International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

Analytical Method Development and Validation for the Estimation of Pantoprazole by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

Y. Krishna Reddy*, D. Ramesh, D. Kowshik, E. Manisha, K. Manisha, G. Kiranmayi

Bomma Institute of Pharmacy, Khammam, Telangana, India

A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Pantoprazole by using thermosil C_{18} 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 4.35 mins. The % purity of Pantaprazole was found to be 99.87%. The system suitability parameters for Pantaprazole such as theoretical plates and tailing factor were found to be4146, 1.23, the resolution was found to be 5.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Pantaprazole was found in concentration range of 30µg-150µg and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilty.LOD value was 2.97 and LOQ value was 9.92.Hence the suggested RP-HPLC method can be used for routine analysis of Pantaprazole in API and Pharmaceutical dosage form.

Keywords: Pantoprazole, HPLC, LOD, LOQ

A R T I C L E I N F O

CONTENTS

Article History: Received 19 March 2016, **Accepted** 10 May 2016, **Available Online** 27 July 2016

***Corresponding Author**
Y. Krishna Reddy
Bomma Institute of Pharmacy,
Khammam, Telangana, India
Manuscript ID: IJCPS3042

PAPER-QR CODE

Citation: Y. Krishna Reddy, *et al.* Analytical Method Development and Validation for the Estimation of Pantoprazole by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form. *Int. J. Chem, Pharm, Sci.*, 2016, 4(7): 371-375.

1. Introduction

Analytical methods: Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision

and ruggedness [1, 2]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate[7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC.

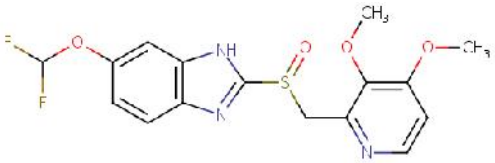

Figure 1: Pantoprazole

2. Experimental

Apparatus: The instrument used for the study was Waters

Apparatus: The instrument used was WATERS HPLC Auto Sampler, separation module 2695, photo diode array detector 996, Empower-software version-2. The solvents used were Potassium dihydrogen orthophosphate, Orthophosphoric acid, Methanol, Acetonitril, and Water

Selection of detection wavelength:

10 mg of was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained .The overlay spectrum was used for selection of wavelength for Pantaprazole [10].

Selection of mobile phase

Water: Methanol (35:65) as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved [11].

Optimization Chromatographic trials for Estimation of Pantoprazole by RP- HPLC.

Optimization Chromatographic conditions

Column : Thermosil C18 4.6×150mm 5.0µm
Mobile phase ratio : methanol: water (65: 35 % v/v)
Detection wavelength : 265 nm
Flow rate : 1 ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature: Ambient
Run time : 6.0mins
Retention time : 4.5

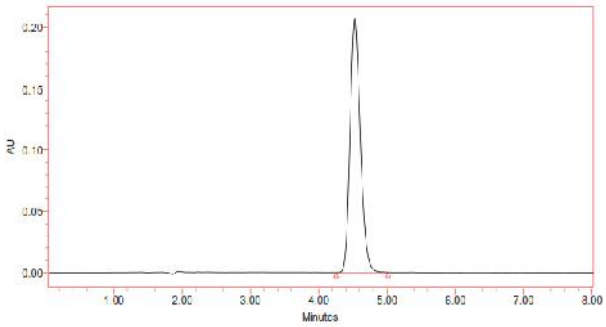

Figure 2: Optimization Chromatogram

Observation: The chromatogram is perfect with clear

Observation: The retention of peak was good, resolution was good, tailing factor was less than 2, theoretical plates were more than 2000, and this trial was taken as optimized method.

Procedure

Preparation of the individual standard preparation

10 mg of working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.9 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluents [12].

Preparation of the Pantaprazole sample solution

Sample solution preparation

10 mg equivalent Pantaprazole capsule powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 1ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluents [13].

