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Research Article

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Method development and validation of related substances in Pantoprazole Sodium by RP HPLC

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ABSTRACT

A simple, sensitive and reproducible Reverse phase high performance liquid chromatography (HPLC) coupled with a photodiode array detector; method was developed for the quantitative determination of related substances in API. Chromatographic separation was achieved on Hypersil ODS (125 X 4.0) mm, 5µm, and the gradient eluted with runtime, 50.0 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 of Pantoprazole and three impurities was greater than 2.0 for all pairs of components. The high correlation coefficient (r^2> 0.9990) values indicated clear correlations between the investigated compound concentrations and their peak areas within the test ranges. The repeatability and intermediate precision, expressed by the RSD, were less than 10%. The accuracy evaluated by performing recovery studies via a spike method, was in the range 80.0-120.0%. The performance of the method was validated according to the present ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.

Keywords: Pantoprazole sodium, Impurity, Validation

INTRODUCTION

Pantoprazole sodium is proton pump inhibitor, belonging to benzimidazole group of drug [1]. The drug used for short term treatment of erosion and ulceration of esophagus caused by gastro esophageal reflux disease. Pantoprazole sodium is the prototype members of substituted benzimidazoles which inhibits the final common step in gastric acid secretion & have over taken H_2 blocker for acid peptic disorders [2, 3]. It is a newer H^+K^+ATPase inhibitors, similar in potency & clinical, efficacy to omeprazole but is more acid stable and less active at higher pH. It is only PPI available for administration; particularly employed in bleeding peptic ulcer & for prophylaxis of acute stress ulcer. It has lower affinity for cytochrome P450 than omeprazole. Pantoprazole is extensively metabolized in the liver through the cytochrome P450 (CYP) system. Pantoprazole metabolism is independent of the route of administration (intravenous or oral). The main metabolic pathway is demethylation, by CYP2C19, with subsequent sulfation; other metabolic pathways include oxidation by CYP3A4. There is no evidence that any of the pantoprazole metabolites have significant pharmacologic activity. Pantoprazole is indicated for the treatment of Peptic ulcer, Gastro esophageal reflux disease (GERD) as well as Zollinger-Ellison syndrome. These are minimal: nausea, diarrhoea headache, abdominal pain, muscle and joint pain, dizziness are complained by 3-5%. Rashes,

leucopenia and hepatic dysfunction are infrequent. Pantoprazole inhibits oxidation of certain drugs: diazepam, phenytoin and warferin levels may be increased. Pantoprazole may degrade and convert into many intermediates and process impurities which may be encountered as related substances. So the study of raw materials and the intermediate products are necessary for identifying impurities and developing the Analytical Method for Pantoprazole. [4-5]

EXPERIMENTAL SECTION

Chemicals and reagents

Pantoprazole Sodium provided by Zydus Cadila Healthcare, India, API, working standard, as a gift Sample. HPLC grade acetonitrile was purchased from Rankem, High purity water was prepared by using Millipore Milli Q plus purification System.0.45 pump nylon filter was obtained from Advanced Micro device Pvt. Ltd. (Ambala Cantt, India).

HPLC instrument and chromatographic conditions

A chromatographic system used was Waters 2695 separations module with 2487 dual wavelength absorbance detector and 2996 Photodiode array detector equipped with Empower chromatographic software. HYPERSIL ODS3V, (125X4.0mm), 5.0 µ, maintained at 40° C using column oven, eluted with mobile phase at the flow rate of 1.0 ml/min. The mobile phase consists of aqueous solution of 1.74 gm K_2HPO_4 adjusted the pH 7.0 with OPA and acetonitrile at the 0.01 min mobile phase A (Buffer) mobile phase B (acetonitrile) in the ratio of 80:20 v/v after the 40 min mobile phase A and mobile phase B in the ratio of 20:80 v/v again 45 min mobile phase A and mobile phase B in the ratio of 80:20 v/v and 50 min mobile phase A and mobile phase B in the ratio of 80:20v/v. The mobile phase filtered through 0. 45 µm nylon membranes filter and degassed in ultrasonic bath prior to use. Measurements were made with injection volume 20 µL and UV detection at 290 nm.

System Suitability:-

Selection of system suitability solution

The resolution between sulfone and Pantoprazole was considered and other factors which were included were tailing factor, theoretical plates and %RSD (6 injections) based on diluted standard of the standard solution.

