International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 737-741, April-June 2011

Development of Spectrophotometric methods for the estimation of Pyrazinamide in Bulk and Pharmaceutical formulations

Ganga Prasad Chenna*, Sathish Kumar Shetty, Jyoti B.Pai, Gopinath B, Manzoor Ahmed**

Department of Pharmaceutical Analysis, National College of Pharmacy, Balarj Urs Road, Shimoga (District), Karnataka, India – 577 201.

Corres.authors: drskshetty@rediffmail.com ** ,gangaprasadchenna@gmail.com* Cell:09449100007,Fax:08182 273796.

Abstract: Two simple, accurate and precise spectrophotometric methods have been developed for Pyrazinamide in bulk and pharmaceutical dosage form. Pyrazinamide has absorbance maxima at 269nm.Method A is area under curve (AUC) method, which involves the calculation of integrated value of absorbance with respect to wavelength between 264-274nm. Method B involves the derivatisation of the primary absorption spectra to second order and by the examination of the second derivative spectra of Pyrazinamide, 270 nm (λ) was selected as working wavelength. Linearity range was found to be 2-16 µg/ml for method A and Beer's law is obeyed in the concentration range of 2-16µg/ml for method B. The results of analysis were found to be satisfactory by validation with recovery studies. The additives and common excipients did not interfere in their determination.
Keywords: Pyrazinamide, AUC, Derivative.

Introduction

Pyrazinamide is chemically pyrazine-2-carboxamide and the structural formula is shown in Fig: 1.The molecular formula is $C_5H_5N_3O$ and molecular weight is 123.1g/mol. It occurs as a white crystalline powder that is soluble in water, chloroform, slightly soluble in ethanol (95 percent) and very slightly soluble in polar organic solvents. Pyrazinamide has been elevated to first-line status in short-term tuberculosis treatment regimens because of its tuberculocidal activity and comparatively low short-term toxicity.[1-4] It is an official drug in Indian Pharmacopoeia 2007,[5] British Pharmacopoeia 2005,[6] and United state pharmacopoeia 2007.[7] From the literature survey, it was found that Pyrazinamide was estimated by analytical methods such as few UV-Visible methods[8-10] , Reverse-phase high-performance liquid chromatographic (RP-HPLC) method [11-15],gas chromatography[16] and UPLC method[17]. The present developed method was simple, precise, specific and accurate.

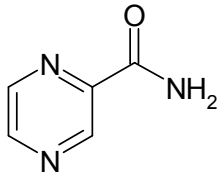

Fig: 1. Chemical Structure of Pyrazinamide

Experimental

Instrument used and reagents

For both the methods, Shimadzu model 1700 double beam UV-VIS spectrophotometer with spectral

bandwidth of 1.8nm, wavelength accuracy of 2nm and a pair of 1 cm matched quartz cells of 10 mm optical path length was used as an instrument for spectral measurements. An analytically pure sample of Pyrazinamide was procured as a gift sample from Arch pharmalabs Limited, Mumbai, India. Double distilled water was used as solvent. Tablet formulation of Pyrazinamide (500 mg) was collected from local market.

Preparation of working standard drug solution

Pure Pyrazinamide powder equivalent to 100 mg was accurately weighed and dissolved in 40 ml of water in a 100 ml volumetric flask and the volume was made up to the mark with water to obtain a final concentration of 1000 µg/ml (stock A solution). From stock 'A', 10 ml of aliquot was pipetted out in a 100 ml volumetric flask and the volume was made up to the mark to obtain a final concentration of 100 µg/ml (stock B solution).

Preparation of marketed formulations

Twenty tablets of Pyrazinamide each containing 500 mg were accurately weighed, average weight was determined and crushed into fine powder and a quantity equivalent to 100 mg of Pyrazinamide was transferred into 100 ml volumetric flask and dissolved in 40 ml of water and sonicated for 5 min. The solution was filtered through Whatmann filter paper no.41. The residue was washed with 10 ml portions of water, three times and the total volume of the filtrate was made up to 100 ml with water to obtain 1000 µg/ml (stock A' solution). From the above stock A' solution 10 ml of aliquot was pipetted out in a 100 ml volumetric flask and the volume was made up to the mark to obtain the final concentration of 100 µg/ml (stock B' solution). From the stock solution B', various dilutions of the sample solution were prepared and analysed.

Calibration curve

Method A: Area Under Curve Method[17]

The AUC (Area Under Curve) method involves the calculation of integrated value of absorbance with respect to wavelength between 264nm to 274 nm (Fig: 2). The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area and concentration. Suitable dilutions of standard stock solution (100µg/ml) of the drug were scanned in the wavelength range 400-200 nm and the calibration curve was plotted (Fig: 3).