3. Results and Discussion

Method Validation Parameters

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of

any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

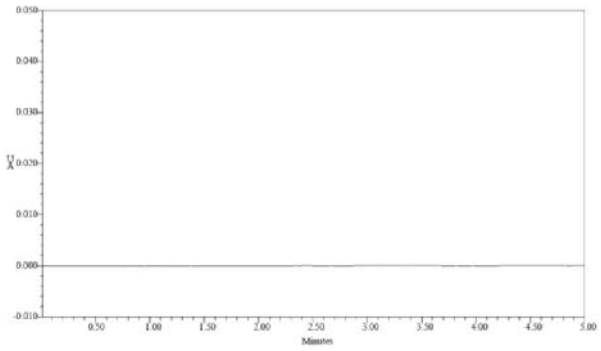

Figure 3: Chromatogram of Blank

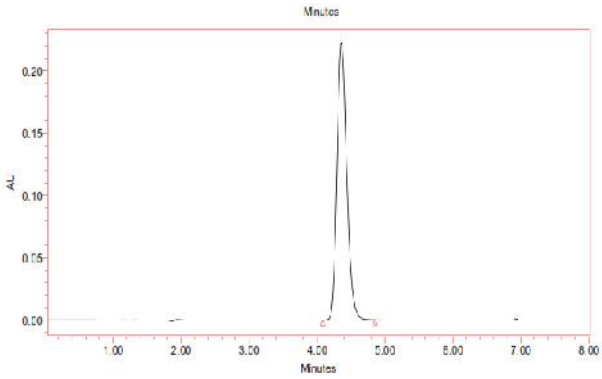

Figure 4: Chromatogram of Sample

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity study was performed for the concentration of 30 ppm to 150 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient [15].

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

The linearity study was performed for the concentration of 30ppm to 150ppm. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient [16].

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

Standard addition method:

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

Percentage method:

For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively [17].

Acceptance criteria:

The mean % recovery of the Pantoprazole 100.8 should be not less than 95.0% and not more than 105.0%.

5. Precision

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported [18].

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Validation of the Method

Linearity: The linearity study was performed for the concentration of 30ppm to105 for Pantoprazole and chromatograms are shown below.

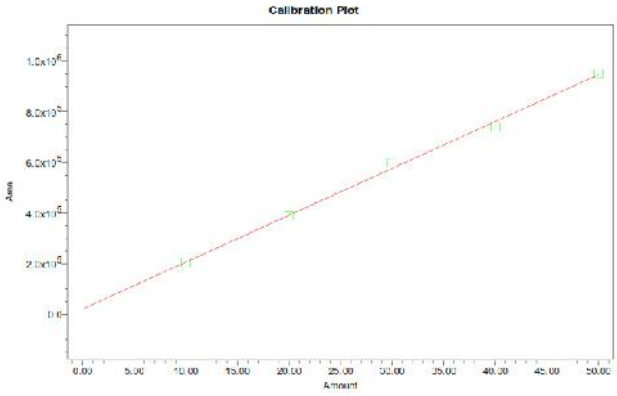

Figure 5: Calibration graph of Pantoprazole

Recovery studies

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Detection Limit

The LOD was performed for Ceritinib was found to be 2.97 respectively

Quantitation Limit

The LOQ was performed for Ceritinib was found to be 9.92 respectively.

Table 1: Calibration data of Pantoprazole

Name	Rt	Area
Pantaprazole	5.745	201932
Pantaprazole	6.019	338071
Pantaprazole	5.891	597859
Pantaprazole	6.030	740654
Pantaprazole	6.177	950396
Co efficient of correlation (R^2)		0.997

Table 2: Accuracy results for Pantoprazole

% Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1508773	5	5.14	100.2%	100.4%
100%	1866573	10	10.01	98.8%	
150%	1942321	15	15.2	96.5%	

Table 3: System suitability results for Pantoprazole (Flow rate)

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	10 % less	4331	1.20
2	*Actual	4024	0.87
3	10% more	3693	1.26

