.

ii. The tailing factor not more than 2.0 for Pantoprazole peak in standard solution.

Reporting of unknown impurities for quantification purpose

a) Known organic impurities of Pantoprazole will be reported using diluted standard solutions.

b) Other unknown organic impurities for which is not corresponding with the Placebo data shall be identified by spectra using Photo diode array detector and reported accordingly.

c) Any other unknown organic impurities which are unaccountable shall be reported using diluted standard.

Analytical method validation

Pantoprazole Sodium Lyophilized Injection 40mg / vial chosen for the method. Specificity, Forced degradation, Precision, Ruggedness, Sensitivity (Limit of detection and Limit of Quantification), Linearity, Range, Accuracy, solution stability, and Robustness were all validated in accordance with ICH parameters.

Specificity- The Specificity of organic impurities method was determined by using injecting the diluents, Standard, Placebo, individual impurities and spiked sample was prepared in the presence of finished product at the concentration of 400 µg/mL and its consequent degradation performed on drug product and drug substance. Pantoprazole sodium to study its impurity profile, degradation pathway and to facilitate the development of a stability-indicating method. In addition, knowledge obtained from the forced degradation studies was used during formulation and process design and development to prevent impurities from being generated. The following stress conditions were conducted Acid degradation (1 ml 5N HCl and heated at

80℃ for 60 mins), Base degradation (2 ml 5N NaoH were added and Heated at 80°C for 120 mins). Peroxide degradation (500 µL H2O2 and heated at 80℃ for 20 Min), Photo stability (Sun light exposure for 6 Hours), U.V light (24 hrs), Thermal Degradation (6 Hrs at 105°C).The Data was represented in below section.8 and 9.

Determination of Limit of Quantification and Limit of Detection

Limit of detection and quantitation calculated by using Residual standard deviation method. Limit of detection and quantitation performed by analyzing. A series of mixture of Organic impurities solutions were prepared from 1% to 150% with respect to impurity test concentration. The limit of detection and limit of quantitation results are summarized in Table-10 to 12. **Linearity, Relative response factor and Range:L**inearity curves were drawn from the finalized LOQ concentration to 150 percent of the impurity specification level. All impurities' correlation coefficients, slopes, and Y-intercepts were calculated. For RRF was calculated slope against organic impurities against and Pantoprazole sodium. Method precision, Linearity, and Accuracy as illustrated concentrations were used to determine the method's range. The resulted data was reported in Table 13 and 14.

Method precision

The precision of the method was determined by analyzing a series of samples spiked with known impurities. Data obtained is summarized in Table-15.The percentage of relative standard deviation was calculated for % of all impurities. As well as to determine the % RSD for at precision a LOD and LOQ. The resulted data was reported in Table 10 and 12.

Accuracy

A study for accuracy of Pantoprazole and its known impurities has been carried out by preparing three replicates at approximately 50 % and 150% of the specification level (0.2%). The percentage recovery results obtained for each specified impurity are summarised in Table-25 to Table-16.

Solution stability

Solution stability performed by analysing standard solution and spiked sample solution periodically in to HPLC system. Measure the stability of the standard andsample solution by keeping at 25°C and 5°C. To determine the percent difference in peak area between the standard and all known impurities, use the generated data. The resulted was reported in Table 17 and 18.

Robustness

Prepared standard, spiked sample preparation and perform robustness parameter by variation in chromatographic conditions like flow rate (±10.0%), column oven temperature (±5℃) and wavelength (±2nm), change in organic phase of mobile phase(methanol &acetonitrile) A and B (±5%) and buffer pH (±0.2 units). The data was reported in

RESULT AND DISCUSSION

Risk Assessment of critical quatlity attributes on formulation studies

As discussed in above section-2.3 following parameters Process-I and II , Temperature (25°C and 5°C), Light and Nitrogen purging results comparison given below Table 5and 6.

Analytical results of Order of addition–I and Order of addition–II, temperature, light intensity and nitrogen purging data was concluded below.

1. Analytical results of Order of addition–I and Order of addition–II there is no significant change in the results comparing with the initial batch processing results
2. At room temperature shows significant high level Any unspecified degradation product (%) as compare to batch manufactured at 5°C±3°C. All other quality parameters are within proposed specification.
3. The analytical results of light exposure study show there is considerable impact of light intensity on Assay, Impurity and Colour of solution. Room light and sun

light exposed bulk sample shows decrease in assay value compared to sodium light exposed sample .Sun light exposed sample shows significant increase in level of total Impurity and colour of solution value compared to sodium light exposed sample. Considering above analytical results and light sensitivity of formulation it is recommended to manufacturing bulk solution under sodium vapour lamp or avoid exposure to direct room light using closed manufacturing vessel.

4. Review of analytical results show that there is no significant difference between impurity profile of Batch without nitrogen purging and Batch with nitrogen purging.

Review of forced degradation data of Pantoprazole sodium API and Pantoprazole sodium for injection, 40mg/vial shows that it is highly sensitive to oxygen and undergo degradation in presence of oxygen. Hence, nitrogen purging is recommended as precautionary measure during manufacturing of drug product.

Data comparison of developed formulation product Vs RLD Product

Test product and RLD Product was analyzed as per the above mentioned analytical method the results comparison was given below.

Specificity

Based on the reviewed data it was demonstrated that there is No interference found at the retention time of main peak (Pantoprazole) and known impurities due to blank and placebo.Peak purity for the Pantoprazole peak and known impurities peak should be pass in As such sample and in spiked sample.RT and RRT of specified impurities shall be comparable with developed analytical method.

