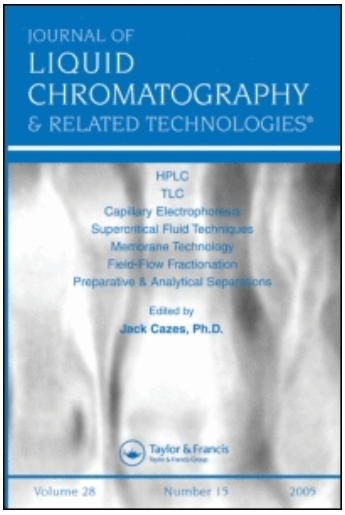

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Simple and Sensitive HPLC Method for Antipyrine in Plasma

J. Shen[a]; S. Wanwimolruk[a]
[a] School of Pharmacy Univeristy of Otago, Dunedin, New Zealand

To cite this Article Shen, J. and Wanwimolruk, S.(1991) 'A Simple and Sensitive HPLC Method for Antipyrine in Plasma', Journal of Liquid Chromatography & Related Technologies, 14: 14, 2801 — 2808
To link to this Article: DOI: 10.1080/01483919108049357
URL: http://dx.doi.org/10.1080/01483919108049357

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(14), 2801-2808 (1991)

A SIMPLE AND SENSITIVE HPLC METHOD FOR ANTIPYRINE IN PLASMA

J. SHEN AND S. WANWIMOLRUK*
School of Pharmacy
Univeristy of Otago
P.O. Box 913
Dunedin, New Zealand

ABSTRACT

A rapid, simple and sensitive high-performance liquid chromatographic (HPLC) method was developed for the determination of antipyrine in small volume (50 μl) of plasma samples. Aminopyrine was employed as the internal standard. The sample preparation is a direct plasma protein precipitation procedure so is less tedious and rapid. The assay employs a column packed with a C_{18} reversed-phase material (5 μm Nucleosil) with an isocratic mixture of acetonitrile and water (25:75, v/v) as the mobile phase. The eluant was detected at 254 nm. The assay achieves the level of sensitivity (0.5 μg/ml) and accuracy required to obtain meaningful data about the single-dose pharmacokinetics of antipyrine in guinea pig and rat. The method gave high reproducibility with coefficients of variation less than 5%.

* Corresponding Author

INTRODUCTION

Antipyrine is used as a model drug in studying the influence of genetic factors, disease, drug and environmental factors on the rate of hepatic drug metabolism in humans and laboratory animals (1). It is also used as a reference compound in the investigation of placental drug transfer *in vitro* and *in situ* (2, 3). Antipyrine was selected for these studies on the basis of its simple pharmacokinetic behaviour: first-order kinetics, no first-pass effect and practically no protein binding (4). Many HPLC assays for the determination of antipyrine in biological samples have been published (5-10). However, these methods require an organic solvent extraction and time-consuming preparation of samples. Shargel *et al* (6) described a reversed-phase HPLC method for antipyrine in 0.1 ml of rat plasma samples with methanol-water as mobile phase. These procedures had a problem regarding tailing peak and sensitivity of the assay was only 1 μg/ml. In order to study the placental drug transfer *in situ* and pharmacokinetics in animals with small volume of samples, a rapid, simple and sensitive method of analysis was needed. In this report we have developed a sensitive assay to overcome most of above inconveniences and to minimize peak tailing as much as possible.

MATERIALS AND METHODS

Chemicals

Antipyrine and aminopyrine (the internal standard) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). The HPLC-grade acetonitrile was purchased from BDH Chemicals Ltd. (Poole, UK). Water was double glass distilled and MilliQ^{R} filtered.

Chromatographic Conditions

The HPLC system was consisted of an M6000A Waters pump (Waters Asso., Milford, MA, USA), a 7125 injector (Rheodyne, Cotati, CA, USA) equipped with a 20-µl injection loop. The column was 4.6 mm I.D. x 150 mm stainless steel tubing, packed with 5 µm C_{18} stationary phase, Nucleosil (Shandon, London, UK). An in-line guard column (3.2 x 15 mm), RP-18 Newguard (Brownlee Labs Inc., Santa Clara, CA, USA), was used to protect the analytical column. The absorbance was measured at 254 nm using a Waters Model 441 absorbance detector. A mobile phase of acetonitrile-water (25:75, v/v) was used and run at a flow-rate of 1 ml/min. The chromatogram was recorded on a R-02 model chart recorder (Rikadenki Kogyo Co. Ltd, Japan).

