WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 2315-2326. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF PANTOPRAZOLE IN PURE FORM AND ITS TABLET DOSAGE FORM

Puppala Sindhu*[1], Dr. Gampa Vijayakumar[1], Ramya Sri Sura[2]

[1]CVM College of Pharmacy, Velichala, Karimnagar District, T.S. India.

[2]SURA LABS, 4 th Floor, S.S Towers , Beside Chandana Brothers, Dilsukhnagar, Hyderabad.

Article Received on
19 June 2015,

Revised on 10 July 2015,
Accepted on 02 Aug 2015

***Correspondence For Author**
Puppala Sindhu
CVM College of Pharmacy, Velichala, Karimnagar District, T.S. India.

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Pantoprazole, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6×250mm) 5µ column using a mixture of Methanol: TEA Buffer pH 4.0 (70:30 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 280nm. The retention time of the Pantoprazole was 2.302 ±0.02min respectively. The drugs were exposed to thermal, photolytic, acid, alkali, and oxidative stress and the stressed samples were analyzed by use of the proposed method & chromatograms from the stressed samples, obtained by use of the photodiode-array detector. The method produce linear responses in the concentration range of 10-50mg/ml of Pantoprazole. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Pantoprazole, RP-HPLC, validation.

INTRODUCTION

Pantoprazole,[13-17] or pantoprazol (also called Pantoprazolum) is a proton pump inhibitors, a type of, Anti-Ulcer Agents used for the treatment of Gastric Ulcer.it is chemically 6-(difluoromethoxy)-2-{[(3,4-dimethoxypyridin-2-yl)methane]sulfinyl}-1H-1,3- benzodiazole.

The author have developed a liquid chromatographic and validated, sensitive and reproducible method for the determination of Pantoprazole in bulk and pharmaceutical dosage forms.

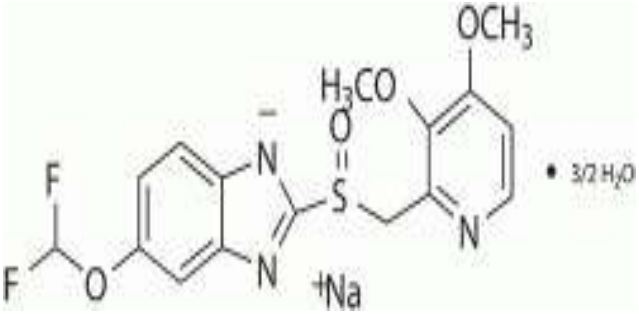

Fig.No.Chemical structure of Pantoprazole

MATERIALS AND METHODS

Chromatographic conditions

A prominence isocratic HPLC system (waters high performance liquid chromatography with auto sampler and PDA detector) column Phenomenex Gemini C18 (4.6×150mm, 5µm). A 10µL Waters injection syringe was used for sample injection. HPLC grade methanol and Triethylamine Buffer were used for the preparing the mobile phase. A freshly prepared Methanol: Triethylamine Buffer (p^H-4.0) (70:30 v/v) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicate before use. The flow rate of the mobile phase was maintained at 1mL/min., column temperature was maintained at 35ºC temperature and the detection of the drug was carried out at 280nm.

Preparation of Triethylamine buffer (pH-4.0)

Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.0 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase

Mix a mixture of above buffer 300mL (30%) and 700mL of Methanol HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Diluent preparation

Mobile phase as diluent.

Standard solution preparation

Accurately weigh and transfer 10mg of Pantoprazole working standard in to 10mL volumetric flask add about 10ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) further pipette 0.3ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Sample solution preparation

Weigh 10 Pantoprazole tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Pantoprazole into a 10ml volumetric flask. Add about 10ml of diluent and sonicate to dissolve it completely and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and through 0.45µm filter.

