Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, Nos 10–12, pp. 977–979, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

The determination of antipyrine elimination in saliva by liquid chromatography*

J. GARTZKE† and H. JÄGER

Bundesanstalt für Arbeitsmedizin, 0-1134 Berlin, Germany

Abstract: A simple, fast and reliable liquid chromatographic method for the determination of antipyrine in saliva is described. The elimination of antipyrine is a good indicator for general evaluation of the liver function for dispositional purposes for example in occupational and environmental medicine. The described LC method was compared with a more extensive photometric procedure. The results obtained from both methods show very good correlation. Only one measurement is necessary to determine the antipyrine clearance. Furthermore the antipyrine dosage can be minimized, because of the sensitivity of the HPLC-method.

Keywords: *Antipyrine elimination; LC determination in saliva; comparison of methods.*

Introduction

The antipyrine elimination is the most important indicator of metabolic functions of microsomal liver enzymes in man [1]. Variation in antipyrine clearance is caused by differences in disposition [2, 3], occupational exposures [4–7], by environmental factors [8, 9] nutritional [8, 10, 11] factors and drugs [12]. Antipyrine clearance may also provide information on health risks caused by xenobiotics such as cancer [13, 14].

The elimination of antipyrine has been determined by photometry [15], GC [16], HPLC [17, 18] TLC [19, 20] and the [14 C]-antipyrine breath test [21].

An isocratic reversed-phase LC method for antipyrine with direct analysis of saliva has been developed and compared with a photometric method [15].

Experimental

Chemicals and reagents

Antipyrine (Ferak, FR), phenacetin (AWD, GDR) and all other reagents were of analytical reagent grade and have been used without further purification. All solvents were HPLC grade. The reversed-phase HPLC glass cartridge columns Separon SGX-C 18™ (150 × 3 mm, 5 μm), the guard columns Separon SGX-C 18™ (30 × 3 mm, 7 μm) and the solid phase extraction cartridges (Silica cart-C 18, 60 μm) were purchased from Tessek Ltd, Prague.

Standards and solutions

The antipyrine stock solution was prepared by dissolving 100 mg antipyrine in a small volume of methanol and diluting with potassium phosphate buffer pH 3.0 to 100 ml (1.0; g l^{-1}). For the standard curves, the stock solution was diluted with buffer into the range 0.1–30 mg l^{-1}.

The internal standard was prepared by dissolving 10 mg phenacetin with water or buffer to 1000 ml.

Solid phase extraction

Silica cart-C 18 (60 μm) cartridges, previously conditioned with methanol, were used for antipyrine extraction. After washing with 2 ml water, containing 100 μl *n*-hexane, and drying under vacuum for 2 min, antipyrine from saliva was eluted with 2.0 ml methanol. The methanolic solution was used without any further concentration steps.

Antipyrine elimination test

Antipyrine administered orally (10 mg kg^{-1}) and one or more saliva samples were collected between 2 and 24 h after drug administration, with the help of Salivetten collection devices (Sarstedt, Nümbrecht, Germany). After

* Presented at the "Third International Symposium on Pharmaceutical and Biomedical Analysis", April 1991, Boston, MA, USA.

† Author to whom correspondence should be addressed.

centrifugation the saliva was diluted (1:5, v/v) either with the internal standard or, if only external calibration was used, with water.

Equipment

An isocratic Shimadzu HPLC-Pump LC6A (Shimadzu Europa Ltd, Germany) fitted with a sample loop injector LCI 30, a fixed wavelength detector LCD 2563 and a computing integrator CI-100 (Laboratorni Pristroje, Prague) were used.

Liquid chromatography

A 20 µl volume of the extract or sample were injected onto a Separon SGX-C18 column (5 µm, 30 × 3 mm). The separation was carried out, isocratically with methanol–10 mM potassium phosphate buffer (pH 3.0) (40:60, w/w) at a flow rate of 0.5 ml min^{-1}. The detection was at 254 nm. Caffeine did not interfere with the analytical determination (Fig. 1).