Sample Preparation

To a 50 µl of plasma sample in a 0.5-ml polypropylene Eppendorf® centrifuge tube was added 50 µl of acetonitrile containing 20 µg/ml of aminopyrine as the internal standard. The content was briefly vortexed and then centrifuged for 3 min at 10,000 g. The resultant supernatant of 20 µl was injected onto the HPLC column.

RESULTS AND DISCUSSION

In this study a reversed-phase HPLC method has been developed for the determination of antipyrine in plasma. A mobile phase of acetonitrile-water mixture (25:75, v/v) gave well resolved, sharp peaks of antipyrine and the internal standard (aminopyrine). The retention times of antipyrine and the internal standard were 3.8 and 6.6 min, respectively (Figure 1). Three chromatograms, one obtained from guinea

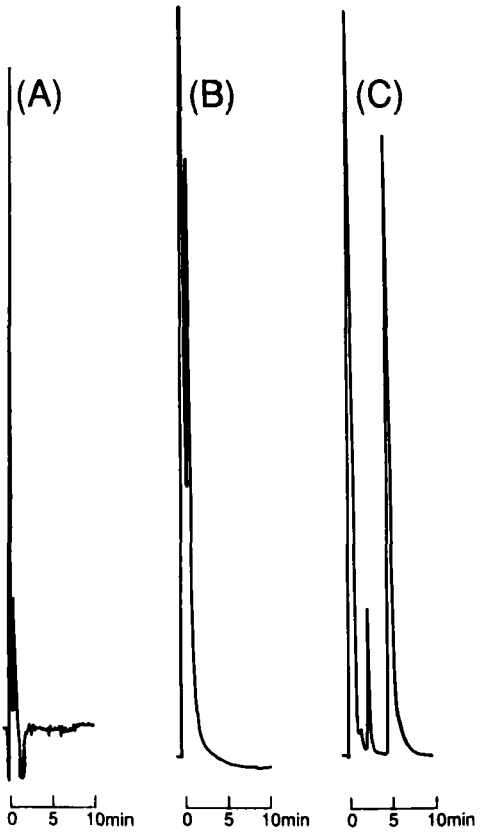

Figure 1

Chromatograms of guinea-pig blank plasma (A); rat blank plasma (B); and rat plasma taken at 5 hr after an intravenous bolus dose of antipyrine (40 mg/kg) containing 4.2 μg/ml of antipyrine (C).

Peaks: AP = antipyrine; I/S = internal standard (aminopyrine)

pig blank plasma (*i.e.* drug-free plasma), a second from rat blank plasma and a third from plasma taken from a rat after an intravenous dose of antipyrine are presented in Figure 1. Under these chromatographic conditions, there was no endogenous interference from plasma. Human blank plasma samples were also tested and had shown no source of endogenous interference. 4-Hydroxyantipyrine, the major metabolite of antipyrine was tested for interference with the drug and the internal standard. The peak corresponding to 4-hydroxyantipyrine was well resolved with a retention time of 2.4 min. All the plasma samples treated with acetonitrile could be injected on the HPLC system successively without disturbing the baseline.

The use of aminopyrine as internal standard allowed the simultaneous determination of both antipyrine and 4-hydroxy antipyrine. Compared to other published HPLC assays, the present method has the advantages of rapid analysis, using a straightforward sample treatment procedure, and lower limits of assay sensitivity.

The absolute analytical recovery of antipyrine from plasma was 9.8 $\pm$ 3.3% (S.D.) at 1 μg/ml and 99.6 $\pm$ 0.2% at 50 μg/ml of antipyrine (n = 5). The recovery of the internal standard, aminopyrine was also good at the concentration used with a recovery of 99.5 $\pm$ 0.4% (n = 5).

The calibration curves constructed from the peak height ratios versus antipyrine concentrations were linear ($r > 0.995$) over the concentration range examined (0.5 to 100 μg/ml). The limit of detection was 0.5 μg/ml of plasma, based on a signal-to-noise ratio of 4:1. This sensitivity appears to be sufficient for the determination of the drug levels usually encountered in the pharmacokinetic study of antipyrine.

