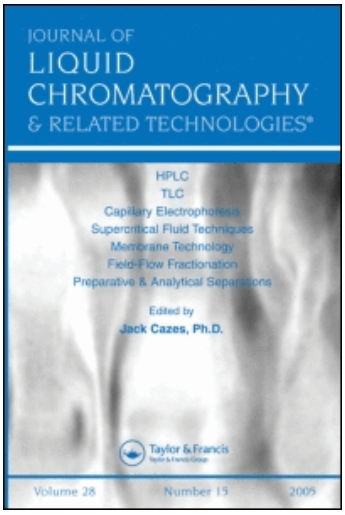

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Rapid Simplified Analysis of Pyrazinamide in Rat Plasma by HPLC

J. S. Kaka[a]
[a] Department of Clinical Pharmacy, College of Pharmacy King Saud University, Riyadh, Saudi Arabia

To cite this Article Kaka, J. S.(1994) 'Rapid Simplified Analysis of Pyrazinamide in Rat Plasma by HPLC', Journal of Liquid Chromatography & Related Technologies, 17: 17, 3793 — 3801
To link to this Article: DOI: 10.1080/10826079408013994
URL: http://dx.doi.org/10.1080/10826079408013994

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(17), 3793-3801 (1994)

RAPID SIMPLIFIED ANALYSIS OF PYRAZINAMIDE IN RAT PLASMA BY HPLC

J. S. KAKA
Department of Clinical Pharmacy
College of Pharmacy
King Saud University
P.O. Box 2457
Riyadh 11451, Saudi Arabia

ABSTRACT

An HPLC method for quantitative estimation of pyrazinamide in rat plasma has been developed. The procedure consists of precipitation of plasma proteins with methanol containing 5-fluorouracil as an internal standard. The mixture was vortexed for 1 min. and centrifuged at 4000 rpm for 10 min. Twenty-five microliters of the supernatant was eluted on μ-Bondapack C_{18} column at 269 nm. The mobile phase consisted of 2% v/v acetonitrile in 5 mM potassium phosphate buffer at pH 4. The flow-rate was set at 1 ml per min. The retention times for 5-fluorouracil and pyrazinamide were 4 and 9 min. respectively with a detection limit of 0.5 μg/ml for pyrazinamide.

INTRODUCTION

The increase in acquired immunodeficiency syndrome (AIDS) has made tuberculosis an increasing worldwide problem(1,2). The effectiveness of modern chemotherapy

is also blunted by high incidence of primary drug resistance, especially in developing countries(3-5). Since 1952, pyrazinamide has been used in the treatment of tuberculosis(6). Excessive doses led to hepatic toxicity, which discontinued its use (7,8). Recently, there has been renewed interest in pyrazinamide use against Mycobacterium tuberculosis and Mycobacterium leprae(9,10). In AIDS and tuberculosis, a combination of drugs are to be used in conjunction with pyrazinamide for an effective outcome(9,11). In addition, pyrazinamide is to be used for at least six months for successful treatment(11,12).

Limited numbers of HPLC analytical methods have been reported for pyrazinamide in biological fluids. Lacroix and Yamamotos' methods require a fluorimetric apparatus(13,14). The Acocelli et al. procedure involves extraction, evaporation and at least 0.5 ml of plasma for analysis(15). The method described by Woo et al. requires extraction, a gradient programmable pump and one of the effluents require 60% acetonitrile (16). The procedure by Hammiche et al. is similar but requires filtration and 5% acetonitrile as mobile phase(17).

The purpose of the present report is to describe a simple and sensitive HPLC technique for quantitative determination of pyrazinamide in rat plasma.

MATERIALS

Pyrazinamide was purchased from Wilson Labs., Bombay, India and 5-florouracil was obtained from H. Roche, Basel, Switzerland. Acetonitrile, methanol and di-potassium hydrogen phosphate were purchased from BDH Chemicals Ltd., Poole, U.K.

METHODS

Stock Solutions:

Pyrazinamide solution was prepared by dissolving 10 mg in 10 ml of methanol. 5-Fluorouracil (5 mg) was dissolved in 100 ml of methanol. Potassium phosphate buffer (5 mM) was prepared and adjusted to pH 4 with phosphoric acid. All stock solutions were stored at $4^{o}C$.

Chromatographic conditions:

The HPLC unit consisted of a Waters Assoc. Model M-45 solvent delivery system, a Waters Assoc. Model 450 variable wavelength detector, a Waters Assoc. Model 46K universal liquid chromatograph injector and BBC Goerz Metrawatt SE120 recorder. The column used was a stainless steel Waters Assoc. μ-Bondapack C_{18} (30 cm x 8mm I.D., 10 μm particle size) column. The mobile phase was 5 mM potassium phosphate buffer-acetonitrile mixture at pH 4, with a final acetonitrile concentration of 2% v/v. The flow-rate was set at 1 ml/min. The

effluent was monitored at 269 nm with a detection scale of 0.1 or higher as needed. All measurements were performed at room temperature.