Forced degradation studies

For typically degraded samples, the Pantoprazole peak should pass the peak purity test.The mass balance should be in the range of 90% to 110%.Forced degradation study was carried out with acidic (HCl), basic (NaOH), oxidation (H2O2) stress condition in solution state and thermal, humidity and photo degradation in fininished product.Stress conditions and data for samples are summarized in below .

(a) Chemical stability of drug substance

Stress testing (Forced degradation) was mentioned in below Table:8 for Pantoprazole sodium to study its impurity profile, degradation pathway and to facilitate the development of a stability-indicating method. In addition, knowledge obtained from the forced degradation studies was used during formulation and process design and development to prevent impurities from being generated.

(b) Chemical stability of Drug substance in Formulation:

Stress testing of Drug substance in Formulation is mentioned in below Table: 9 to study its impurity profile, degradation pathway and to facilitate the development of a stability-indicating method. In addition, knowledge obtained from the forced degradation studies was used during formulation and process design and development to prevent impurities from being generated.

Determination of Limit of Detection and Limit of Quantification

The detection and quantitation limits were calculated using the residual standard deviation and signal-to-noise ratio methods. Limits of detection and quantitation were determined by analyzing Pantoprazole and its known impurities at concentrations ranging from 1% to 150% of the test concentration.The Quantitation limit should be 50% less than level of specification, preferably much less. The Finalized LOD and LOQ concentrations and S/N ratios are summarized in below Table-10 and 11. Based on the Precision at LOQ and LOD the concentrations are finalized.

a) Signal to Noise ratio should be not less than 3 (For LOD determination).

b) Signal to Noise ratio should be not less than 10 (For LOQ determination).
c) The relative standard deviation (RSD) of six injections of impurities and Pantoprazole in LOQ solution should be less than 10.0%.

Linearity and Range

Linearity is determined by plotting a graph of the area response versus concentration and calculating the correlation coefficient using the regression method. Over the calibration ranges tested, there was a linear correlation between peak response and concentration with a correlation coefficient greater than 0.995. Over the calibration range LOQ to 150%, a linear calibration plot for the Organic impurities method is obtained. The linearity results are summarized in Table-13.

Method Precision

The precision of the method was determined by analyzing a sample of Pantoprazole Lyophilized injection 40mg/ vial spiked with specified impurities. at specification level (0.20%).

`The result of % RSD for each impurity was met with the specification limits. The % RSD for each of impurity for six sample solutions should be NMT 10% if impurities are above 0.1% level.

Accuracy:

A study for accuracy of Pantoprazole and known impurities has been carried out by preparing three replicates of level-1 (50%), level-2 (100%) and level-3 (150%) by spiking known amount of impurities. The observed recovery results range in between 90-110 with % RSD Not more than 5.0 signifying that the method is accurate within the preferred range.

The results are as below.

Table: 7 Data comparison of Test and RLD products

Sr. No.	Test	PROTONIX IV (pantoprazole sodium) for injection Batch No.: 395493	Pantoprazole sodium for injection, 40mg/vial
1	Description	White lyophilized cake filled in glass vial	White lyophilized cake filled in glass vial
2	Assay of Pantoprazole (%)	99.1	100.9
3	Related substances		
	Related compound A (%)	0.026	0.040
	Related compound B (%)	0.000	0.017
	Related compound C (%)	0.079	0.012
	Related compound D&F (%)	0.2750	0.087
	Related compound E (%)	0.024	0.030
	Any unspecified degradation products (%)	0.066 (RRT 0.24)	0.021(RRT 1.143)
	Total degradation product (%)	0.510	0.234
4	Reconstitution time (Seconds)	21	15
5	pH of reconstituted solution	10.08	9.83
6	Osmolality (mOsm/kg)	306	330
7	Water content (%)	3.50	0.90

Based on the above data it can be concluded that both the Test and RLD results shown nearer to similar.