Preparation of system suitability solution

Weight accurately about 2.3 mg of impurity sulfone reference/working standard into a 50 ml volumetric flask. Dissolve and dilute to volume with diluent. Into a separate 50 ml volumetric flask, weigh accurately about 23 mg of pantoprazole sodium reference/working standard. Dissolve in about 10 ml of diluents. Add 1.0 ml of Imp Stock Sol. into this flask and diluted to volume with diluent.

Preparation of sample solution

About 23 mg of the sample into 50 mL volumetric flask was weighed accurately. Dissolved and diluted to volume with diluent.

Validation Parameters

Accuracy

System suitability solution was prepared as given above. Blank, system suitability solution was injected as per injection sequence and the acceptance criteria for system suitability was checked. Accuracy (recovery) was carried out at QL level, 50%, 100% and 120% of target limit.

Precision

System suitability solution was prepared as given above. Blank and six replicate injections of system suitability solution were injected. Resolution was checked between Pantoprazole and impurity A peaks and % RSD of peak areas was calculated for Pantoprazole peak and impurity A in the chromatogram obtained with six replicates of system suitability solution.

Linearity and Range

System suitability solution was prepared as given above. Blank, system suitability solution was injected as per injection sequence. The linearity of response was determined for all known impurities and Pantoprazole in the range

from QL to 120% of considered target limit. Response should be linear over the specified range. Linearity levels for impurity A, impurity B, impurity C, and Pantoprazole are as follows:

Table-1: Linearity levels for different components

Linearity levels	Concentration (%)
Quantitation level	At QL level
Linearity at 50 % level	0.050
Linearity at 80 % level	0.080
Linearity at 90 % level	0.090
Linearity at 100 % level	0.100
Linearity at 110 % level	0.110
Linearity at 120 % level	0.120

Limit of Detection and Limit of Quantitation

Determination of LOD and LOQ, all known impurities and Pantoprazole solutions at 0.01%, 0.03% of sample concentrations were prepared.

Table2. Preparation of solutions for the determination of DL and QL

Sr. no.	Concentration (%)	Volume of DL-QL stock solution (mL)	Diluted to (mL)
1	0.01	1	100
2	0.03	3	100

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Data from six injections of system suitability solution were utilized for calculating parameters for system suitability. The resolution between Pantoprazole and sulfone was resolved. It showed that the proposed method is precise. Hence, it can be concluded that the system suitability parameter meets the requirement of method validation. All peaks are observed to be well resolved and Relative retention time is shown in Table 4.

Table 3. Chromatographic Conditions for final optimized method

Column	Hypersil ODS(125X4.0)mm,5 µm		
Column Temperature	40° C		
Flow Rate	1.0 mL per minute		
Gradient Program	Time (Min)	M P-A, %	M P-B, %
	0.01	80	20
	40	20	80
	45	80	20
	50	80	20
Injection Volume	20 µL		
Detector wavelength	290 nm		
Run Time	50 min		

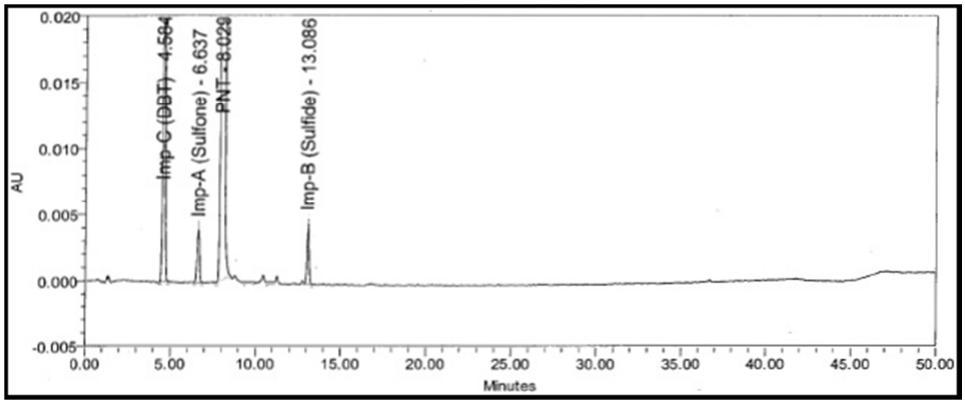

Fig. 1.Typical Chromatogram of Pantoprazole sodium spiked with impurities

Table 4. Relative retention times of different components

Component	Relative retention time(min)
Pantoprazole Sodium	1.0
Impurity DBT	0.57
Impurity Sulfone	0.80
Impurity Sulfide	1.60