Method B: Second Order Derivative Spectroscopic method[17]

The standard drug solution was prepared and scanned between the wavelength range of 400 – 200 nm in the concentration range of 2-16µg/ml(Fig: 4 and 5) where the second order derivative spectra showed sharp dip at 270 nm when n=2. The calibration curve of $d^2A/d\lambda^2$ against concentration was plotted. Similarly absorbances of sample solutions were measured and the amount of Pyrazinamide was determined from standard calibration curve.

Validation of methods[18]

The above methods were satisfactory in accordance to the ICH guidelines. Accuracy studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to previously analyzed tablet sample and the percentage recovery was calculated **(Table 1).**

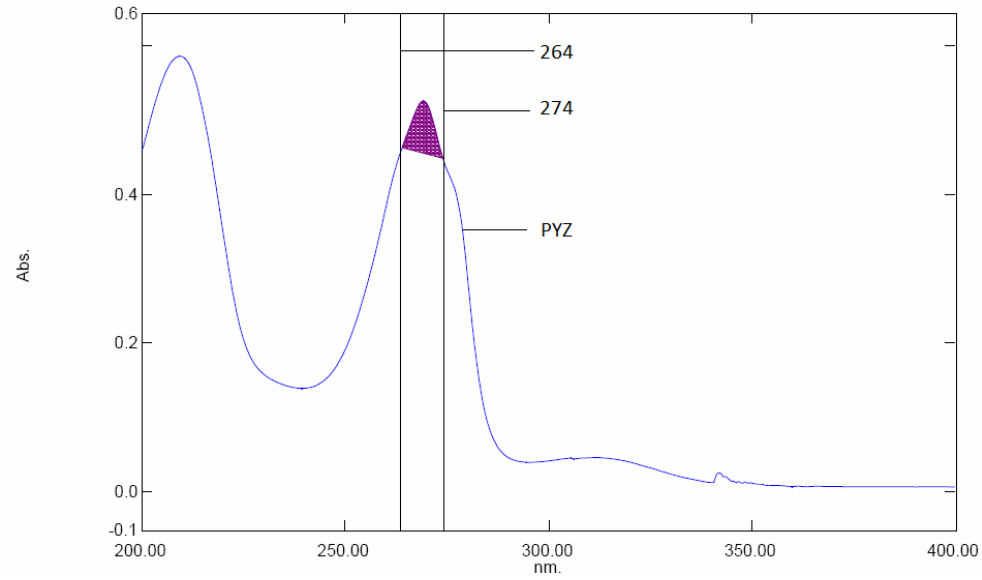

Fig: 2. Area under curve spectra of Pyrazinamide

Table 1: Optical characteristics and Other Parameters

PARAMETERS	RESULTS	
	METHOD A	METHOD B
Absorption Maxima (nm)	264-274	270
Beer's-Lambert's range (µg/ml)	2-16	2-16
Regression equation (y)* Slope (m) Intercept (c)	 0.052 0.001	 -0.001 0.0002
Correlation coefficient	0.9998	0.9995
Sandell's sensitivity (mcg / cm^2-0.001 absorbance units)	0.019	-0.7352
Molar absorptivity (L mol^{-1} cm^{-1})	0.0645×10^3	-0.0167×10^3
Precision (% RSD) Intraday precision Interday precision	 0.690 0.757	 0.47 1.09
Accuracy(% RSD) 80% 100% 120%	 0.0417 0.0382 0.0327	 0.1527 0.1577 0.2466
Limit of detection (µg / ml)	0.03	0.25
Limit of quantification (µg / ml)	0.11	0.76

*y = mx + c; when x is the concentration in mg/ml and y is absorbance unit.

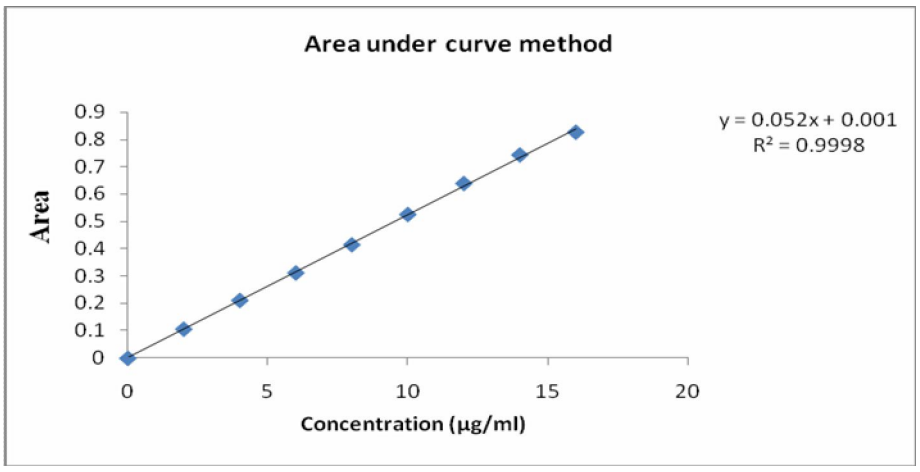

Fig: 3. Calibration curve for Pyrazinamide between 264-274 nm in water by Area under curve method.