Method validation

Linearity

The linearity of the method was demonstrated over the concentration range of 10-50 µg/ml of the target concentration. Aliquots of 10,20,30,40 and 50µg/ml were prepared from above stock solution. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Pantoprazole was constructed by plotting peak area vs applied concentration of Pantoprazole.[1-10] A typical chromatogram is shown in Fig 1. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in fig:2. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table:1 and their calibration parameters were shown in Table:2.

SAMPLE PREPARATIONS TO PERFORM FORCED DEGRADATION STUDIES

Acid degradation

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Pantoprazole sample into a 10mL clean dry volumetric flask and add about 3mL of 5 N Hcl and kept a side for 3hours and add 3mL of 5 N NaOH solution to

neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely and was analyzed as per the test method.

Alkaline degradation

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Pantoprazole sample into a 10mL clean dry volumetric flask and add about 3mL of 5 N NaOH and kept a side for 3hours and add 3mL of 5 N Hcl solution to neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely and was analyzed as per the test method.

Peroxide degradation

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Pantoprazole sample into a 10mL clean dry volumetric flask and add about 3mL of Hydrogen peroxide solution and kept a side for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely and was analyzed as per the test method.

Thermal degradation

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Pantoprazole sample into a 10mL clean dry volumetric flask and expose to heat at 80-90ºc for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely and was analyzed as per the test method.

Photolytic degradation

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Pantoprazole sample into a 10mL clean dry volumetric flask and expose to sunlight for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely and was analyzed as per the test method.

The results are shown in “Fig:4” to “Fig:8” and “Table:6”

Precision method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and% RSD were calculated and present in Table 3. The chromatogram was shown in Fig 3. In the inter-day variation studies, six repeated injections of standard

solution were made for six consecutive days and response factor of drug peak and %RSD were calculated shown in Table3. From the data obtained, the developed method was found to be precise.

Accuracy

A study of recovery of Pantoprazole from spiked placebo was conducted at three different spike levels i.e.50, 100 and 150 samples were prepared with Pantoprazole raw material equivalent to about the target initial concentration of Pantoprazole. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table4. The mean recoveries of Pantoprazole from spiked were found to be in the range of 99.1-100.33%.

LOD and LOQ

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ (Table no.2)

System suitability

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 100mcg/ml. The results given in Table5. Were within acceptable limits.

Table 1. Linearity results for Pantoprazole

Conc. (µg / ml)	**10**	**20**	**30**	**40**	**50**
Avg. area	111022	232738	358996	476130	584909
Correlation	0.999				

Table: 2 Characteristic parameters of Pantoprazole for the proposed RP-HPLC method

Parameters	**RP-HPLC**
Calibration range (µg/ml)	10-50
Detection wavelength	280nm
Mobile phase (Methanol: Buffer)	70:30
Retention time	2.302±0.02
Regression equation(Y*)	Y=11846x-2186
Slope (b)	11846
Intercept (a)	2186

Correlation coefficient (r^2)	0.999
Intraday precision (%RSD*) Interday precision (% RSD*)	0.78 0.61
Limit of detection (µg/ ml)	1.6
Limit of quantitation(µg/ml)	5.0

Table 3. Precision results for Pantoprazole

S.No	Concentration (µg /ml)	Intraday precision (area)	Interday precision (area)
1	30	359018	354835
2	30	354707	355270
3	30	356238	359122
4	30	359990	356405
5	30	358262	354250
6	30	352671	352699
Mean		356814.3	355430.2
Std Dev		2792.023	2182.287
% RSD		0.782	0.613

Table 4. Accuracy results for Pantoprazole

Sample No	Spike level	Amount (µg / ml) added	Amount (µg / ml) found	% Recovery	Mean % Recovery
1	50%	14.8	15	99.1	99.1
	50%	15.0	15	100.0	
	50%	14.75	15	98.3	
2	100%	30.5	30	101.6	100.5
	100%	30.2	30	100.9	
	100%	29.7	30	99.1	
3	150%	45.0	45	100.1	100.3
	150%	45.1	45	100.3	
	150%	45.2	45	100.5	

Table:5 system suitability studies of Pantoprazole by RP-HPLC method

Property	Values	Required limits
Retention time (R_t)	2.301±0.02	RSD ≤ 2%
Theoretical plates (N)	5307	N > 2000
Tailing factor	1.7	T≤ 2

Table: 6 Data for forced degradation studies of Pantoprazole.