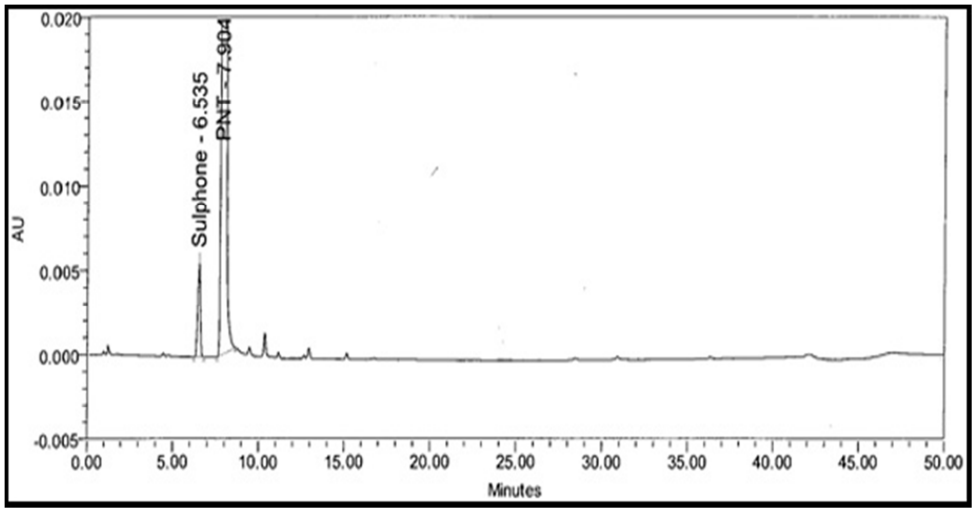

Fig. 2. Chromatogram of System Suitability working standard of Pantoprazole and Sulfone

Table 5: Accuracy studies for Impurity- DBT

Recovery Level	Conc. (µg/mL)	Amount Added (%w/w)	Amount Recovered (%w/w)	% Recovery	Mean	SD	% RSD
LOQ	0.134	0.029	0.027	93.10	**95.32**	**1.923**	**2.02**
		0.028	0.027	96.43			
		0.028	0.027	96.43			
50%	0.223	0.048	0.047	97.92	**98.61**	**1.201**	**1.22**
		0.048	0.047	97.92			
		0.048	0.048	100.00			
100%	0.447	0.097	0.095	97.94	**98.97**	**1.030**	**1.04**
		0.096	0.095	98.96			
		0.096	0.096	100.00			
120%	0.536	0.116	0.114	98.28	**98.85**	**0.497**	**0.50**
		0.116	0.115	99.14			
		0.116	0.115	99.14			
Overall					**97.94**	**1.919**	**1.96**

Table 6: Accuracy for Impurity- Sulfone

Recovery Level	Conc. (µg/mL)	Amount Added (%w/w)	Amount Recovered (%w/w)	% Recovery	Mean	SD	% RSD
LOQ	0.138	0.030	0.033	110.00	**110.23**	**0.196**	**0.18**
		0.029	0.032	110.34			
		0.029	0.032	110.34			
50%	0.461	0.099	0.108	109.09	**109.12**	**0.052**	**0.05**
		0.098	0.107	109.18			
		0.099	0.108	109.09			
100%	0.921	0.199	0.210	105.53	**106.05**	**0.881**	**0.83**
		0.198	0.209	105.56			
		0.198	0.212	107.07			
120%	1.105	0.239	0.250	104.60	**105.16**	**0.970**	**0.92**
		0.239	0.250	104.60			
		0.239	0.254	106.28			
Overall					**107.64**	**2.260**	**2.10**

Accuracy: Accuracy for individual and mean at each level between 50 % to 120% as the data shown in table 5, 6, 7 for Impurity DBT, Impurity- Sulfone and Impurity- Sulfide.