Table 2: Analysis of tablet formulation

METHOD	Tablet	Label claimed (mg)	Amount found (mg)	%Recovery ± SD**
A	BRAND I	500	499.01	99.01±1.13
	BRAND II	500	498.89	99.77±0.35
B	BRAND I	500	499.91	99.98±0.041
	BRAND II	500	499.96	99.99±0.382

**Average of six determinations

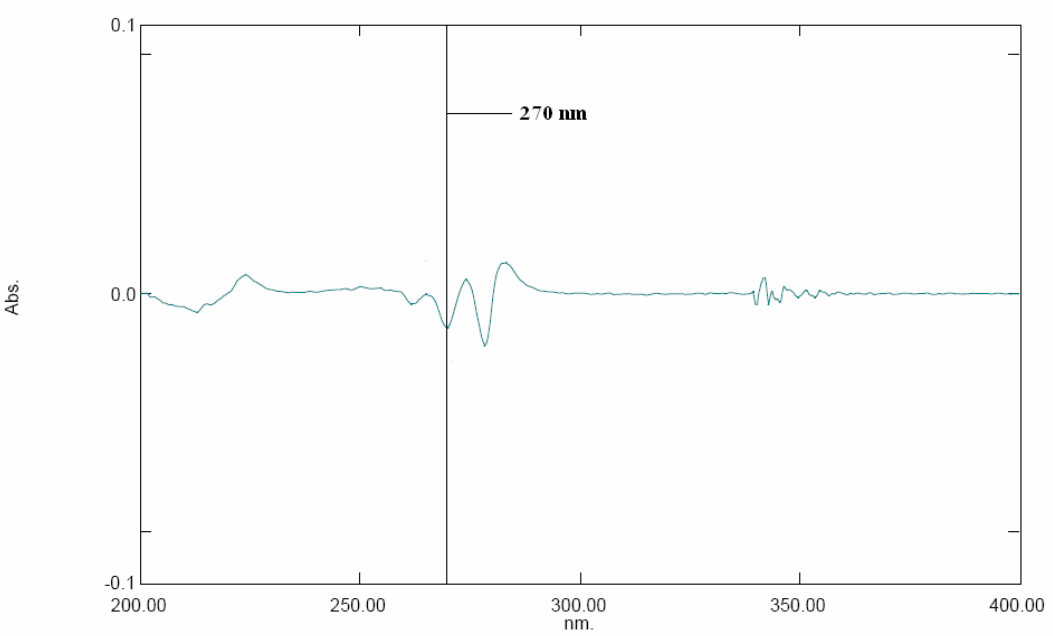

Fig: 4. Second order derivative spectra of Pyrazinamide.

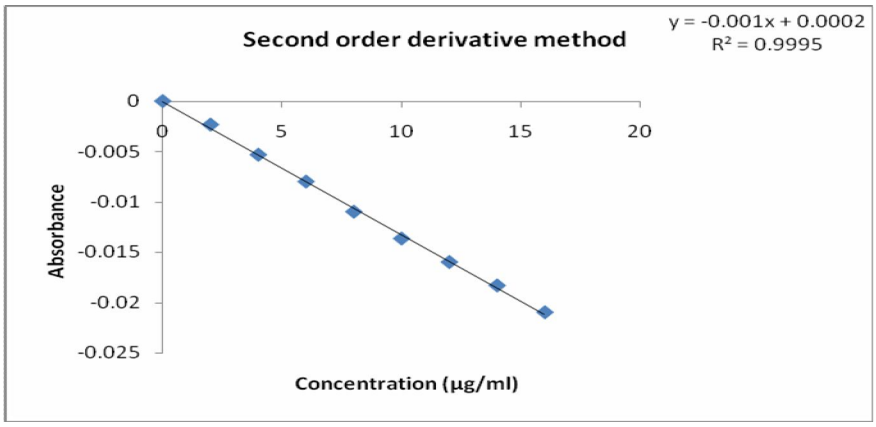

Fig: 5. Calibration curve for Pyrazinamide at 270 nm in water by Second order derivative method.