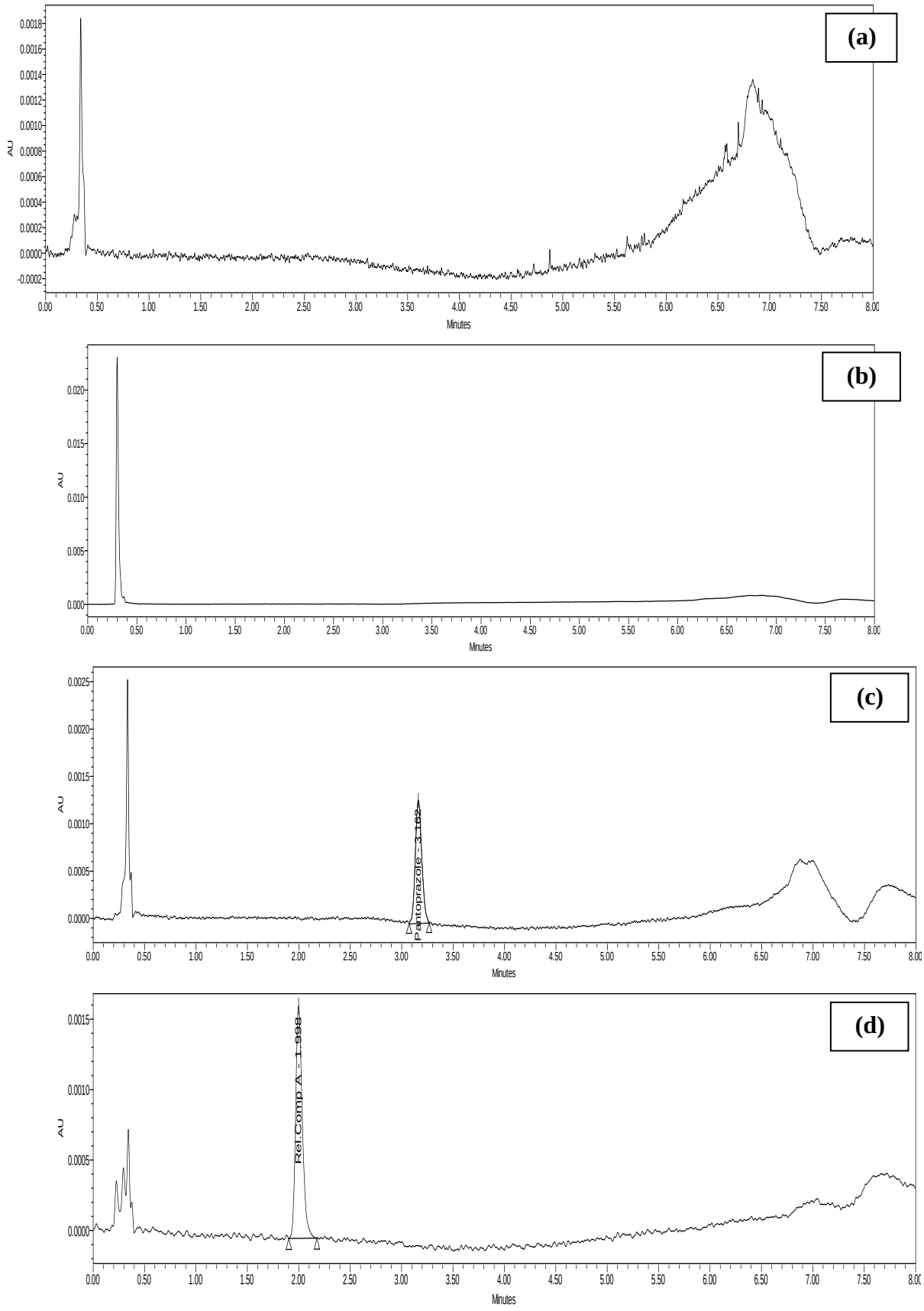
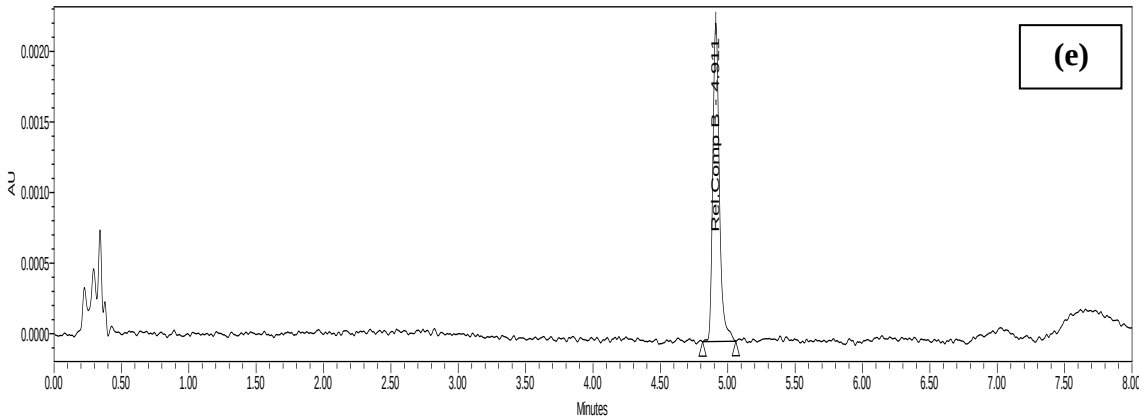
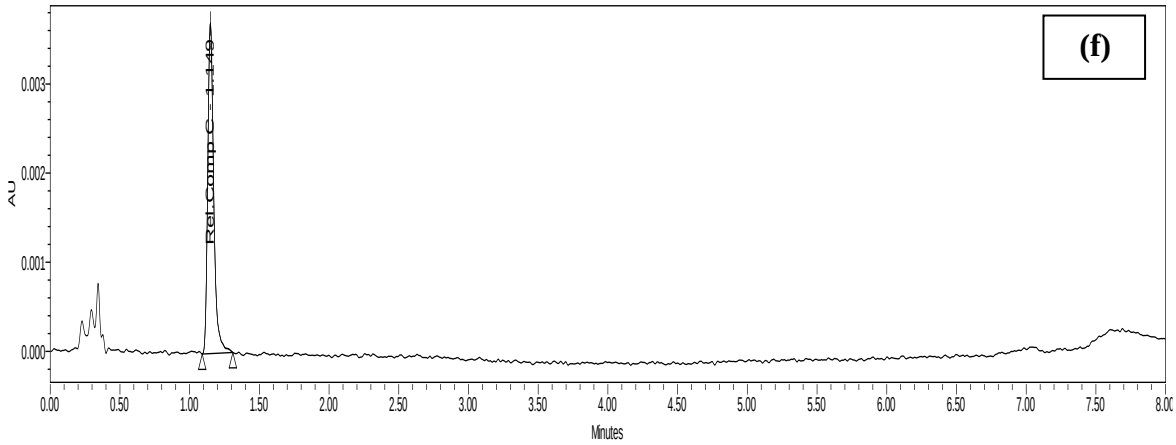
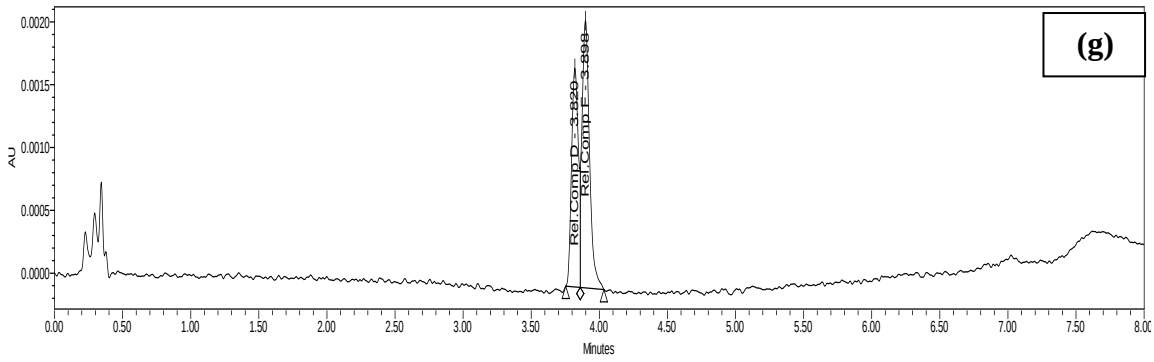


