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Research Article

A validated stability-indicating HPLC assay method to investigate stability of Pantoprazole injection with injectable solutions (5 % Dextrose injection and 0.9% Sodium Chloride injection)

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ABSTRACT

Intravenous Pantoprazole is an alternative short-term treatment for patients with gastro esophageal reflux disease (GERD) who cannot take pantoprazole tablets. The stability of reconstituted solutions of Pantoprazole Injection in 5% Dextrose and 0.9% Sodium Chloride is critical before intravenous infusion, Physical stability (by pH measurement) and chemical stability (by measuring the concentration) in solution of Pantoprazole was assessed. A stability-indicating reversed phase High-Performance liquid chromatographic (HPLC) assay method was developed and validated. The reconstituted solutions were kept under storage at room temperature (25°C ±2°C) and tested as per method at 0,4,8,12,18 & 24 hours. The validation study concluded that assay method is suitable to investigate the stability of Pantoprazole injection in injectable solutions (5% Dextrose injection and 0.9% Sodium Chloride injection) and solutions are stable for up to 24 hours at 25°C.

Keywords: Pantoprazole injection; 5% Dextrose injection; 0.9% Sodium Chloride injection; Physical and Chemical Stability; Stability-indicating HPLC Assay

INTRODUCTION

Pantoprazole is a substituted benzimidazole, sodium 5-(difluoromethoxy)-2-[[(3, 4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole, a compound that inhibits gastric acid secretion. Its empirical formula is $C_{16}H_{14}F_2N_3NaO_4S$, with a molecular weight of 405.4. (Fig. 1), A compound that inhibits gastric acid secretion in the stomach by specific action on the proton pumps of the parietal cells. Pantoprazole sodium is a white to off-white crystalline powder and is racemic. Pantoprazole has weakly basic and acidic properties. Pantoprazole sodium is freely soluble in water, very slightly soluble in phosphate buffer at pH 7.4, and practically insoluble in n-hexane.

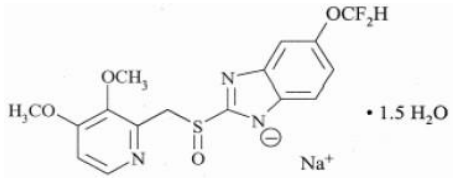

Figure 1: Structure of Pantoprazole Sodium

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The concentration of Pantoprazole injection after reconstitution to Dextrose and Sodium chloride solutions is extremely low. The literature survey resulted into some published methods for solid dosage forms and injection solutions like Pantoprazole determination in human plasma by HPLC (Ramakrishna NVS et al, 2005), Identification and characterization of potential impurities of Pantoprazole sodium (Reddy GM et al, 2007), Chiral separation of enantiomers of Pantoprazole and other benzimidazole sulfoxides using cellulose-based chiral stationary phases (Tanaka Makoto et al, 1995), Determination of Four PPIs, Omeprazole, Pantoprazole, Lansoprazole and Rabeprazole in Human Plasma (Noubarani Maryam et al, 2010), Simultaneous determination of Pantoprazole and Domperidone from their Combination Drug Product (Thanikachalam Siva Kumar et al, 2008), Simultaneous Determination of Pantoprazole and its two metabolites in Dog Plasma (Xie Zhiyong et al, 2005), Chiral resolution of Pantoprazole sodium and related sulfoxides by complex formation with bovine serum albumin in capillary electrophoresis (Ebarle Daniele et al, 1997), Determination of Pantoprazole, Rabeprazole, Esomeprazole, Domperidone and Itopride in Pharmaceutical Products (Patel B.H.et al, 2007), Stability of Pantoprazole for injection in 0.9% Sodium Chloride or 5% Dextrose (Walker Scott et al, 2009), Stability of Pantoprazole Sodium in Glass Vials, Polyvinyl Chloride and Polypropylene Syringes (Donnelly Ronald F et al, 2011), Stability of Omeprazole Sodium and Pantoprazole Sodium

Diluted for intravenous infusion (Carpenter Jane F et al, 2006), Physical and Chemical Stability of Esomeprazole Sodium Solutions (Kupeic Thomas C et al, 2008). The published methods are with different column chemistries, Flow rates, detection wavelength, sample concentrations and composition of mobile phase eluent.