Result and Discussion

The absorption spectra of Pyrazinamide were recorded in the wavelength range of 200-400 nm for both the methods. The spectra were reported as Fig: 2 and 3.The optical characteristics such as Beer's law limits, molar absorptivity, sandell's sensitivity, percentage relative standard deviation and range of errors in each method were calculated and the results were reported in Table [1]. Also the regression characteristics like slope (m), intercept (c), and correlation coefficient (r) were calculated and are presented in Table 1. The results showed that the methods have reasonable precision. The assay results obtained for the both the methods are summarized in Table 2 and the accuracy of the methods were confirmed by the recovery studies by adding known amount of the pure drug to the pharmaceutical formulation previously analyzed by this method.

Conclusion

Thus, it can be concluded that the method developed in the present investigation was economical, simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Pyrazinamide in pharmaceutical dosage forms..

Acknowledgement

We would like to thank Arch pharmalabs Limited Mumbai, India, for providing reference sample of Pyrazinamide to facilitate this work.

References

1. Block J.H. and Beale J.M., Wilson and Gisvold's Textbook of Organic Medicinal & Pharmaceutical Chemistry, 11th ed, Philadelphia, Lippincott Williams & Wilkins, Wolters Kluner Company, 2004, 254-256.
2. Neil M.J., The Merck Index-an Encyclopedia of Chemicals, Drugs and Biologicals, 13th ed, New Jersey, Merck & Co, 2001, 8046.
3. Sweetmann S.C., Martindale-The Complete Drug Reference, 33rd ed, London, Pharmaceutical press, 2002, 913.
4. Joel G.H., Lee E.L., Perry B.M., Raymond W.R. and Alfred C.G., Goodman Gilman's the pharmacological basis of therapeutics, 10th ed, New york, McGraw Hill medical publishing, 2001, 1281.
5. Indian Pharmacopoeia, Ghaziabad, The Indian Pharmacopoeia Commission, 2007, Vol III, 1622-1623.
6. British Pharmacopoeia, Her Majesty's Stationery Office, London, 2005, Vol II, III, 1697, 2761.
7. The United States Pharmacopoeia, 23 NF 18. Rockville, MD, United States Pharmacopoeial Convention, Inc, 1995, 1344.
8. Khuhawar M.Y. and Rind F.M.A., Liquid chromatographic determination of Isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood, J. Pak Pharm Sci 2002,766(2):357-363.
9. Héctor C., Goicoechea and Alejandro C., Simultaneous determination of rifampicin isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration. J. Pharm. & Bio. Analysis, 1999, 20(4):681-686.
10. Martin A., Takiff H., Vandamme P., Swings J., Carlos J., Palominol and Portaels F.C., A new rapid and simple colorimetric method to detect Pyrazinamide resistance in Mycobacterium tuberculosis using nicotinamide, J . Antimicrobial Chemotherapy, 2006 June 2.
11. Calleri E., Lorenzi E.D., Furlanetto S., Massolini G and Caccialanza., Validation of a RP-LC method for the simultaneous determination of isoniazid, pyrazinamide and rifampicin in a pharmaceutical formulation, J. Pharm. & Biomed. Analysis, 2002 Aug 1, 29(6):1089-1096.
12. Mariappan T.T., Singh B., Singh S.A.,Validated Reversed-Phase (C_{18}) HPLC Method for Simultaneous Determination of Rifampicin, Isoniazid and Pyrazinamide in USP Dissolution Medium and Simulated Gastric Fluid, Pharmacy and Pharmacology Communications, 2000 Aug 6(8):345-349.
13. Panchagula R., Sood A., Sharda N., Kaur K. and Kaul C.L., Determination of rifampicin and its main metabolite in plasma and urine in presence of pyrazinamide and isoniazid by HPLC method, J. Pharm. & Biomed. Analysis, 1999 Jan,18(6):1013-1020.
14. Glass B.D., Kustrin A., Chen Y.J. and Wisch M.H., Optimization of a Stability-Indicating HPLC Method for the Simultaneous Determination of Rifampicin, Isoniazid, and Pyrazinamide in a Fixed-Dose Combination using Artificial Neural Networks, J. Chrom. Sci., 2007 Jan, 45(1):38-44.
15. Husain S., Sarma P.N., Sajjad S.M., Narsimha R. and Subrahmanyam M., Determination of 2-methylpyrazine and pyrazinamide in reaction mixtures by gas and high-performance liquid chromatography, J. Chrom. A,1990 Mar 13, 83-90.
16. Nguyen D.T., Guillarme D., Rudaz S., Veuthey J., Validation of an ultra-fast UPLC-UV method for the separation of antituberculosis tablets, J. separation Sci., 2008 Apr 31(6-7):1050-1056.
17. Beckett A.H., Stenlake J.B., Practical pharmaceutical chemistry, 1st ed, Delhi, CBS Publishers and Distributors, 1988, 296-300.
18. ICH Harmonised Tripartite Guideline, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October1994, 1-5.

*****