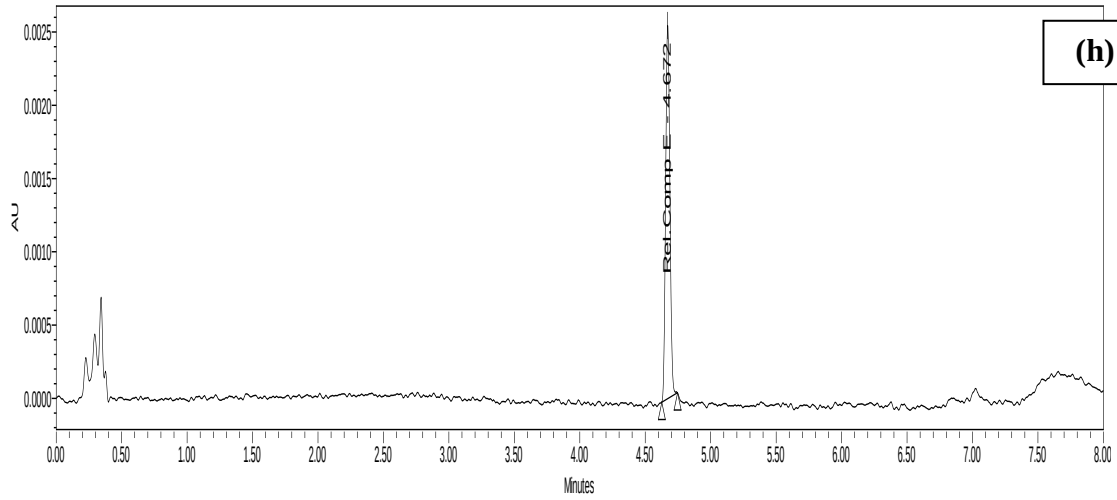
Table: 6 Data comparison of the critical quality attributes

S.No.	Test	Tentative Specification	Initial	Order of Addition Process-I	Order of addition Process-II	25°C±5°C)	5°C ±3°C	Sodium vapour light (Exposed for 8 hrs) (NMT 400Lux)	Room light (Exposed for 8 hrs) (Fluorescent light)	Sun light (Exposed for 8 hrs)
01	Description	Clear Pale yellow to brown colour solution	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
02	Assay of Pantoprazole (%)	95 to 105 %	101.3	98.6	99.0	101.3	99.8	102.96	98.04	98.71
03	pH	10.0 to 12.0	10.78	10.48	10.59	10.48	10.59	10.17	10.32	10.46
04	Related substance									
	RC A (%)	NMT 0.15%	0.063	0.088	0.08	0.086	0.088	0.063	0.064	0.115
	RC B (%)	NMT 0.15%	ND	ND	ND	ND	0.006	ND	ND	0.010
	RC C (%)	NMT 0.15%	ND	ND	ND	ND	0.003	ND	ND	0.010
	RC D&F (%)	NMT 0.4%	0.045	0.024	0.03	0.027	0.020	0.045	0.043	0.036
	RC E (%)	NMT 0.15%	0.027	ND	ND	ND	0.025	0.027	0.026	0.018
	Any Unknown Impurity (%)	NMT 0.15%	0.012 (RRT1.270)	0.134	0.143	0.146	0.060	0.011 (RRT1.260)	0.011 (RRT 1.620)	0.711 (RRT0.360)
	Total Impurities%	NMT 1.5 %	0.183	0.297	0.312	0.321	0.295	0.182	0.174	1.397
05	Color of solution (APHA units)	NMT 200 APHA units	154.2	155.3	158.4	162.5	155.8	160.90	157.00	>500
ND: Not detected										