Pantoprazole for Injection is indicated for short-term treatment (7 to 10 days) of patients with gastro esophageal reflux disease (GERD) and a history of erosive esophagitis. Pantoprazole for Injection is indicated for the treatment of pathological hypersecretory conditions associated with Zollinger-Ellison Syndrome or other neoplastic conditions. Pantoprazole for Injection may be administered intravenously through a dedicated line or through a Y-site.

Pantoprazole injection is currently marketed in vials at dosage strength 40mg. A series of stability studies were conducted to evaluate the stability of Pantoprazole injection after its dilution with commonly used diluents as 5% Dextrose injection and 0.9% Sodium chloride injection. The reversed phase High-Performance liquid chromatographic (HPLC) Assay method was developed and validated as per ICH guideline (ICH Q2 (R1), 2005) over the concentration range after dilution in the diluents (i.e. concentration of 0.4 mg/ml which was further diluted to 0.04mg/ml for analysis purpose).The method development, method validation and stability testing experiments are described in this paper.

MATERIALS AND METHODS

Materials and Instruments

Pantoprazole, Impurity A, Impurity B, Impurity C and Pantoprazole injection were obtained from Cadila Healthcare ltd (Ahmedabad, Gujarat). All chemicals required to perform the analytical research were obtained as HPLC grade reagents from various sources. The HPLC column of brand Phenomenex Luna C8 with 150mm length and 4.6mm diameter was used. HPLC instrument of Agilent 1200 Series with VWD detector and Shimadzu 2010 with UV detector was used.

Preparation of 5% Dextrose injection and 0.9% Sodium chloride injection solutions

Two vials of Pantoprazole injection were taken and 10ml of 5% Dextrose injection and 0.9% Sodium chloride injection was added in each separately and reconstituted. This reconstituted solution then transferred to two separate 100 ml volumetric flasks and diluted to volume with respective diluents. The concentration of Pantoprazole in each solution was approximately 0.4 mg/ml. This solution was subsequently diluted to obtain concentration of Pantoprazole for assay method as 0.04 mg/ml. A quantitation of Pantoprazole was made immediately after the preparation of each solution and subsequent assays were performed after storage for 2, 4, 8, 12, 18 & 24 hours at room temperature 25°±2°C.

Method development of assay method

A novel assay method was developed by taking into consideration the Pantoprazole aqueous solubility and UV absorbance maxima at 290 nm. The reverse phase High-Performance liquid chromatography (HPLC) was preferred as an elution technique (Snyder Lloyd R. et al, 1988). The commonly employed Dipotassium hydrogen phosphate solution (0.02M) was selected as a buffer system and the pH of buffer solution was adjusted to 7.5 by using 10% Orthophosphoric acid solution. Acetonitrile was used as an organic solvent to modify the polarity of mobile phase. The reversed phase chromatography uses non polar stationary phase. The octysilane (C8) brand Phenomenex Luna C8 with 150mm length and 4.6mm diameter was used. The column temperature was kept at 30°C. The flow rate was kept as 1 ml/min. Injection volume of 20µl was used. Different compositional trials of buffer and acetonitrile were taken to optimize the elution pattern of Pantoprazole peak. The optimized mobile phase consists of Acetonitrile: 0.02M phosphate buffer (35:65 v/v).

Analysis and Calculations

The Pantoprazole content in each solution was quantitated using an isocratic reversed phase High-Performance liquid chromatography (HPLC) method. The working standard injection was injected five times to check the system suitability performance. In this the Pantoprazole peak area response for five injections should be precise with 2 % Relative Standard deviation. The standard injections were followed by Pantoprazole injections in 5% Dextrose injection and 0.9% Sodium chloride injection for the respective storage stability time points of 0, 2, 4, 8, 12, 18 & 24 hours.

