Journal of Chromatography, 307 (1984) 475—480
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2057

Note

Simple reversed-phase high-performance liquid chromatographic determination of antipyrine in rabbit plasma for pharmacokinetic studies

JACQUES O. DE BEER*, GUIDO A. JACOBS and MARK A. MARTENS

Instituut voor Hygiëne en Epidemiologie, J. Wytsmanstraat 14, B-1050 Brussels (Belgium)

(First received October 7th, 1983; revised manuscript received December 15th, 1983)

In order to study the influence of dilution on the pharmacokinetic behaviour of antipyrine after oral administration, a rapid, sensitive, precise and accurate method of analysis was needed. The sensitivity required had to be at least 0.1 μg/ml antipyrine in rabbit plasma for sample volumes ranging from 0.25 to 0.5 ml.

Antipyrine, which is normally used as an indicator of hepatic drug metabolism [1—5], was selected for this experiment on the basis of its simple pharmacokinetic behaviour: first-order kinetics, no first-pass effect, one-compartment distribution [6] and practically no protein binding [7].

Eichelbaum and Spannbrucker [8] were the first to present a rapid and sensitive method of analysis for antipyrine by high-performance liquid chromatography (HPLC) on silica gel. The most prevalent disadvantage of this type of chromatography, however, especially in bioanalyses, is column inactivation. Usually the presence of water in the injection sample or in the mobile phase is responsible for this. As a result retention times of the compounds decrease and resolution is lost. According to our experience with the above method, a change of retention volume was often observed after the ammonia-containing mobile phase was renewed. More recently, Guinebault and Broquaire [9] described an interesting straight-phase HPLC method in which neither mobile phase nor injection sample contained any water and no variation of retention times of antipyrine and phenacetin was observed.

We also considered reversed-phase HPLC methods to resolve our problem. Campell et al. [10] reported a reversed-phase HPLC method. Remarkable in their method is the ten-fold dilution of the plasma samples, taking into account the injection of only 25 μl of filtrate. They thus have to select a maximum detector sensitivity to measure even high concentrations. As to the choice of

0378-4347/84/$03.00

internal standard, benzoic acid is used, but a structurally related compound would be more convenient. Shargel et al. [11] described a reversed-phase HPLC method for antipyrine in 0.1 ml of rat plasma samples with methanol—water as mobile phase. Too much peak tailing was observed, however, while the sensitivity was not higher than 1.0 μg/ml.

In this paper we have tried to overcome most of these inconveniences. Special attention was paid to a solvent combination to minimize peak tailing as much as possible.

MATERIALS AND METHODS

Chemicals

When available, all the chemicals and solvents were of analytical grade (Merck, Darmstadt, F.R.G.). Antipyrine and aminopyrine (aminophenazone), conforming to the European Pharmacopoeia, were used as standard and internal standard, respectively. The HPLC solvents were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.).

Apparatus

The samples were analysed on an HPLC system consisting of a Pye Unicam LC3-XP pump (Cambridge, U.K.), an Altex Model 210 injection valve, fitted with a 20-μl loop and a Pye Unicam PU 4020 ultraviolet detector operated at 254 nm with the sensitivity range at 0.08 a.u.f.s. A reversed-phase 10-μm μBondapak C_{18} column (300 × 3.9 mm I.D., stainless steel) (Waters Assoc., Milford, MA, U.S.A.) was used at ambient temperature. Isocratic elution was performed with a mixture of acetonitrile—water (32.5:67.5, v/v) containing 0.5% acetic acid at a flow-rate of 0.75 ml/min. The chromatograms were recorded at a chart-speed of 120 mm/h.

Procedure

In a glass tube 0.1—0.25 ml of plasma, 0.1 ml of sodium hydroxide (0.1 mol/l) and 0.1 ml of internal standard solution of aminopyrine in water (25 μg/ml) were mixed on a vortex mixer. Then 1 ml of dichloromethane was added, the mixture shaken on a IKA-Schüttler S50 apparatus for 5 min and the two liquid phases were separated by centrifugation (1500 *g*) for 5 min. By means of an eppendorf pipette, 500 μl of the dichloromethane phase were transferred into a test tube, evaporated under nitrogen and the residue was dissolved in 100 μl of water, 20 μl of which were injected into the HPLC system. At the same time 0.1 ml of sodium hydroxide (0.1 mol/l), 0.1 ml of internal standard solution and 0.1 ml of aqueous solutions of increasing amounts of antipyrine (0.223 μg, 0.446 μg, 0.892 μg, 1.784 μg, 2.230 μg, 4.460 μg and 7.805 μg) were mixed with 0.25 ml of water and analysed together with the plasma samples.