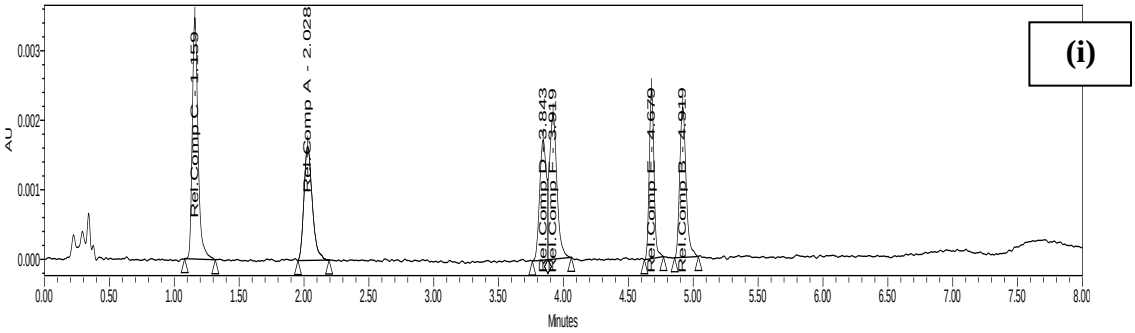


Table: 8 Forced degradation data of Pantoprazole sodium Drug substance

Limit in API (%)	**INITIAL**	**Thermal (heating at 75°C for 24 hrs.)**	**High humidity (90% RH for 24 hrs.)**	**Photolytic (under UV light at 254 nm for 24 hrs.)**	**Acid 0.04N (HCl at RT for 30 min.)**	**Alkali (2N NaOH at 60°C for 1 hr.)**	**Oxidation (0.1% H_2O_2 at RT for 1 hr.)**
Assay % (98-102)	99.9	99.9	99.9	97.0	83.7	97.8	81.7
% Degradation	-	-	-	2.9	16.2	2.1	18.2

Conclusion: Based on forced degradation data, Pantoprazole sodium is highly sensitive to Acidic, and oxidation with selected test condition.

Table: 9 Forced degradation data of Pantoprazole sodium for injection, 40mg/vial

Name of the impurity	As such	Thermal 6 hours at 105°C	Base 2ml of 5N NaOH added and heated at 80°C for 120min	Sunlight 6hours	Acid 1ml of 5N HCL added and heated at 80°C for 60min	Peroxide 0.5mL of H2O2 and Heated at 80°c for 20min	UV 24 hours
Pantoprazole related compound A	0.091%	0.337	ND	0.468	0.25	1.692	ND
Pantoprazole related compound B	0.024%	0.049	0.01	0.022	0.019	0.025	0.013
Pantoprazole related compound C	0.008	0.256	1.237	0.373	0.448	ND	0.074
Pantoprazole related compound D&F	0.089	1.144	ND	0.805	0.353	0.026	0.061
Any unspecified degradation products	0.093 (RRT 1.13	0.168 (RRT 0.18)	0.408 (RRT 1.15)	0.674 (RRT 0.18)	2.385 (RRT 1.54)	0.164 (RRT 0.32)	1.464 (RRT 0.45)
Total degradation product	0.395	2.468	2.167	3.119	4.989	2.133	3.21

The chromatographic data was given below

Conclusion: Forced degradation study data shows that Pantoprazole sodium for Injection is sensitive to Acidic, alkali, thermal, oxidative and UV-visible light conditions.