Calculation of the antipyrine elimination in saliva

As well as the antipyrine clearance (Cl_{AP}), the antipyrine elimination constant (k) and the antipyrine half-life ($t_{0.5}$) are indicators for the hepatic function and its changes. For calculation of the antipyrine elimination, knowledge of the possible distribution volume (V_d) is necessary ($Cl_{AP} = kV_d$). Either a mean value or separate values for males and females can be used [22]. For single measurement, the antipyrine concentration in saliva is determined after 24 h (t = 24 h), following a dose of 1.0 g. A mean distribution volume of 40 l is assumed and the antipyrine clearance (Cl_{AP}) is calculated from

$$Cl_{AP} = (3.22 - \ln c_{24h}) \times 28 \text{ [ml min}^{-1}\text{]}, \quad (1)$$

the elimination constant (k' one compartment model) is calculated from

$$k' = \frac{Cl_{AP}\ (24\ h)}{40}\ [h^{-1}] \quad (2)$$

and the half-life ($t_{0.5}$) and is calculated from

$$t_{0.5} = \frac{\ln 2}{k'}\ [h]. \quad (3)$$

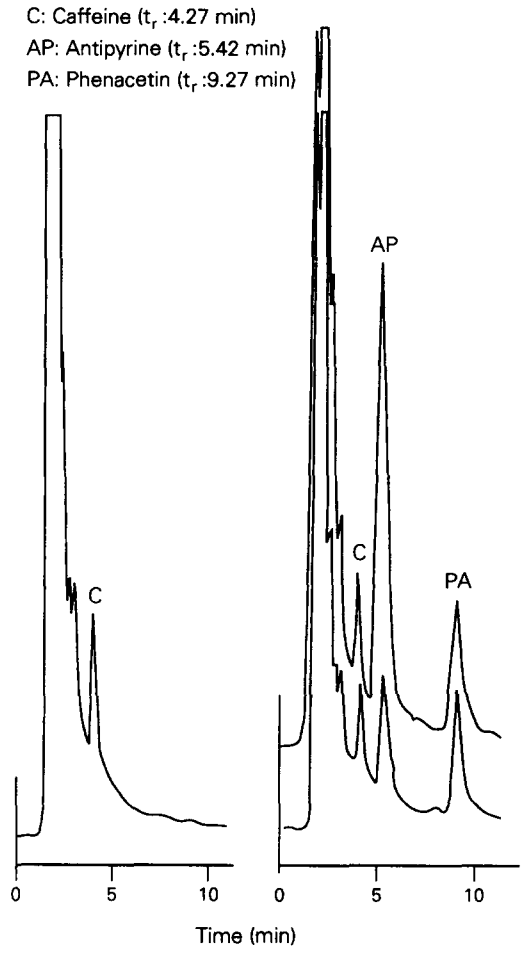

Figure 1
LC separation of unspiked and spiked (right) saliva. LC conditions: Column Separon SGX-C18, (5 µm, 150 × 3 mm) fitted with a guard column Separon SGX-C18, (7 µm, 30 × 3 mm); mobile phase methanol–potassium phosphate buffer (pH 3.0, 40:60, w/w), flow rate 0.5 ml min^{-1}, pressure 18 bar, detection 254 nm. Peaks (from left): unknown from saliva (t_R: 1.5 – 2.92 min), caffeine (C) (t_R: 4.27 min), antipyrine (AP) (t_R: 5.27 min) and phenacetin (PA) (t_R: 9.27 min).

Results and Discussion

Antipyrine is metabolized more than 90% by the liver. The antipyrine elimination is, therefore, a good reflection of liver function [23] (Fig. 2). Because of genetic polymorphism and of exogenous influences a reliable method of prediction of changes in hepatic function by a single test is not possible. Only by estimating the antipyrine clearance in a cross-section of the population can environmental hazards that cause changes in liver function be detected [24]. The antipyrine half-life estimated by the described method was 11.7 ± 3.64 and 13.1 ± 5.38 h for males and females, respectively.

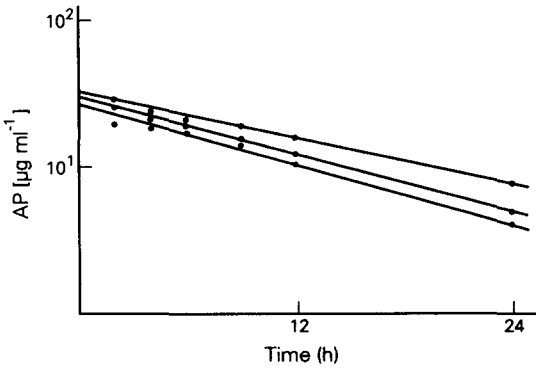

Figure 2
Elimination curve of an actual antipyrine elimination test sample as a function of dilution, from top to bottom: original sample, 1 + 4 diluted and 1 + 10 diluted (v/v) with buffer.