Parameter	Area	%label	% Degradation
Acid	344532	97.5%	2.5
Alkali	354540	100.3%	-
Peroxide	344518	97.5%	2.5
Thermal	349614	98.9%	1.1
Photolytic	319454	90.5%	9.5

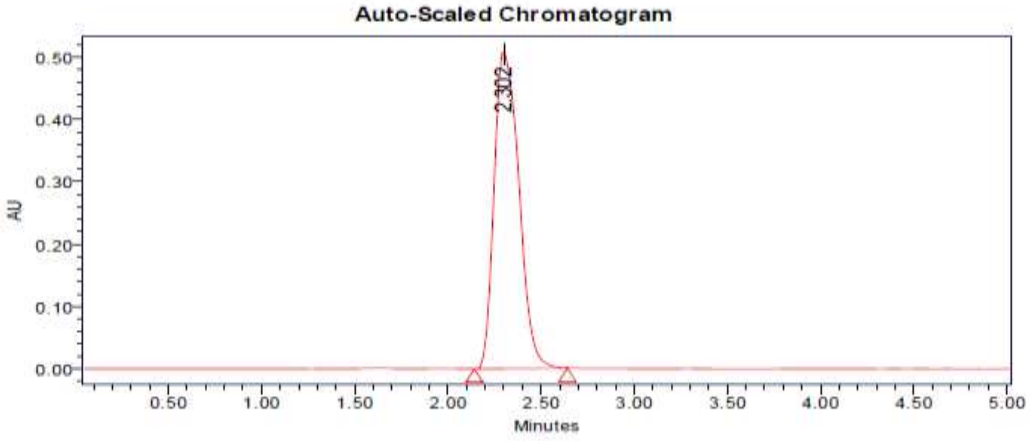

Fig: 1. Chromatogram of Pantoprazole at 280nm

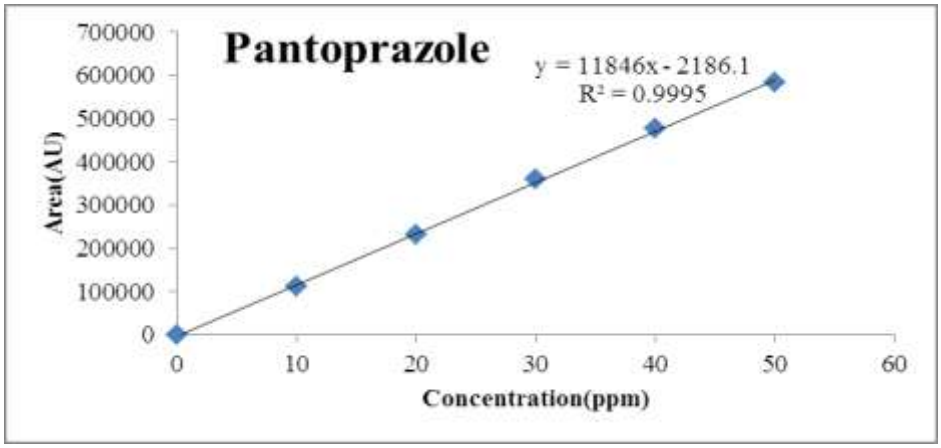

Fig:2. Calibration curve of Pantoprazole

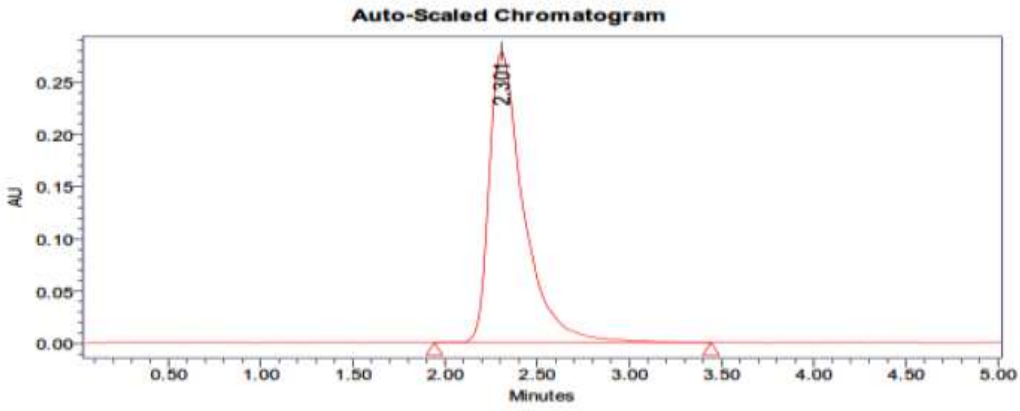

Fig:3. Chromatogram of precision

FORCED DEGRADATION STUDIES

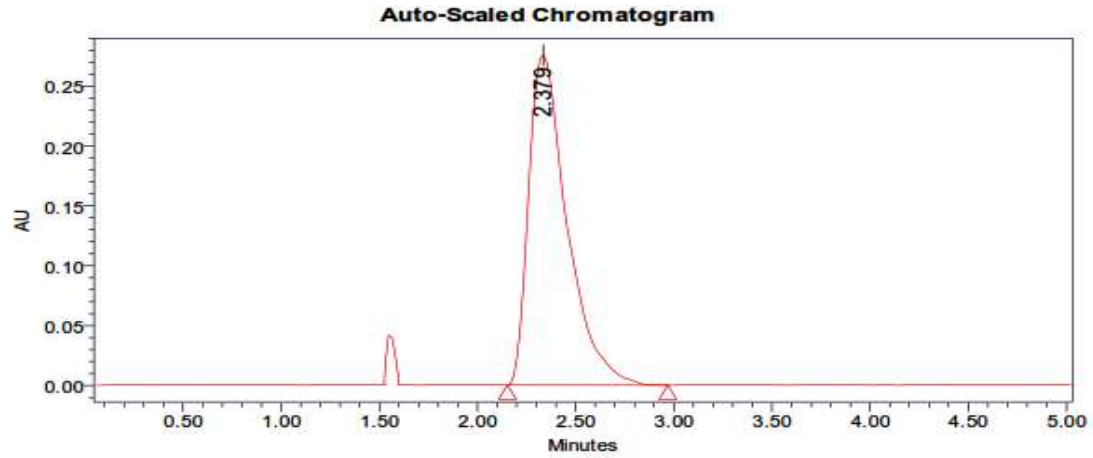

Fig: 4. ACID

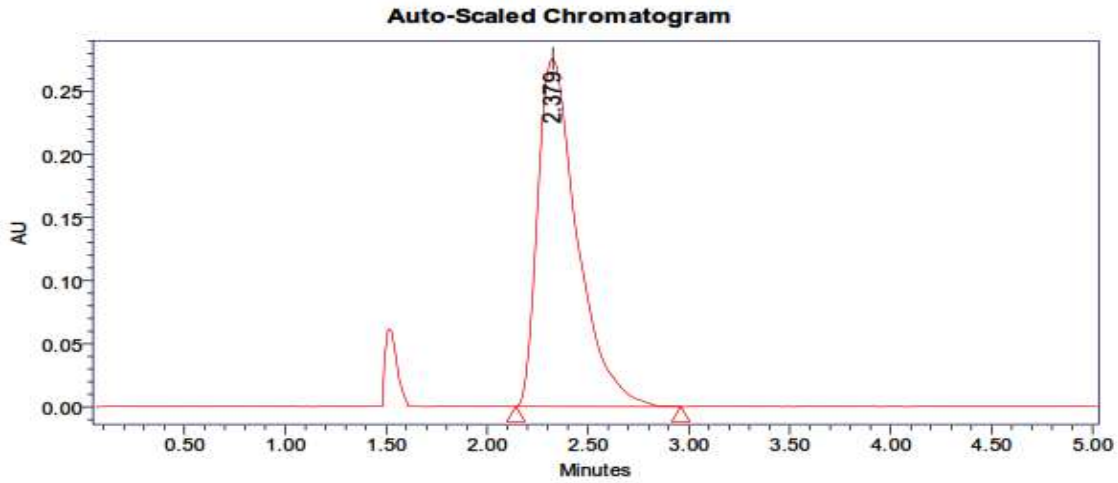

Fig: 5. BASE

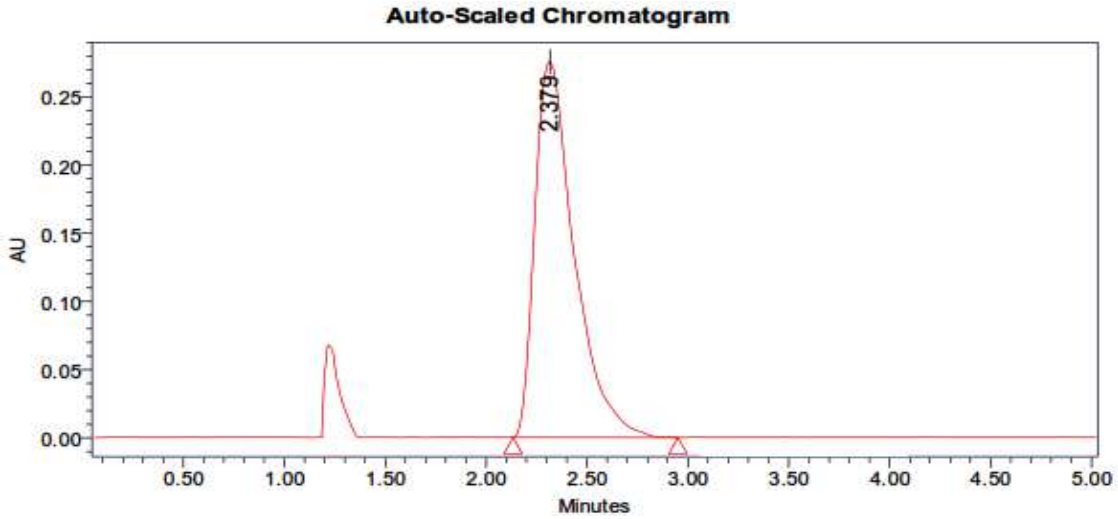