The within-day reproducibility and accuracy of the antipyrine assay in guinea pig plasma is shown in Table 1. The

TABLE 1

Within-day Reproducibility and Precision of the Assay for Antipyrine in Guinea Pig Plasma

Spiked Concentration (μg/ml)	n	Observed Concentration[1] (μg/ml)	C.V. (%)	Accuracy[2] (%)
1	5	0.99 ± 0.04	3.6	99.4 ± 3.7
50	5	49.8 ± 0.28	0.6	99.6 ± 0.5

[1] Results given are mean ± S.D.

[2] Accuracy (%) = $\frac{\text{Observed Concentration}}{\text{Spiked Concentration}} \times 100$

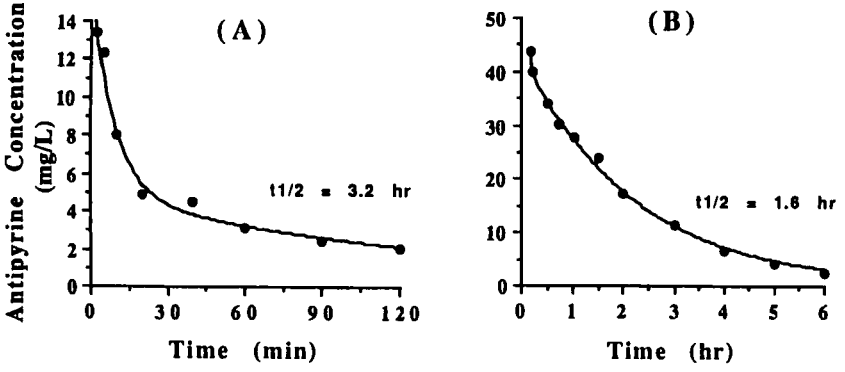

Figure 2

Plasma antipyrine concentration-time profiles in a representative of guinea pig (A) and rat (B). Antipyrine was given as an intravenous bolus dose to guinea pig (3 mg/kg) and rat (40 mg/kg). $t_{1/2}$ is the elimination half-life of antipyrine.

coefficient of variation (C.V.) values obtained at two different concentrations studied were less than 5% with a good accuracy. These results indicate the good precision of the assay.

The day-to-day C.V. of the slope of the calibration curves of antipyrine was 1.5 (n = 9).

The applicability of the assay described was demonstrated by the analysis of plasma samples obtained from pharmacokinetic studies in guinea pigs and rats and also of the samples from placental transfer study of β-blockers. In the particular studies carried out in guinea pigs and rats, venous blood samples were drawn into a heparinized tubes at different times. Plasma concentrations of antipyrine in all samples were determined using the described method. The plasma drug concentration-time profiles of antipyrine in guinea pig and rat are illustrated in Figure 2. The results demonstrated that this method is useful for pharmacokinetic and placental drug transfer studies with small volume of blood samples. The assay sensitivity probably could be improved by injection of larger sample volume on the chromatography.

In summary, the HPLC assay reported affords a simple, rapid and sensitive method for the determination of antipyrine in plasma suitable for use in pharmacokinetic studies. The procedure is a direct plasma protein precipitation therefore is less tedious and rapid.

REFERENCES

1. Vesell, E. S. Fed. Proc. 31, 1253, 1972
2. Schneider, H., Panigel, M. and Dancis, J. Am. J. Obstet. Gynecol. 114, 822, 1972
3. Hamshaw-Thomas, A., Rogerson, N. and Reynolds, F. Placenta, 5, 61, 1984

4. Soberman, R., Brodie, B. B., Levy, B. B., Axelrod, J., Hollender, V. and Steele, J. M. J. Biol. Chem. 179, 31, 1949
5. Chang, S., Emmick, K. and Wedlund, P. J. Pharm. Sci. 75, 456, 1986
6. Shargel, L., Cheung, W. and Yu, A. B. C. J. Pharm. Sci. 68, 1052, 1979
7. De Vries, J. X., Staiger, C., Wang, N. S., Schlicht, F. and Koroknay, B. J. Chromatogr. 309, 219, 1984
8. De Beer, J., Jacobs, G. A. and Martens, M. A. J. Chromatogr. 307, 475, 1984
9. Danhof, M., der Vis, E. de G. and Breimer, D. D. Pharmacol. 18, 210, 1979
10. Eichelbaum, M. and Spannbrucker, N. J. Chromatogr. 140, 288, 1977