RESULTS AND DISCUSSION

Aminopyrine (internal standard) and antipyrine exhibited two symmetrical, well resolved peaks under the described chromatographic conditions (Fig. 1).

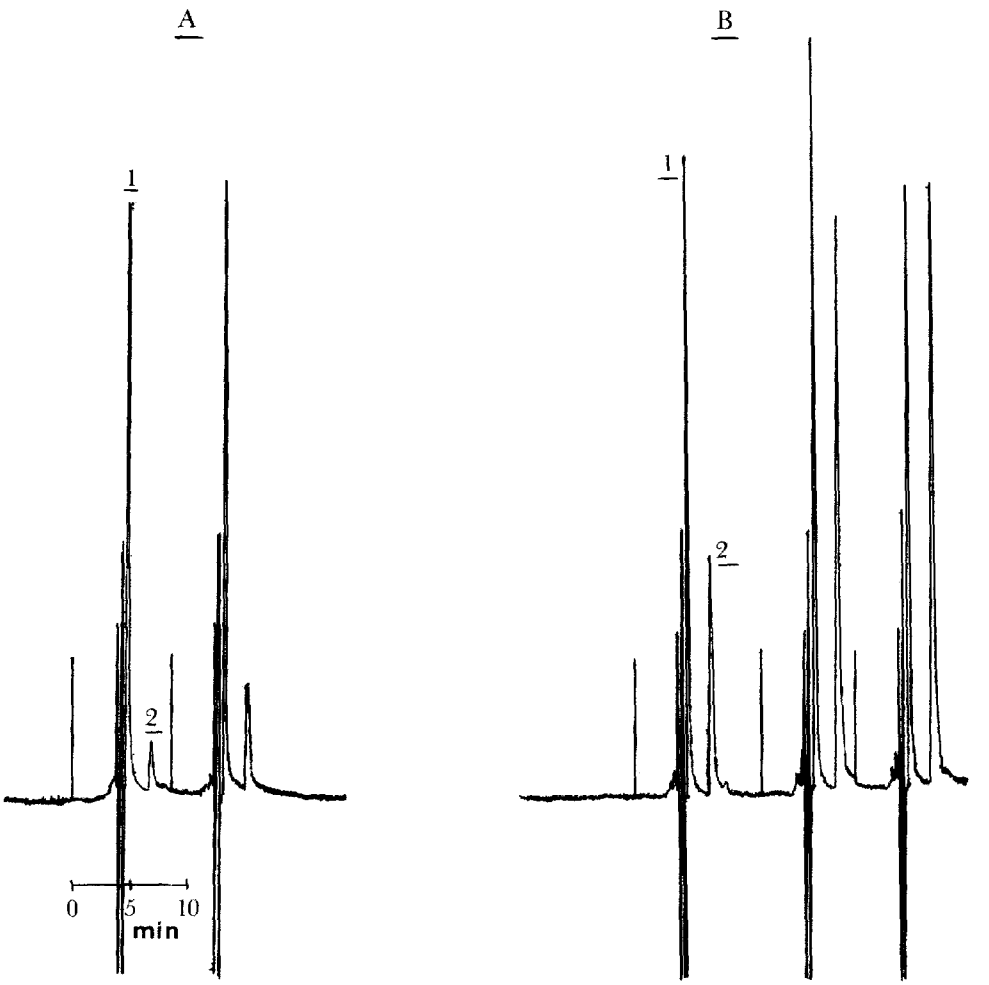
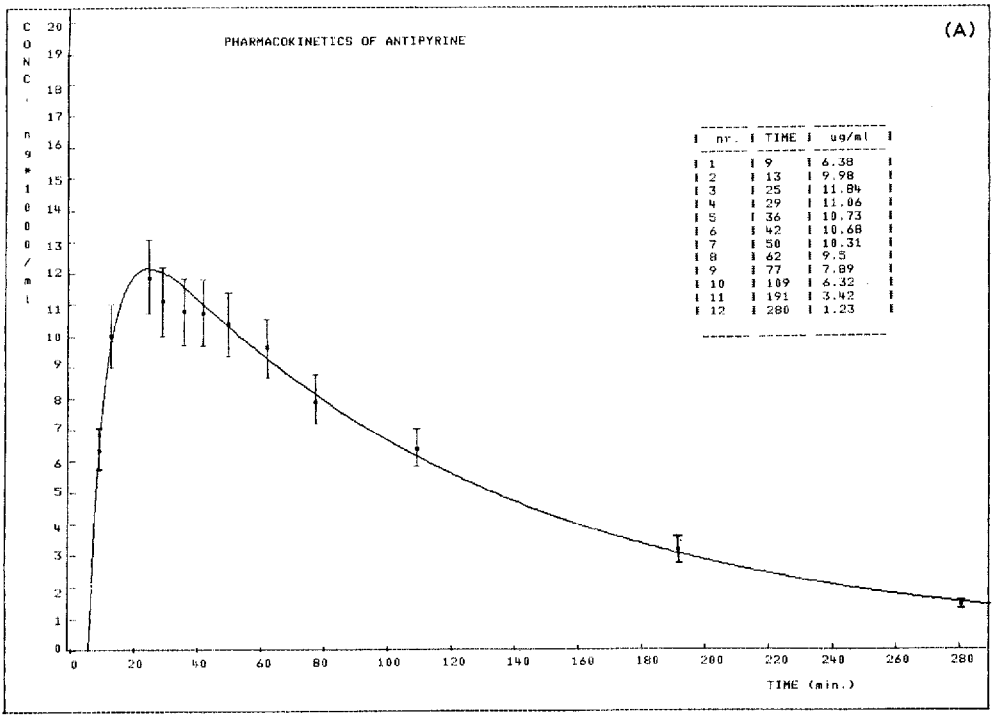