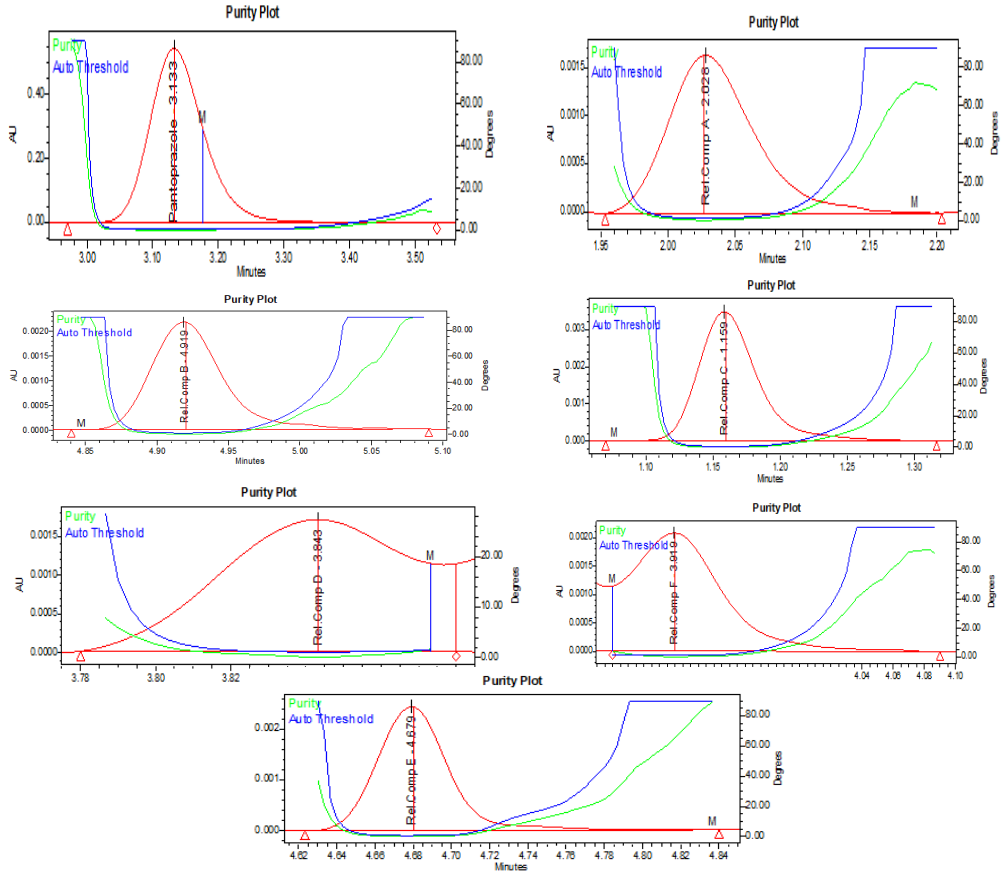
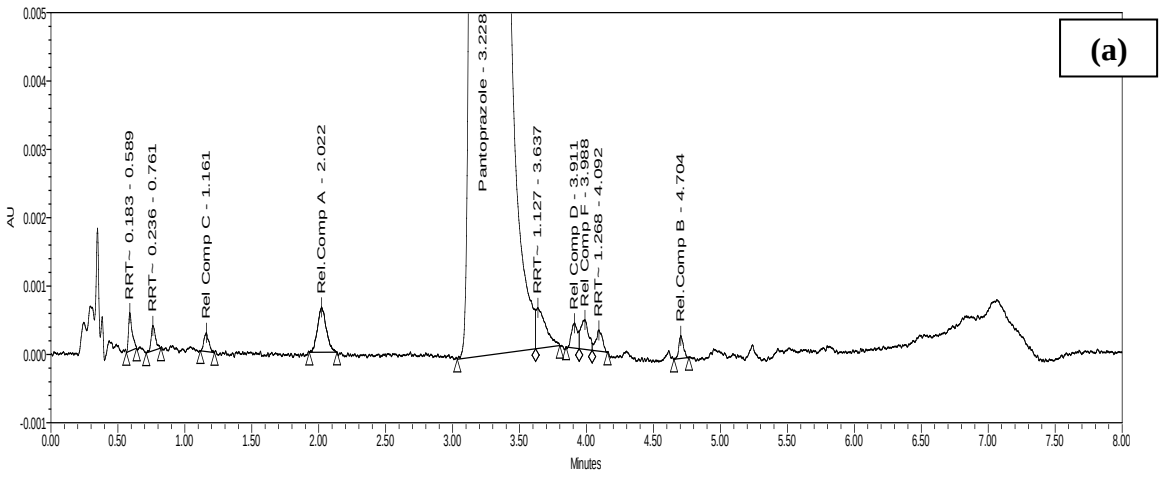
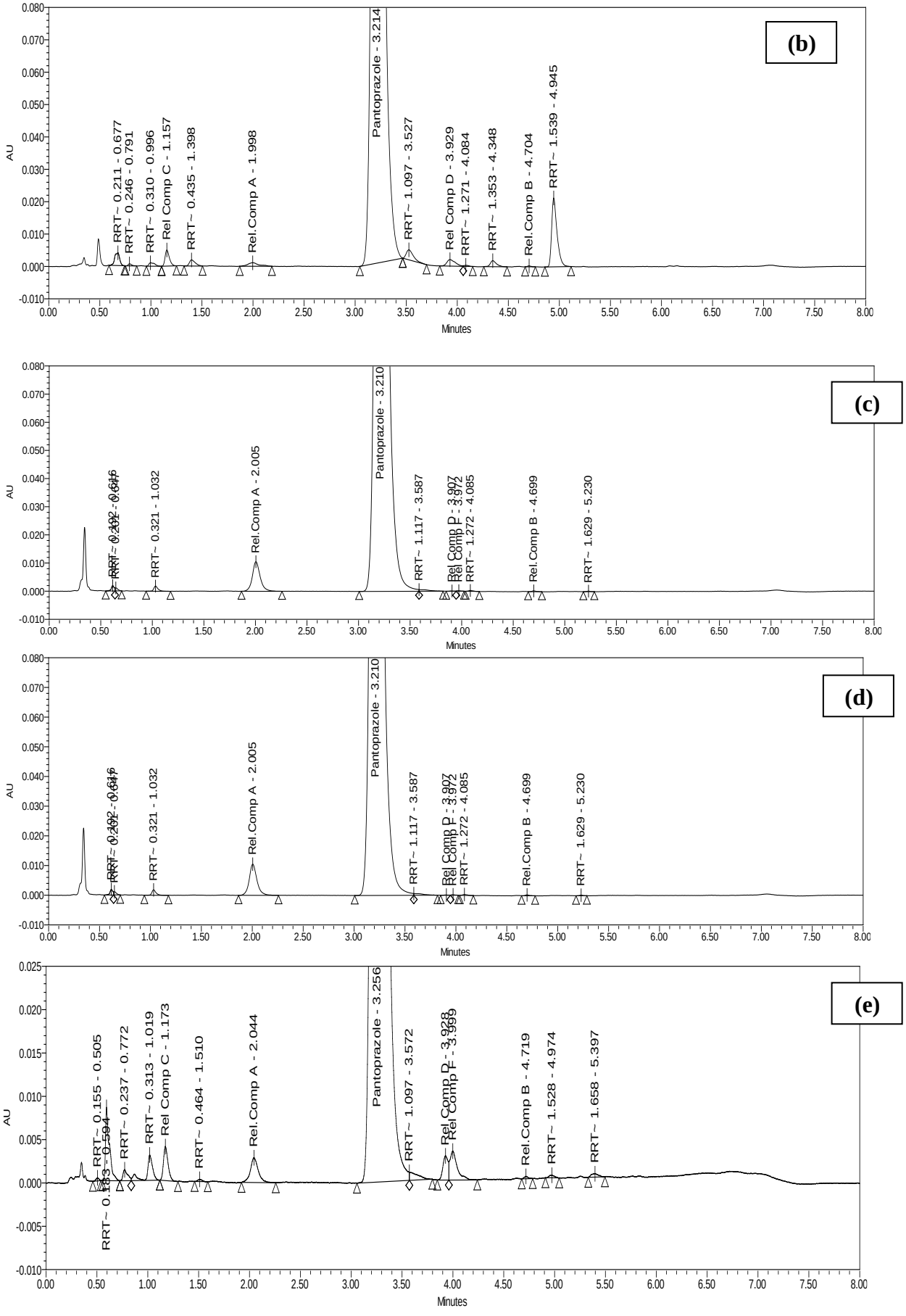


Fig: 2 (a) Chromatogram of the Blank, (b) Chromatogram of the Placebo, (c) Chromatogram ofthe Standard,(d) Chromatogram of the Pantoprazole related compound A, (e) Chromatogram of the Pantoprazole related compound B, (f) Chromatogram of the Pantoprazole related compound C (g) Chromatogram of the Pantoprazole related compound D and F, (h) Chromatogram of the Pantoprazole related compound E, (i) Chromatogram of the all known impurities in Pantoprazole(J) Purity plots of all known impurities.