Sample Preparation:

To a 100 μl of plasma aliquot, 100 μl of methanol containing 5-fluorouracil was added. The mixture was vortexed for 1 min and centrifuged at 4000 rpm for 10 min. Twenty-five microliters of supernatant was injected onto the column.

Standard Calibration Curve:

Blank rat plasma aliquots were spiked with a pyrazinamide standard solution to get concentrations ranging from 2.0 to 40 μg/ml. 5-Fluorouracil in methanol was used as an internal standard. Each point was an average of four replicates.

Precision studies:

Rat plasma samples at concentrations 2, 20, and 40 μg/ml were analyzed on five separate days in order to determine the between day-to-day coefficient of variation. For elucidation of the within-day variability, five specimens of the same sample were analyzed on each day at the above mentioned concentrations.

Animal study:

Pyrazinamide disposition was assessed in a pilot study, where a single rat received pyrazinamide (25

mg/kg) by iv administration via the tail vein. A polyethylene cannula (1 mm O.D.) was implanted in the left femoral artery and exteriorized from the neck of the animal with enough length for safe handling. Approximately, 0.25 ml of blood was taken before and 10, 20, 30, 45 min. and 1, 2, 3, 4, 6, and 8 hour, respectively after iv administration. Heparinized blood was centrifuged, plasma separated, and aliquots were kept at -20°C until analysis.

RESULTS AND DISCUSSION

Using the chromatographic procedure described, the separation of pyrazinamide and 5-fluorouracil in rat plasma is shown in Fig. 1. The retention times for 5-fluorouracil and pyrazinamide were 4 and 9 min. respectively, with no interfering peaks from the plasma in this region. There was a good separation between 5-fluorouracil and pyrazinamide. Each sample was completely eluted in 10 min.

The calibration curve for pyrazinamide was linear over the range investigated with a correlation coefficient of 0.997. The limit of sensitivity was at least 0.5 μg/ml which was defined as a measure of peak height of 0.5 mm of pyrazinamide by the described procedure. Similar limits of sensitivity were reported by others authors. There is no extraction process in this method, thus recovery studies were not needed.

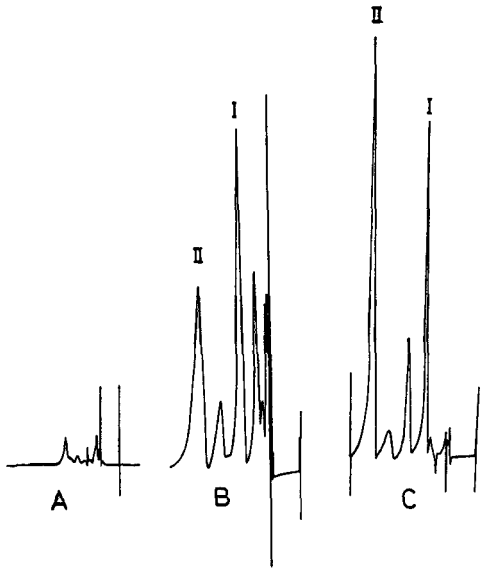

Figure 1. High-pressure chromatographs for a blank rat plasma (A), standard sample (B) containing 5-fluorouracil (I) and pyrazinamide (II) and rat sample (C) after 5 hours of pyrazinamide i.v. administration containing 5-fluorouracil (I) and pyrazinamide (II).

The precision of pyrazinamide assay was assessed by five replicate aliquots of rat plasma spiked with pure pyrazinamide at concentrations 2, 20, and 40 μg/ml respectively. The coefficient of variation for the within-day assay was less than 5%. The coefficient of variation for the day-to-day assay over a week at the above mentioned concentrations were less than 6%.

The assay was applied to determine pyrazinamide plasma concentrations obtained from a pilot

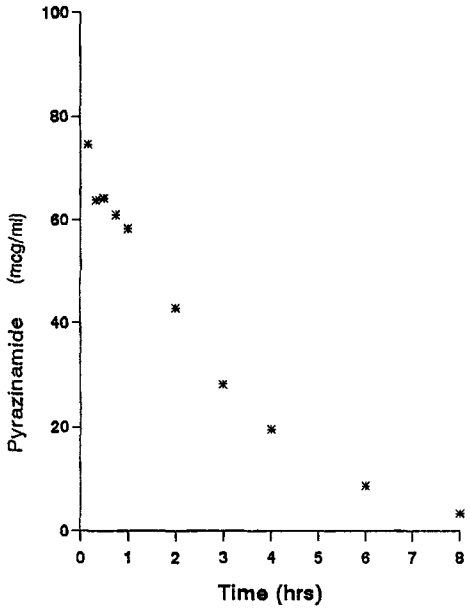

Figure 2. Plot of plasma pyrazinamide concentration after iv administration of pyrazinamide (25 mg/kg) to rat.

pharmacokinetic rat study. Fig. 2. shows the plot of pyrazinamide plasma concentrations following iv administration.