Fig: 6. PEROXIDE

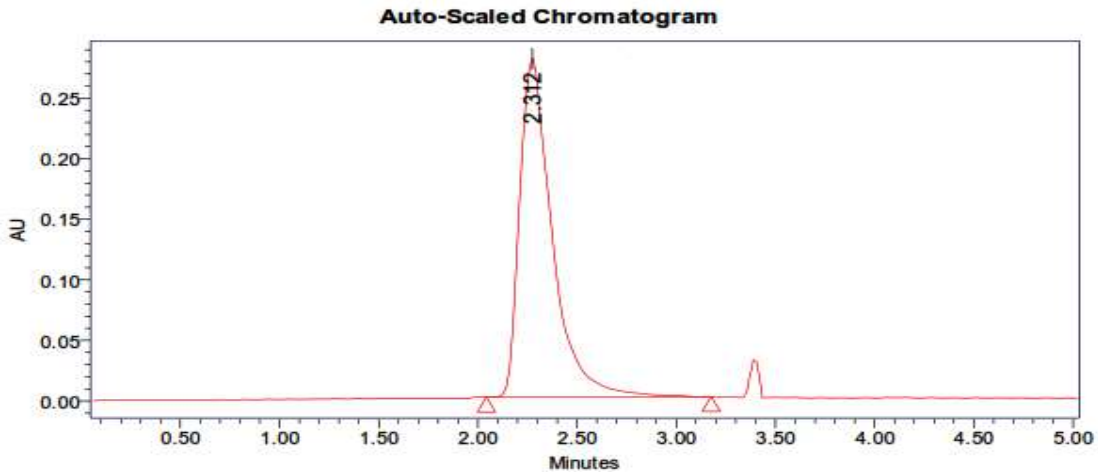

Fig: 7. THERMAL

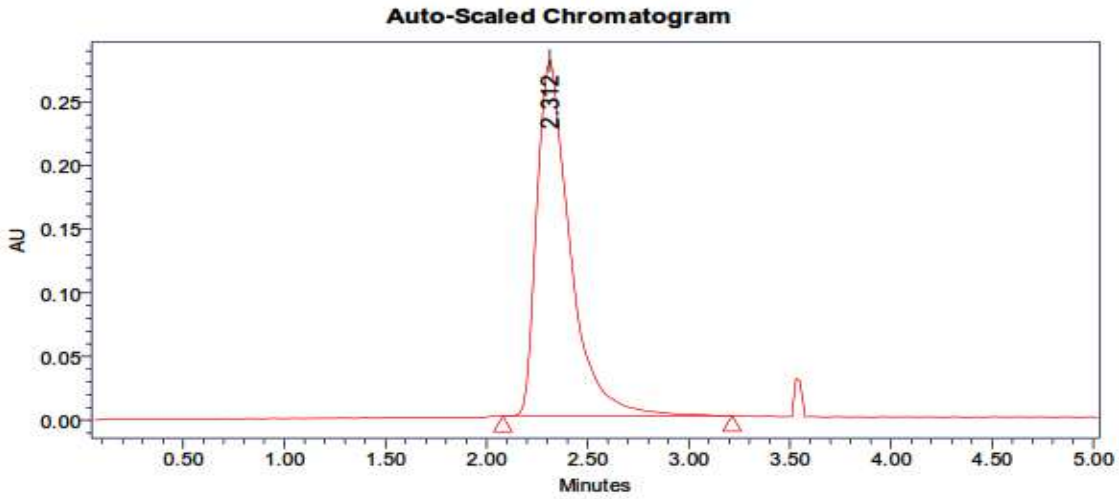

Fig: 8. LIGHT

RESULTS AND DISCUSSION

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Pantoprazole in bulk dug and pharmaceutical dosage form by using the most commonly employed RP C-18 column with PDA-detection.

The run time was set at 5min and the retention time for Pantoprazole was 2.302±0.2 min. Each sample was injected 5 times and the retention times were same. When the concentrations of Pantoprazole and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2=0.999) was observed between the concentration of Pantoprazole and the respective peak areas in the range 10-50µg/ ml. The regression equation was used to estimate the amount of Pantoprazole, either in tablet

formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table: 2.

To analyse tablet formulations, RP-HPLC method has been developed. Pantoprazole tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries found in the range of 99.1-100.33%%. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

CONCLUSION

The proposed RP-HPLC method was also validated for intra and inter-day variation. When the solution containing 30µg/ml of Pantoprazole was repeatedly injected on the same day, the % RSD in the peak area for six replicate injections was found to be 0.78%. Also the inter day variation (6 days and six injections) was found to be 0.61%. The results are presented in Table:3. The % RSD values were within 2 and the method was found to be precise. It can concluded the proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Pantoprazole and can be reliably adopted for routine quality control analysis of Pantoprazole in Bulk and its pharmaceutical formulations.