The concentration of Pantoprazole (expressed as %) in each sample was calculated by using the formula.

$$\% \text{ of Pantoprazole} = (A_{sample} / A_{standard}) \times (W) \times (D_{sample} / D_{standard}) \times (P/100) \times 100/\text{Dose strength}$$

Where, A_{sample} & $A_{standard}$ are the peak areas of Pantoprazole in sample and standard preparations respectively. W is the mg of Pantoprazole sodium standard taken, D_{sample} & $D_{standard}$ are the dilutions of sample and standard preparations respectively, P is the % potency of Pantoprazole sodium and Dose strength is 40 mg

Measurement of pH

The pH of the solution was determined using a glass combination electrode, which was calibrated for analysis with standard pH solutions. The pH measurement was performed immediately after preparation of the solutions and on each storage time point sample for 24 hours.

METHOD VALIDATION

Validation of the assay method

The assay method to quantitate Pantoprazole in 5% Dextrose injection and 0.9% Sodium chloride injection was validated for performance characteristics like accuracy, precision, specificity, linearity and robustness. The method was not evaluated for its Limit of Detection and Limit of Quantitation as it determines the major constituent in a solution (ICH, Q2 (R1), 2005).

Method Precision

The precision of the assay method was evaluated through multiple analyses of the each diluent's solutions prepared at 0.4mg/ml concentration level and further diluted to obtain concentration of Pantoprazole for assay method as 0.04 mg/ml. Six samples were independently prepared and analyzed according to the method. The precision of the assay values (as measured by the relative standard deviation of the various independent preparations) was well within 2% for each diluent.The results of precision study of Pantoprazole injection in 5% Dextrose injection and 0.9% Sodium chloride injection solution are summarized in Table 1.

Table 1: Precision of Pantoprazole Injection in 5% Dextrose and 0.9% Sodium chloride solution

Precision	% Average/ % RSD
Assay in 5% Dextrose injection	99.8/0.6
Assay in 0.9% Sodium Chloride injection	99.4/0.6

Accuracy of the method

The Accuracy solutions of the Pantoprazole injection were prepared by spiking with known concentrations of Pantoprazole at target concentrations of 50, 100 & 150% of the intended working concentration of the method (0.04 mg/ml). The solutions were injected on HPLC and concentration of each solution was determined according to the method procedure. The accuracy was evaluated as the percentage recovery from these solutions. These accuracy results are summarized in Table 2 & 3. The average recovery observed as between 98.0- 102.0% over the range.

Linearity

Linearity of the method was evaluated by performing the linear regression analysis between the analytical results and known concentrations of Pantoprazole in test solutions prepared for linearity studies. The results are summarized in Table 4 and 5. The linearity graphs are shown in Fig 2 and 3. The correlation coefficient of 0.9999 in each of 5% Dextrose injection and 0.9% Sodium chloride injections obtained during validation indicates that the assay method has an acceptable linearity.

Robustness

The robustness of the method was determined by small deliberate changes in flow rate, pH of mobile phase, temperature and mobile phase composition. The peak area of five replicate injections in all these modified conditions remained within the system precision limit of % RSD of NMT 2.0% indicated that the method is robust. The robustness results are summarized in Table 6.

Specificity

Specificity of method is to prove that the quantification of Pantoprazole is not interefere by co-elution of any impurity. Known impurities like 5-(difluoromethoxy)-2-[[(3,4-dimethoxypyridin-2-yl)methyl]sulphonyl]-1*H*-benzimidazole (Impurity A), 5-(difluoromethoxy)-2-[[(3,4-dimethoxypyridin-2-yl)methyl]thiol]-1*H*-benzimidazole (Impurity B) and 5-(difluoromethoxy)-2-mercapto-1*H*-benzimidazole (Impurity C) were checked for elution in assay method. All impurities are well separated from Pantoprazole peak. Hydrolytic degradation studies performed to prove the peak purity of Pantoprazole peak and thus ensuring the specificity of proposed assay method.

Table 2: Accuracy of Pantoprazole injection in 5% Dextrose injection solution

Accuracy level	Amount Spiked (mg)	Amount Recovered (mg)	% Recovery
Level 1(50)(n=3)	20.13	19.93	99.1
Level 2(100)(n=3)	39.75	39.49	99.3
Level 3(150)(n=3)	59.79	59.29	99.2
Mean % Recovery(n=9)	99.18±0.363		
% R.S.D	0.363		

Table 3: Accuracy of Pantoprazole injection in 0.9% Sodium chloride injection solution