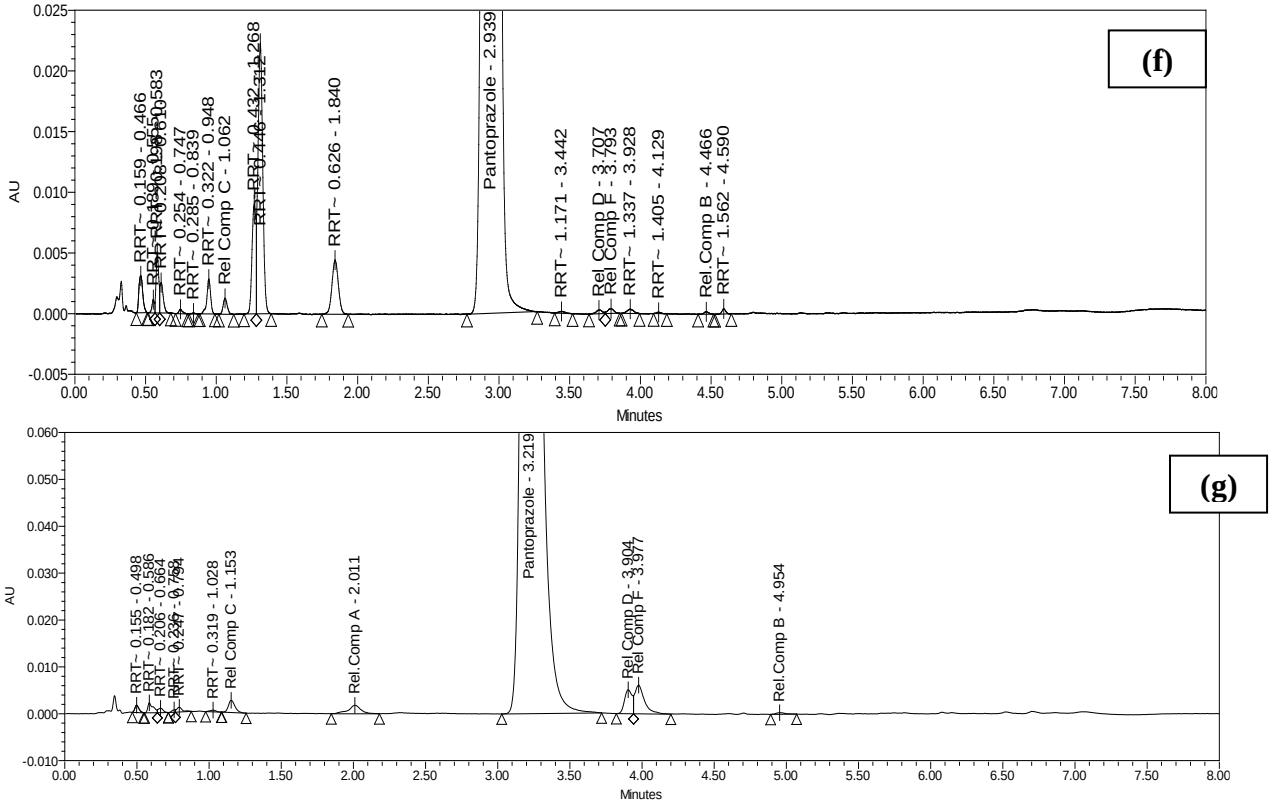


Fig: 3 (a) Chromatogram of control sample, (b) Chromatogram of Acid Degradation sample (c) Chromatogram of Base Degradation sample, (d) Chromatogram of Peroxide Degradation sample, (e) Chromatogram of Sun light Degradation sample, (f) Chromatogram of UV light stressed sample, (g) Chromatogram of Thermal Degradation sample

Table 13 Linearity Table

Name of the component	Trend line equation	Range	Correlation equation	Intercept
PRC A	y=8820.80x-101.67	0.084-1.257	0.9999	-101.67
PRC B	y=8739.54x-42.94	0.041-1.234	0.9998	-42.94
PRC C	y=10355.59-61.36	0.041-1.235	1.0000	-61.36
PRC D & F	y=3917.16-181.91	0.309- 3.089	0.9999	-181.91
PRC E	y= 7105.86-83.73	0.058-1.240	0.9998	-83.73
Pantoprazole	y=8265.51-2241.60	0.8-90.0	0.9999	-2241.60