Table 4: Repeatability results for Pantoprazole

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	pantaprazole	4.333	2230902	224720	4386	1.2
2	pantaprazole	4.333	2220937	223716	4368	1.2
3	pantaprazole	4.332	2218145	222122	4326	1.2
4	pantaprazole	4.331	2209284	221845	4369	1.2
5	pantaprazole	4.330	2230758	224645	4385	1.2
6	pantaprazole	4.327	2236792	226354	4428	1.2
Mean		4.3	2224470			
Std. Dev.		0.0	10157.3			
% RSD		0.1	0.5			

Table 5: Intermediate precision for Pantoprazole

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	pantaprazole	4.333	2230902	224720	4386	1.2
2	pantaprazole	4.333	2220937	223716	4368	1.2
3	pantaprazole	4.332	2218145	222122	4326	1.2
4	pantaprazole	4.331	2209284	221845	4369	1.2
5	pantaprazole	4.330	2230758	224645	4385	1.2
6	pantaprazole	4.327	2236792	226354	4428	1.2
Mean		4.3	2224470			
Std. Dev.		0.0	10157.3			
% RSD		0.1	0.5			

Table 6: The LOD was performed for Pantoprazole was found to be 2.97 respectively.

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Pantaprazole	371827.90	563365963	2.97

Table 7: The LOQ was performed for Pantoprazole was found to be 9.92 respectively.

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Pantaprazole	371827.90	563365963	9.92

4. Conclusion

A new method was established for estimation of Pantaprazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Pantaprazole by using thermosil C18 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 4.35 mins. The % purity of Pantaprazole was found to be 99.87%. The system suitability parameters for Pantaprazole such as theoretical plates and tailing factor were found to be4146, 1.23, the resolution was found to be 5.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Pantaprazole was found in concentration range of 30µg-150µg and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilty.LOD value was 2.97 and LOQ value was 9.92.Hence the suggested RP-HPLC method can be used for routine analysis of Pantaprazole in API and Pharmaceutical dosage form.

5. References

[1] Becket And Stenlake, Practical Pharmaceutical Chemistry, Part 24th Edition Cbs Publications And Distributors, 2005, 157-168.

[2] P.D. Sethi, Hplc Quantitative Analysis of Pharmaceutical Formulations CBS Publications and Distributors, 1st Edition, 2001, 69-70.

[3] B.K Sharma, Instrumental Method of Chemical Analysis, 23rd Edition, Goal Publishers 2004.

[4] Practical Hplc Method Development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, Second Edition, 1, 420-430,686-704.

[5] Validating Chromatographic Methods, David M. Bliesner. 1-4.

[6] International Conference on Harmonization**:** ICH Q2 (R1) Validation of Analytical Procedures: Text and Methodology 1995.

[7] Indian Pharmacopeia 2007 Vol –I Pg.No-715.

[8] British Pharmacopeia 2007 Vol-I Pg.No-136.

[9] Martindale the Complete Drug Reference, Thirty Sixth Editions. Merck Index, 12th Edition.

[10] N. Sreekanth, International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Suppl 1, Nov.-Dec. 2009.

[11] Ch. Naveen Kumar et al, Scholars Research Library Der Pharmacia Lettre, 2014, 6 (5):339-351)

[12] Olivier Heudi et al, Anal Bioanal Chem 2014 Nov 26; 406 (28):7389-96. Epub 2014 Sep 26.)

[13] Heudi O, Anal Bioanal Chem. 2014 Nov; 406(28):7389-96.

[14] Hardik Patel et al (2012). (*International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Suppl 1, Nov.-Dec. 2009)

[15] Ms Palled, M Chatter, Pmn Rajesh, And Ar Bhatt "Difference Spectrophotometric Determination of Pantoprazole In Tablet Dosage Forms,Indian J Pharm Sci 2006;68:685-686.

[16] K Raja Rajeswari, Gg Sankar, Al Rao, and Jvln Seshagirirao "RP-HPLC Method for the Determination Of Pantoprazole In Tablet Dosage Form", Indian J Pharm Sci 2006; 68: 275-277.

[17] A. Zarghi, S.M. Foroutan, A. Shafaati And A. Khoddam "Validated Hplc Method For Determination Of ceritinib In Human Plasma and Its Application to Pharmacokinetic Studies" Il Farmaco. 2005, 60(9): 789-792.