Accuracy level	Amount Spiked (mg)	Amount Recovered (mg)	% Recovery
Level 1(50)(n=3)	20.13	20.08	99.8
Level 2(100)(n=3)	40.29	39.89	99.0
Level 3(150)(n=3)	60.13	59.82	99.5
Mean % Recovery(n=9)	99.44±0.582		
% R.S.D	0.582		

Table 4: Linearity of Pantoprazole injection in 5% Dextrose injection solution

Linearity (n=5)	Results
Range (mg/mL)	0.0196061-0.0588182
Slope	46732.99427
Intercept	4909.68383
Correlation Coefficient	0.9999

Table 5: Linearity of Pantoprazole injection in 0.9% Sodium Chloride injection solution

Linearity (n=5)	Results
Range (mg/mL)	0.0189766-0.0569298
Slope	47138.91033
Intercept	2820.88362
Correlation Coefficient	0.9999

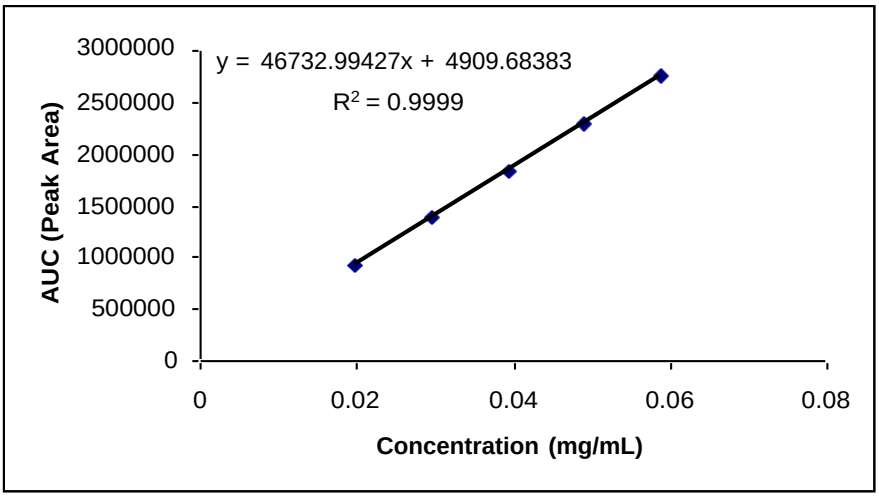

Figure 2: Linearity of Pantoprazole Sodium in 5% Dextrose injection

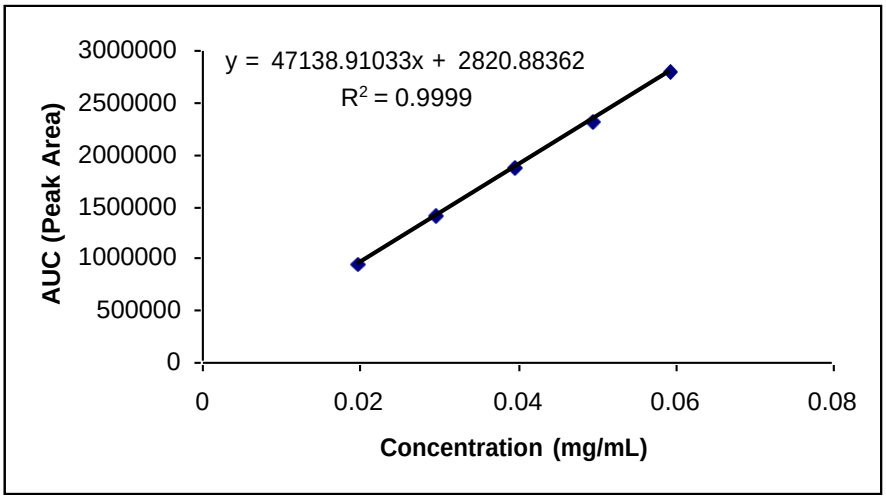

Figure 3: Linearity of Pantoprazole Sodium in 0.9% Sodium Chloride injection

RESULTS AND DISCUSSION

pH measurements

The pH of each solution remained stable within 0.2 units of the initial value in all solutions. The results of pH measurement of Pantoprazole injection in 5% Dextrose injection and 0.9% Sodium Chloride injection are summarized in Table 7.