ACKNOWLWDGEMENT

I would like thank to my Guide and Principal Dr. Gampa Vijayakumar and Sura Labs, Dilsukhnagar, Hyderabad, T.S for providing reference sample of Pantoprazole to facilitate this work and also for providing facilities to carried my work.

REFERENCES

Journal References

1. B. Siddartha and I. Sudheer Babu. Analytical method development and method validation for the estimation of pantoprazole in tablet dosage form by RP-HPLC. Scholars Research Library. Der Pharma Chemica., 2013; 5(4): 99-104.
2. Kareti Srinivasa Rao1, Nargesh Kumar Keshar1,Prasenjit Roy Choudhury and Ajay Kumar Pattnaik. RP-HPLC Method for the Estimation of Pantoprazole Sodium.International journal of pharma medicine and biological sciencs. Vol. 1, No. 1, July 2012.

3. M. Rama Chandraiah and Y.V Rami Reddy. Method Development and Validation of HPLC for the Determination and Quantification of Pantoprazole. Bulletin of Environment, Pharmacology and Life Sciences., 2012; 1[8]: 39 – 42.
4. G. Manasa, n. Anusha development and validation of rp-hplc method for the simultaneous estimation of pantoprazole and mosapride in capsule dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 4, Suppl 5, 2012
5. Prasanna Reddy.B, Mathsa.Jayaprakash, Kotta.Sivaji, G.T.Jyothesh Kuamr, E.C.Surendranath Reddy4 and B.Ravindra Reddy. Determination Of Pantoprazole Sodium And Lansoprazole In Individual Dosage Form Tablets By Rphplc Using Single Mobile Phase. International Journal of Applied Biology and Pharmaceutical Technology, Volume: I: Issue-2: Aug-Oct -2010.
6. Krishna R. Gupta, Rajesh B. Chawala, Sudhir G. Wadodkar. Stability indicating RP-HPLC method for simultaneous determination of pantoprazole sodium and itopride hydrochloride in bulk and capsule, Orbital - The Electronic Journal of Chemistry.Vol 2 No. 3 July-Seo 2010
7. G. Srinivasa Rao,A. B. N. Nagewara Rao, G. Rohini Reddy,MD. Abdul Shoeb, Ojeyemi M. Olabamiwo1 and J. V. L. N. Seshagiri Rao. RP-HPLC method for simultaneous estimation of domperidone and pantoprazole in capsule dosage form. Scholars Research Library. Der Pharmacia Lettre., 2012; 4(6): 1712-1720.
8. Prasanna Reddy.Battu Simultaneous HPLC Estimation o Pantoprazole and Domperidone from Tablets, International Journal of ChemTech Research. Vol.1, No.2, pp 275-277
9. Patel BH , Suhagia BN, Patel MM, Patel JR. Simultaneous estimation of pantoprazole and domperidone in pure powder and a pharmaceutical formulation by high-perfomance liquid chromatography and high-performance thin-layer chromatography methods. Simultaneous estimation of pantoprazole and domperidone in pure pow... - PubMed – NCBI, Jan-Feb; 90(1): 142-6.
10. Sivakumar Thanikachalam, Manavalan Rajappan, Valliappan Kannappan, Stability-Indicating HPLC Method for Simultaneous Determination of Pantoprazole and Domperidone from their Combination Drug Product, Chromatographia. Volume 67, Issue 1-2, pp 41-47

Books References

11. Government of India, Ministry of health and family welfare, Indian Pharmacopoeia, Vol-I & II, The Controller of Publication, New Delhi, 2010.

12. The United States pharmacopoeia, USP 34 NF 29, Vol- III United States pharmacopoeia convention, lnc, Rockville, 2011.

Websites

13. http://www.drugbank.ca/drugs/DB00213
14. https://en.wikipedia.org/wiki/Pantoprazole
15. http://www.medicinenet.com/pantoprazole/article.htm
16. http://www.rxlist.com/protonix-drug.htm
17. http://www.scbt.com/datasheet-204831-pantoprazole-sodium.html