Table-14 RRF for all known impurities

It can be calculated by dividing the Impurity slope and main peak slope

Table:15 Method precision results

Sample ID	P.R.A %w/w	P.R.B %w/w	P.R.C %w/w	P.R.D&F %w/w	P.R.E %w/w	Total impurities %w/w
Sample-1	0.30	0.25	0.50	0.42	0.18	1.65
Sample-2	0.30	0.25	0.51	0.41	0.18	1.62
Sample-3	0.30	0.24	0.51	0.40	0.17	1.62
Sample-4	0.30	0.25	0.52	0.40	0.18	1.65
Sample-5	0.30	0.24	0.51	0.40	0.17	1.62
Sample-6	0.31	0.24	0.52	0.42	0.18	1.67
Average	0.30	0.25	0.51	0.41	0.18	1.64
% RSD	1.4	2.2	1.5	2.4	2.9	1.30

Table: 16 Accuracy data of all known impurities

Pantoprazole				**P.R. A**			
mg added (ppm)	mg found (ppm)	% Recovery	%RSD	mg added (ppm)	mg found (ppm)	% Recovery	%RSD
0.412	0.402	102.7	3.1	0.413	0.419	98.6	3.1
0.823	0.803	102.5	2.1	0.848	0.838	101.1	1.3
1.229	1.205	102	1	1.266	1.257	100.7	2
P.R. B				**P.R. C**			
mg added (ppm)	mg found (ppm)	% Recovery	%RSD	mg added (ppm)	mg found (ppm)	% Recovery	%RSD
0.434	0.411	105.6	1.5	0.398	0.412	96.6	0.2
0.868	0.811	105.5	1.2	0.809	0.824	98.3	1.3
1.292	1.234	104.8	1.3	1.241	1.235	100.6	1.5
P.R. D and F				**P.R.E**			
mg added (ppm)	mg found (ppm)	% Recovery	%RSD	mg added (ppm)	mg found (ppm)	% Recovery	%RSD
1.005	1.030	97.6	1.1	0.394	0.413	95.4	1.2
1.958	2.059	95.1	3.5	0.789	0.827	95.4	0.7
2.991	3.089	98.8	0.7	1.215	1.24	97.9	2.9

Range

The method is Linear, accurate and precise from LOQ to 150% levels of specification with respect to test concentration of Pantoprazole finished dosage form. (Refer to Linearity, Accuracy and Precision data)

Solution Stability

As per the literature, Pantoprazole is unstable at 25°C. Hence, solution stability experiments were performed at 5°C

Solution stability performed by analysing standard solution and sample solution periodically in to HPLC system. The standard solution stored 5°C for 24 hrs, and 16 hrs spiked sample solution respectively. The test and standard solutions are considered stable with respect to the time interval if the cumulative % RSD for peak area of analyte peak in standard solution and peak area of specified impurity, any other impurity and total unknown impurity in sample solution is NMT 10 %. The known and unknown impurities in the sample solution at a level of below 0.1 % should be monitored and any significant change should be evaluated for defining solution stability.Results are summarised in Table-17 and 18

Robustness

A close study of the analytical results for deliberately varied chromatographic conitions such as flow rate, column temperature, wave length, pH, and modification of organic component in gradient programme indicated that there is no significant change in the relative retention time of the main analyte and their corresponding impurities, demonstrating the robustness of the established approach.

Table:17 Stability of Standard solution at 5°C

Time point (Hrs)	Area of Pantoprazole peak	% Difference
0	7646	-
6	7314	4.54
12	7570	0.99
18	7458	2.46
24	7590	0.73

Table: 18 Stability of the Sample solution at 5°C

Name of Impurity	% Impurity difference 4 Hrs	% Impurity difference 16 Hrs
Pantoprazole Rel Com A	-3.45	-3.45
Pantoprazole Rel Com B	-4.17	-8.33
Pantoprazole Rel Com C	1.96	1.96
Pantoprazole Rel Com D&F	2.44	-4.88
Pantoprazole Rel Com E	0.00	0.00

Table:19 Results of Robustness parameter

Condition	RT min	Theoretical plates	%RSD	Tailing
As Such 0.4mL/min	3.64	21368	0.7	1.32
Flow 0.45mL/min	3.518	19502	1.6	1.19
Flow 0.35mL/min	3.82	26390	0.8	1.23
Column Temp 45°C	3.42	15355	1.8	1.26
Column Temp 35°C	3.83	27860	1.2	1.34
MeOH +5% (MP:A) (85:10.5:5)	2.95	3099	1.2	1.78
MeOH -5% (MP:A) (85:10.5:4)	3.03	3106	2.0	1.83
ACN +5% (MP:A) (85:10:4.5)	2.98	2964	1.9	1.71
ACN -5% (MP:A) (85:11:4.5)	3.31	5289	1.4	1.83
MeOH +5% (MP:B) (70:31.5)	3.52	6449	0.8	1.84
MeOH -5% (MP:B) (70:28.5)	3.40	5141	1.2	1.83
pH 7.3	3.22	4452	1.3	1.35
pH 7.7	3.05	3047	2.0	1.45

CONCLUSION

The formulation of Pantoprazole Lyophilized injection 40mg/ vial successfully developed by using freeze drying technique and to overcome formulation criticalities with critical quality attributes (CQA). The proposed RP-UPLC method enables the separation and simultaneous quantitative determination of known and unknown impurities of PAN in Pantoprazole Lyophilized injection 40mg/ vial. The developed method is validated accordance with ICH requirements. The stress studies indicated that method is selective, sensitive and stability indicating. UV detection at 290 nm was found to be suitable without any interference from blank and excipients. All the calibration curves obtained were found to linear with values of correlation coefficients greater than 0.999. LOD and LOQ values are the Quantitation limit should be 50% less than level of specification. Recovery study established the accuracy of the method. The proposed RP-UPLC method is fast, precise, accurate, sensitive and efficient. This method was successfully used in the pharmaceutical industry for finished dosage form sample analysis.

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