Stability study results

The stability samples of reconstituted solutions of Pantoprazole Injection (5% Dextrose injection and 0.9% Sodium Chloride injection) were analyzed as per assay method. The concentration of Pantoprazole in both injection solutions has not dropped below 98% by the end of 24 hours storage period. The results of stability study of Pantoprazole injection in 5% Dextrose and

Table 6: Summary of Assay method Robustness

Parameter Modified	Condition	% RSD of Pantoprazole peak	Retention time (minutes) of Pantoprazole peak
Temperature	30°C	0.12	6.06
	25°C	0.15	6.24
	35°C	0.05	5.83
pH	7.5	0.12	6.06
	7.3	0.09	6.46
	7.7	0.23	5.67
Flow rate	1.0 ml/min	0.12	6.06
	0.8 ml/min	0.16	7.47
	1.2 ml/min	0.04	5.06
Mobile phase composition	Buffer 65 : ACN35	0.12	6.06
	Buffer 67 : ACN33	0.11	7.22
	Buffer 63 : ACN37	0.07	5.14

Table 7: Results of pH measurement

Time of Storage (in hours)	pH of Pantoprazole injection in 5% Dextrose	pH of Pantoprazole injection in 0.9% Sodium Chloride
0	9.12	9.10
2	9.10	9.10
4	9.11	9.10
8	9.11	9.09
12	9.11	9.08
18	9.10	9.09
24	9.10	9.08

Table 8: Stability study of Pantoprazole injection

Time of Storage (in hours)	Content of Pantoprazole injection in 5% Dextrose	Content of Pantoprazole injection in 0.9% Sodium Chloride
0	99.8	100.2
2	99.5	99.8
4	99.4	99.7
8	100	100
12	99.5	99.6
18	99.9	99.7
24	99.5	99.7

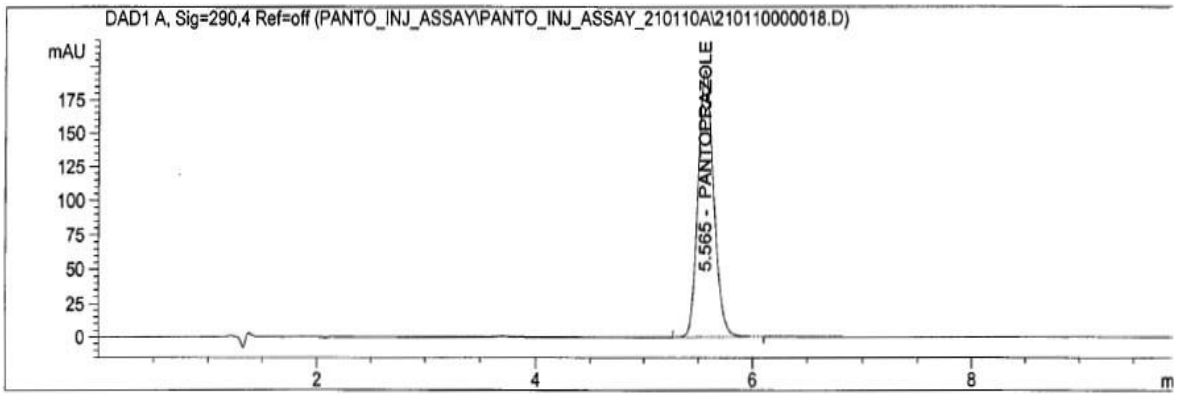

Figure 4: HPLC chromatogram of Pantoprazole injection

0.9% Sodium Chloride injection solution are summarized in Table 8 and the HPLC chromatogram obtained is shown below (Fig 4).

CONCLUSION

As the HPLC method developed has successfully qualified the validation characteristics like specificity, precision, robustness, linearity and accuracy, it has been

concluded that the method is suitable for its intended purpose of assay of Pantoprazole injection in injection solutions (5% Dextrose injection and 0.9% Sodium chloride injection). The stability study revealed that Pantoprazole content in each of 5% Dextrose injection and 0.9% Sodium chloride injection solution over storage period of 24 hours was within the stability acceptance criteria of Not less than 97.0% Pantoprazole content. It was concluded that Pantoprazole is stable in each of 5% Dextrose injection and 0.9% Sodium chloride injection for 24hrs at 25°C.

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