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Letter to the Editor

Simultaneous determination of antipyrine and metabolites in human plasma and urine by high-performance liquid chromatography

Sir,

Antipyrine (AP) is used as a model substance to measure the influence of environmental factors, drugs, and disease states on the activity of the microsomal oxidative degradation enzymes in in vivo systems [1]. After antipyrine administration (oral or intravenous), plasma (or saliva) [2] is analysed for the main substance at various time intervals and pharmacokinetic parameters may be calculated. However, more information may be gained by analysing additionally the metabolites 4-hydroxyantipyrine (OHA), norantipyrine (NAP) and 3-hydroxymethylantipyrine (HMA) in urine [3]. Clinical pharmacological work in this Department on enzyme induction (or inhibition) of drugs [4, 5] prompted us to develop a method for the analysis of antipyrine in blood and saliva and of antipyrine and metabolites in urine involving enzymatic hydrolysis, extraction and gradient elution with reversed-phase high-performance liquid chromatography (HPLC).

Recently, Teunissen et al. [6] described in this journal an HPLC assay for antipyrine and metabolites in blood, saliva and urine. Our procedure was developed independently and is comparable to their method; it differs mainly in the sample work-up and in the chromatographic system.

To 1 ml of plasma (or saliva), 10 μg of phenacetin, 200 μl of 2 *M* sodium hydroxide and 6 ml of dichloromethane were added and the mixture was shaken for 10 min. After centrifugation 5 ml of the organic phase were evaporated to dryness under a stream of nitrogen. Or, 1 ml of urine was hydrolysed for 4 h with 3 ml of 0.1 *M* acetate buffer containing 4 mg of sodium metabisulphite and 40 mg of sulphatase—glucuronidase at 37°C. Then 20 μg of phenacetin were added and the solution was extracted successively with 5 ml of dichloromethane—pentane (3:7, v/v) (A) and after the addition of 600 mg of anhydrous sodium sulphate and 400 μl of 4 *M* sodium hydroxide with 5 ml of dichloromethane (B). The mixture of the organic phases A and B was evaporated under nitrogen.

The HPLC system consisted of two pumps (6000A), an automatic injector (WISP Model 710B), a gradient programmer (M660), an ultraviolet (UV) filter detector (M440) (all from Waters, F.R.G.), a printer plotter (1RB,

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Shimadzu, F.R.G.) and a LiChrosorb RP-2 column (250 × 4 mm I.D., irregular particles, 10 μm; Merck, F.R.G.) preceded by a precolumn filled with Perisorb RP-2 (25 × 4 mm I.D., spherical particles 30—40 μm). For plasma and urine the residue was dissolved in 200 μl of methanol and 10 μl were injected for analysis. The detector was set at 254 nm and 0.1 a.u.f.s. The mobile phase for plasma was phosphate buffer (pH 6.5, 0.05 *M*)—methanol (60:40, v/v) at a flow-rate of 2 ml/min (k' values for antipyrine and phenacetin were 1.66 and 2.34, respectively). Urine was analysed using a linear gradient: solvent A was phosphate buffer (pH 6.5, 0.05 *M*)—methanol (28:72, v/v); solvent B was methanol. The gradient was run from 0% to 100% of solvent B in 15 min at a total flow-rate of 2 ml/min (k' values for HMA, NAP, AP, OHA and phenacetin were 1.81, 3.44, 4.33, 4.95 and 5.73, respectively).

Antipyrine in plasma was analysed in the range 0.1—20 mg/l, and antipyrine and metabolites in urine in the range 1—200 mg/l. Sensitivity was 0.1 mg/l for antipyrine in plasma and 1 mg/l for antipyrine and metabolites in urine. Precision was in the range 1.6—3.8%; the extraction recoveries were between 68% and 91%, depending on the substance. Most of the drugs and metabolites tested did not interfere with the assay. Excellent correlations were found with previously published procedures [7, 8].

The use of the extraction steps described above allowed the simultaneous determination of antipyrine and metabolites in urine, in place of the double-extraction procedure [8] with separate determinations of NAP with OHA, and AP with HMA, affording considerable economy in the hydrolysis, extraction and chromatographic steps. Teunissen et al. [6] have also described a single-step extraction with chloroform—ethanol (9:1, v/v), which in our hands leads to interference by several drugs and metabolites. The use of gradient elution for the analysis of urine produced sharp separations and peaks of antipyrine and metabolites without co-elution of many drugs and their metabolites. The total analysis time is 15 min and the use of an automatic injector permits a large number of assays.

Plasma extraction and chromatography were simple, with short analysis times (5 min) and absence of interference. The chromatographic system is very stable, and frequent changes of the precolumn filling allows a long column life.

The method is comparable to the one described by Teunissen et al. [6]. They used a short column filled with spherical reversed-phase particles and employed isocratic elution for plasma and urine with short analysis times. The analysis of antipyrine and metabolites in the urine of volunteers who had been treated with sulfinpyrazone [5, 9] lead to interference when analysed using a similar isocratic system; this was circumvented by the use of the gradient elution system described above. Theunissen et al. [6] also described the determination of 4,4'-dihydroxyantipyrine, which we did not assay as it is a minor antipyrine metabolite in humans.

The use of the modified conditions for extraction and the use of gradient elution for urine samples present a simple, fast, specific and sensitive method for the analysis of antipyrine in blood and saliva, and its metabolites in urine, adequate for drug-interaction, enzyme-induction or clinical studies where

interference from foreign compounds is to be expected. Similar metabolite profiles to those already published [3] were found with this method.

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1 E.S. Vessell, Clin. Pharmacol. Ther., 26 (1979) 275.
2 P.J. Meffin, R.L. Williams, T.F. Blaschke and M. Rowland, J. Pharm. Sci., 66 (1977) 135.
3 M. Danhof, A. van Zuilen, J.K. Boeijing and D.D. Breimer, Eur. J. Clin. Pharmacol., 21 (1982) 433.
4 E. Walter, C. Staiger, J. de Vries, R. Zimmermann and E. Weber, Eur. J. Clin. Pharmacol., 19 (1981) 353.
5 Ch. Staiger, F. Schlicht, E. Walter, U. Gundert-Remy, R. Hildebrandt, J. de Vries, N.S. Wang, J. Harenberg and E. Weber, Eur. J. Clin. Pharmacol., 25 (1983) 797.
6 M.W.E. Teunissen, J.E. Meerburg-van der Torren, N.P.E. Vermeulen and D.D. Breimer, J. Chromatogr., 278 (1983) 367.
7 Ch. Staiger, J. de Vries and E. Walter, J. Clin. Chem. Clin. Biochem., 18 (1980) 817.
8 M. Danhof, E. de Groot-van der Vis and D.D. Breimer, Pharmacology, 18 (1979) 210.
9 J.X. de Vries, Ch. Staiger, N.S. Wang and F. Schlicht, J. Chromatogr., 277 (1983) 408.

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