Table 7: Accuracy studies for Impurity- Sulfide

Recovery Level	Conc. (µg/mL)	Amount Added (%w/w)	Amount Recovered (%w/w)	% Recovery	Mean	SD	% RSD
LOQ	0.140	0.030	0.030	100.00	**100.00**	**0.000**	**0.00**
		0.030	0.030	100.00			
		0.030	0.030	100.00			
50%	0.234	0.050	0.053	106.00	**104.67**	**1.155**	**1.10**
		0.050	0.052	104.00			
		0.050	0.052	104.00			
100%	0.468	0.101	0.105	103.96	**103.63**	**0.572**	**0.55**
		0.101	0.105	103.96			
		0.101	0.104	102.97			
120%	0.562	0.122	0.126	103.28	**103.55**	**0.473**	**0.46**
		0.122	0.126	103.28			
		0.122	0.127	104.10			
Overall					**102.96**	**1.935**	**1.88**

Precision: Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. According to the ICH, precision should be performed at three different levels: system precision, repeatability and intermediate precision. Individual and cumulative (overall) % of individual impurity found within acceptance limit (%RSD should NMT 10) hence the method is rugged data shown in table 8.

Table 8. Precision Studies

System Precision		Method Precision		Intermediate Precision			
				Impurity-Sulfone (% w/w)		Total Impurities (% w/w)	
Injection No.	Area Counts	Impurity-Sulfone (% w/w)	Total RS (% w/w)	Day 1	Day 2	Day 1	Day 2
1	38726	0.09	0.09	0.09	0.08	0.09	0.08
2	39620	0.10	0.10	0.10	0.09	0.10	0.09
3	39036	0.09	0.09	0.09	0.08	0.09	0.08
4	39874	0.09	0.09	0.09	0.09	0.09	0.09
5	39110	0.09	0.09	0.09	0.09	0.09	0.09
6	39541	0.09	0.09	0.09	0.09	0.09	0.09
Mean	**39318**	**0.09**	**0.09**	**0.09**	**0.09**	**0.09**	**0.09**
S.D.	**429.7**	**0.004**	**0.004**	**0.004**	**0.005**	**0.004**	**0.005**
% RSD	**1.09**	**4.44**	**4.44**	**4.44**	**5.56**	**4.44**	**5.56**

Table 9.Standard calibration curve data for Pantoprazole sodium and related impurities

Conc. Level	Pantoprazole		DBT		Sulfone		Sulphide	
	Conc. (µg/mL)	Area counts (µV*sec.)	Conc. (µg/mL)	Area counts (µV*sec.)	Conc. (µg/mL)	Area counts (µV*sec.)	Conc. (µg/mL)	Area counts (µV*sec.)
LOQ	0.129	5735	0.134	9167	0.138	5964	0.140	7242
50%	0.431	19588	0.223	16412	0.461	19475	0.234	11822
80%	0.689	31620	0.357	26814	0.737	30818	0.374	19145
90%	0.775	34891	0.402	30145	0.829	34543	0.421	21816
100%	0.862	39272	0.447	33295	0.921	38067	0.468	23499
110%	0.948	43328	0.491	37381	1.013	42680	0.515	26302
120%	1.034	48540	0.536	41348	1.105	45354	0.562	27916
Slope		**46517**	**78900**		**41112**		**49906**	
Intercept		**-496**	**-1399**		**422**		**328**	
Correlation coefficient		**0.9994**	**0.9996**		**0.9997**		**0.9991**	
Response Factor		**1.00**	**0.59**		**1.13**		**0.93**	

Linearity and range: The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. Linearity of the proposed method was carried out over the range of LOQ to 120% of considered target limit for all known impurities. Table 9 shows the result for the linearity of the plot of concentration against the peak area. The results indicated that the method is linear in the concentration

range of LOQ 0.129 µg/mL to 1.034 µg/mL for all known impurities with Pantoprazole.