The advantages of this assay over other earlier published methods are that it involves no extraction process, only 2% v/v acetonitrile mobile phase at 1.0 ml/min, and requires only 100 μl of rat plasma without compromising the minimum detectable limits for pyrazinamide.

ACKNOWLEDGEMENTS

The author is grateful to A.R. Quriashi for technical assistance.

REFERENCES

1. Anon. Diagnosis and management of mycobacterial infections and disease in persons with human T-lymphotropic virus type III/lymphodenopathy-associated virus infection. MMWR 35: 448-457 (1986).

2. M.M. Furio, C.J. Wordell, Treatment of infectious complication of acquired immunodeficiency syndrome. Clin. Pharm. 4: 539-554 (1985).

3. Anon. Hong Kong Chest Service/British Medical Research Council: Controlled trial of 6-month and 8-month regimens in the treatment of pulmonary tuberculosis: the results upto 24 months. Tubercle 60: 201-207 (1979).

4. K.D. Strottmeier, R.E. Beam, G.P. Kulsica, The determination of drug susceptibility of mycobacteria by pyrazinamide in 7HIO agar. Am. Rev. Respir. Dis. 96: 1072-1077 (1966).

5. Anon. CDC advices on management of person exposed to multiple-drug-resistant tuberculosis. Clin. Pharm. 11: 991-999 (1992).

6. R.L. Yeager, W.G.C. Munroe, F.I. Dessau, Pyrazinamide (Aldinamide) in the treatment of pulmonary tuberculosis. Am. Rev. Tubercle. 65: 523-546 (1952).

7. C.D. Cohen, A.R. Sayed, R.E. Kirsch, Hepatic complications of antituberculosis therapy revisited. S. Afr. Med. J. 63: 960-963 (1983).

8. G. Danan, D. Pessayre, D. Larrey, J.P. Benhamau, Pyrazinamide fulminant hepatitis: an old hepatotoxin strikes again. Lancet 2: 1056-1057 (1981).

9. Anon. Centers for Disease Control and Prevention. Initial therapy for tuberculosis in the era of multidrug resistance: recommendations of the advisory council for the elimination of tuberculosis. MMWR 42 (RR-7): 1-18 (1993).

10. K. Katoch, G. Ramu, U. Ramanathan, Sreevatsa, Pyrazinamide as a part of combination therapy for BL-LL patients - a preliminary report. Int. J. Leprosy. 56: 1-9 (1988).

11. Anon. Hong Kong Chest Service/British Medical Research Council. Controlled trail of four three-times-weekly regimens and a daily regimen all given for 6-months for pulmonary tuberculosis: second report: the results up to 24 months. Tubercle 63: 89 (1982).

12. Anon. British Thoracic Association.: A controlled trail of six months chemotherapy in pulmonary tuberculosis. First report: results during chemotherapy. Br. J. Dis. Chest. 75: 141-153 (1981).

13. C. Lacroix, P. Poncet, G. Laine, C. Guyonnaud, M. Ray, S. Menager, O. Lafont, Microdetermination du pyrazinamide et de ses metabolites (acide pyrazinoique, acide hydroxy-5-pyrazinamide, acide pyrazinurique) plasmatiques et urinaires par chromatographie en phase liquide. J Chromatogr. 422: 217-225 (1987).

14. T. Yamamoto, Y. Moriwaki, S. Takahashi, T. Hada, K. Higashino, Rapid and simultaneous determination of pyrazinamide and its major metabolites in human plasma by high-performance liquid chromatography. J. Chromatogr. 413: 342-346 (1987).

15. G. Acocelli, R. Conti, M. Luisetti, E. Pozzi, C. Grass, Pharmacokinetic studies in antituberculosis regimens in humans. I. Absorption and metabolism of the compounds used in the initial intensive phase of the short-course regimens: Single administration study. Am. Rev. Respir. Dis. 132: 510-515 (1985).

16. J. Woo, C. 1. Wong, R. Teoh, K. Chan, A liquid chromatographic assay for simultaneous determination of pyrazinamide and rifampin in serum from patients with tuberculosis meningitis. J. Chromatogr. 420: 73-80 (1987).

17. Y. Hammichi, M.H. Guermouche, K. Abdeddaim, M. Daoud-Brixi, HPLC analysis of pyrazinamide in rat serum. Analusis 17: 394-397 (1989)

Received: February 21, 1994
Accepted: May 6, 1994