These values are within the normal ranges [20, 25]. No remarkable differences were found between the present LC procedure and the more complicated photometric method [15] as estimated by linear regression analysis (y_{LC} = 1.78 + 0.82$x_{Photom.}$; r = 0.999, n = 10). The detection limit of the assay at the detection wavelength of 254 nm and with an injection volume of 20 µl was about 50 ng ml^{-1}.

The administration of antipyrine in capsules and a single sampling of saliva after 24 h was found to be acceptable for routine determination of antipyrine clearance.

References

[1] J. Krausz, *Clin. Pharm. Therap. Toxicol.* **18**, 253–258 (1980).
[2] F.P. Guengerich, *Trends Pharmacol. Sci.* **10**, 107–109 (1989).
[3] M.S. Lennard, *Pharmacol. Toxicol.* **67**, 273–283 (1990).
[4] M. Dossing, *Int. Arch. Occup. Environ. Health* **53**, 205–218 (1984).
[5] D.P. Lodovici and M. Buffoni, *Ann. Occup. Hyg.* **25**, 27–32 (1982).
[6] B. Kolmodin-Hedman, B. Alexanderson and F. Sjöqvist, *Toxicol. Appl. Pharmacol.* **20**, 299–307 (1971).
[7] M. Dossing, S. Loft, J. Sonne and E. Schroeder, *Int. Arch. Occup. Environ. Health* **60**, 115–118 (1988).
[8] A.P. Alvares, E.J. Pantuck, K.E. Anderson, A. Kappas and A.H. Conney, *Drug Metab. Rev.* **9**, 185–205 (1979).
[9] J.M. Scavone, D.J. Greenblatt, B.W. LeDuc, G.T. Blyden, B.G. Luna and J.S. Harmatz, *Pharmacology* **40**, 77–84 (1990).
[10] J.F. Schneider, A.L. Baker, N.W. Haines, G. Hatfield and J.L. Boyer, *Gastroenterology* **79**, 1145–1150 (1980).
[11] M. Dossing and P.B. Andreasen, *Clin. Pharmacol. Ther.* **30**, 101–104 (1981).
[12] D.S. Sitar, *Pharmac. Ther.* **43**, 363–375 (1989).
[13] T. Higuchi, T. Nakamura and H. Uchino, *Experientia* **36**, 236–238 (1980).
[14] T. Ohta, Y. Asabe and S. Takitant, *Chem. Pharm. Bull. (Japan)* **34**, 3866–3872 (1986).
[15] B.B. Brodie, J. Axelrod, R. Soberman and B.B. Levy, *J. Biol. Chem.* **179**, 25–29 (1949).
[16] L.F. Prescott, K.K. Adhpon-Yamank and E. Roberts, *J. Pharm. Pharmacol.* **25**, 205–207 (1973).
[17] W.M. Awni and L.J. Bakker, *Clin. Chem.* **35**, 2124–2126 (1989).
[18] M.A. Sarkar and H.T. Karnes, *J. Chromatogr.* **434**, 324–326 (1988).
[19] J. Aarbakke, *Acta Pharmacol. Toxicol.* **43**, 64–68 (1978).
[20] R.M. Welch, R.L. De Angelis, M. Wingfield and T.W. Farmer, *Clin. Pharmacol. Ther.* **18**, 249–257 (1975).
[21] J. Pirotte and D. El Allaf, *Digestion* **28**, 210–215 (1983).
[22] S. Pfeifer and H.H. Borchert, *Pharmacokinetik und Biotransformation — Eine Einführung*, pp. 31–43. VEB Verlag Volk und Gesundheit (1980).
[23] E.S. Vesell, *Clin. Pharmacol. Ther.* **26**, 275–286 (1979).
[24] R. Uppal, P.R. Sharma and R.R. Chandhury, *Human Toxicol.* **1**, 155–158 (1982).
[25] J.C. Mucklow, M.T. Caraher, D.B. Henderson, P.H. Chapman, D.F. Roberts and M.D. Rawlins, *Br. J. Clin. Pharmac.* **13**, 481–486 (1982).

[Received for review 30 April 1991; revised manuscript received 2 July 1991]