Fig. 1. (A) Chromatograms of rabbit plasma extracts spiked with 0.2 and 0.4 μg/ml antipyrine. Detector sensitivity is 0.08 a.u.f.s. Peaks: 1 = aminopyrine; 2 = antipyrine. (B) Chromatograms of rabbit plasma extracts after administration of antipyrine to the rabbit.

Peak height ratio was used to quantitate detector response. A linear relationship was obtained for the range tested (0.2—25 μg/ml).

For spiked plasma the coefficient of variation was ± 4.8% for 2.23 μg of antipyrine per ml of plasma and 5.1% for 0.223 μg of antipyrine per ml of plasma (n = 6). The recovery was 104% and 97%, respectively. The retention times were 4.75 and 6.25 min for aminopyrine and antipyrine, respectively. Day-to-day precision obtained by analysing pooled rabbit plasma, containing 2.23 μg/ml antipyrine, on different days was 4.2% (n = 10). In a sample volume of 0.5 ml plasma, antipyrine concentrations of 0.1 μg/ml could still be detected with a reasonable precision. No interfering peaks of plasma constituents appeared on the chromatograms of blank plasma samples. All the plasma extracts could be injected on the HPLC system successively without disturbing the baseline. Standard curves were drawn daily by linear regression. The correlation coefficients obtained were at least 0.99.



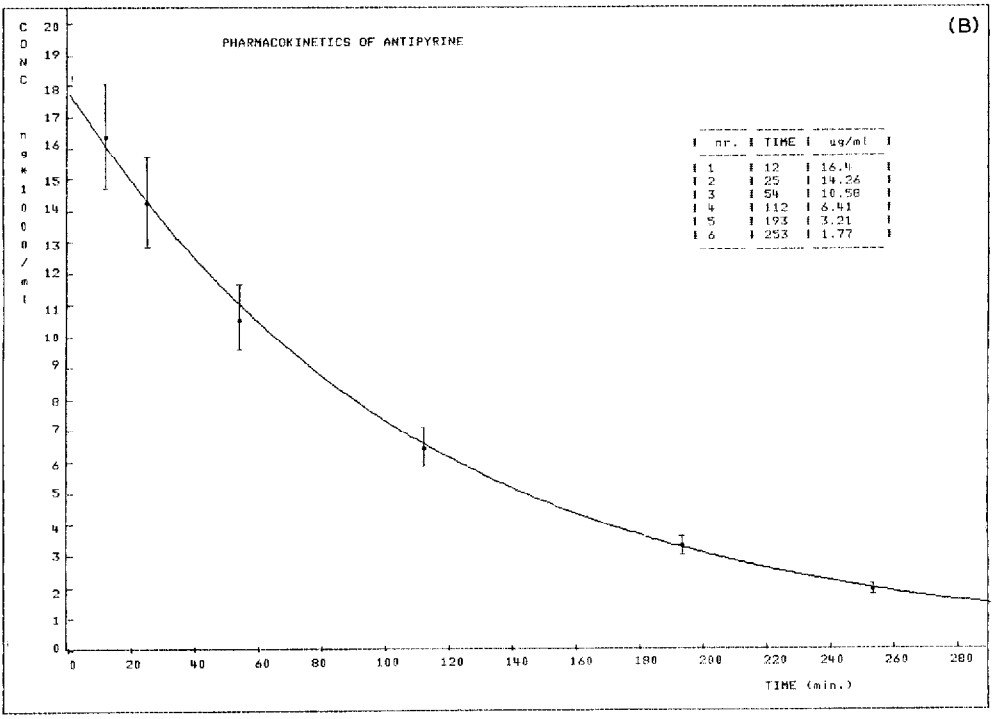


Fig. 2. (A) Rabbit plasma antipyrine concentrations following peroral administration of 12.5 mg/kg as a concentrated aqueous solution. (B) Rabbit plasma antipyrine concentrations following intravenous injection of 12.5 mg/kg as a concentrated aqueous solution.