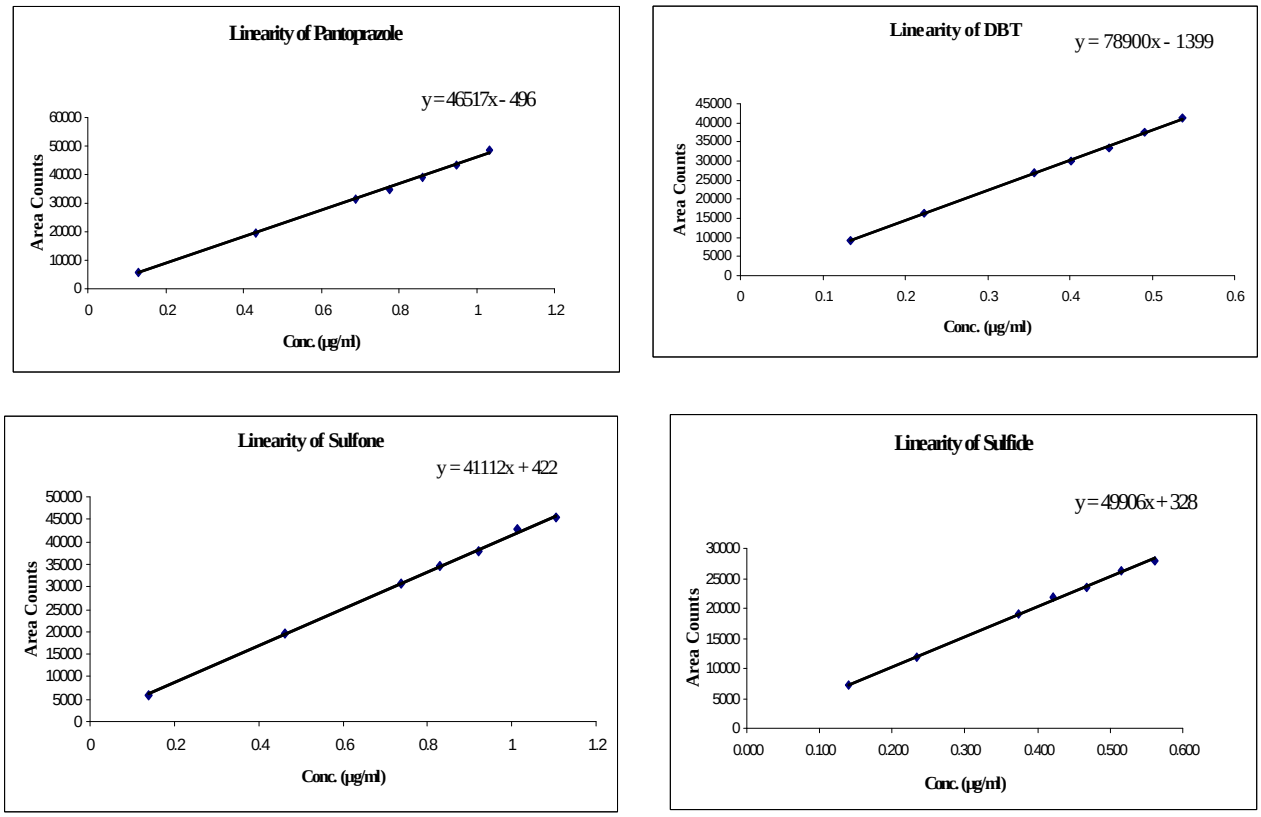

Fig. 3.Calibration curve for all known impurities with Pantoprazole sodium

Limit of Detection and Quantitation

All known impurities and Pantoprazole solutions at 0.01%, 0.03% of sample concentration were prepared. A peak of all known impurities and Pantoprazole was visualized reliably in all six replicate injections for detection limit. % RSD of peak area was found within the limit i.e. should not be more than 10.0 for quantitation limit.

Table 10: Summary of LOD-LOQ

	Pantoprazole		**DBT**		**Sulfone**		**Sulfide**	
Conc.	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
(µg/mL)	0.043	0.129	0.045	0.134	0.046	0.138	0.047	0.140
% w/w	0.009	0.028	0.010	0.029	0.010	0.030	0.010	0.030

CONCLUSION

The RP-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 three potential related substances Impurity DBT, Impurity- Sulfone and Impurity- Sulfide. Chromatographic separation was achieved on Hypersil ODS (125 X 4.0) mm, 5µm, and the gradient eluted with runtime, 50.0 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^{o}C. The resolution of Pantoprazole and three impurities was greater than 2.0 for all pairs of components. The high correlation coefficient (r^2> 0.999) values indicated clear correlations between the investigated compound concentrations and their peak areas within the test ranges. The repeatability and intermediate precision, expressed by the RSD, were less than 10%. The accuracy evaluated by performing recovery studies via a spike method, was in the range 80.0-

120.0%. From the results of related substances of Pantoprazole analysis it can be concluded that the proposed HPLC method is precise, linear and robust that can be used for routine analysis.

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