The HPLC method developed was applied in a pharmacokinetic study of antipyrine, administered by oral intubation and intravenous injection to a rabbit in a dose of 12.5 mg/kg. Venous blood samples were drawn into heparinized tubes by venipuncture at various times for about 6 h after administration. Plasma concentrations of antipyrine in all samples were determined using the described method to demonstrate its performance. Absorption and/or excretion curves obtained are shown in Fig. 2A and B, together with the 95% confidence interval of the plasma concentrations measured.

TABLE I

KINETICS OF INTRAVENOUS AND ORAL ANTIPYRINE

Kinetic variables	Route of administration	
	Peroral	Intravenous
Absorption half-life (min)	4.4	—
Elimination half-life (min)	77.1	76.4
Absorption constant k_a (min^{-1})	0.157	—
Elimination constant k_e (min^{-1})	0.00899	0.009073
Maximum plasma concentration C_{pmax} (μg/ml)	12.55	17.87 ($C_{p,\ t=0}$)
t_{max} (min)	25	—
Lag time t_0 (min)	5.3	—
Distribution volume V_d (ml/kg)	836	700
Area under the curve (AUC) (μg/ml min)	1660	1969.1
Total clearance (ml/min/kg)	—	6.35

In Table I the absorption half-life, the elimination half-life, the absorption constant k_a, the elimination constant k_e, the maximum plasma concentration C_{pmax}, the time t_{max} corresponding to C_{pmax}, the lag time t_0, the distribution volume V_d and the area under the curve (AUC) are listed. All these kinetic variables for antipyrine were determined using standard pharmacokinetic methods [12, 13]. Values of elimination half-life were 77 and 76 min for the peroral and intravenous route of administration, respectively. These values agree with reported values [14]. After a lag time of 5.3 min, oral antipyrine was absorbed with an apparent half-life of 4.4 min. Based on comparison of areas under the curve, following oral and intravenous administration, the fraction of the dose absorbed was 84%. The plasma elimination of antipyrine was first order. Results on the influence of dilution on the pharmacokinetic behaviour after oral administration will be published elsewhere.

CONCLUSION

As illustrated, the described method achieves the level of sensitivity and accuracy required to obtain meaningful data about the single-dose pharmacokinetic behaviour of antipyrine in rabbit.

REFERENCES

1 E.A. Sotaniemi, Pharmacology, 10 (1973) 306.

2 J. Elfström and S. Lindgren, Eur. J. Clin. Pharmacol., 7 (1974) 467.
3 E.S. Vessel, C.J. Lee, G.T. Passanati and C.A. Shively, Pharmacology, 10 (1973) 217.
4 B. Kolmodin, D.L. Azarnoff and F. Sjöqvist, Clin. Pharmacol. Ther., 10 (1969) 638.
5 R.M. Welch, R.L. De Angelis, M. Wingfield and T.W. Farmer, Clin. Pharmacol. Ther., 18 (1975) 249.
6 P.B. Andreasen and E.S. Vessel, Clin. Pharmacol. Ther., 16 (1974) 1059.
7 R. Soberman, B.B. Brodie, B.B. Levy, J. Axelrod, V. Hollender and J.M. Steele, J. Biol. Chem., 179 (1949) 31.
8 M. Eichelbaum and N. Spannbrucker, J. Chromatogr., 140 (1977) 288.
9 P. Guinebault and M. Broquaire, J. Chromatogr., 217 (1981) 509.
10 T.M. Campbell, E.W. Murdaugh and P.G. Killenberg, J. Chromatogr., 163 (1972) 236.
11 L. Shargel, W.M. Cheung and A.B.C. Yu, J. Pharm. Sci., 68 (1979) 1052.
12 R.E. Notari, Biopharmaceutics and Clinical Pharmacokinetics. An Introduction, Marcel Dekker, New York, 3rd ed., 1980.
13 M. Gibaldi and D. Perrier, Pharmacokinetics, Drugs and the Pharmaceutical Sciences, Vol. 15, Marcel Dekker, New York, 2nd ed., 1982.
14 A. van Peer, F. Belpaire and M. Bogaert, Pharmacology, 17 (1978) 307.