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Note

Simultaneous determination of pentoxifylline and its hydroxy metabolite in plasma by high-performance liquid chromatography

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Pentoxifylline [I; 3,7-dimethyl-1-(5-oxohexyl)-xanthine; Fig 1] is a drug used for the treatment of circulatory disorders. Although a number of procedures for the analysis of pentoxifylline and its major metabolite [II; 3,7-dimethyl-1-(5-hydroxyhexyl)-xanthine] in plasma have been developed for metabolic and pharmacokinetic studies, each of these has significant disadvantages. In particular, pentoxifylline has been quantitated spectrophotometrically [1] and colorimetrically [2] but these methods require multiple solvent extractions of plasma and preliminary separation of the drug by thin-layer chromatography. Such methods are inconvenient for processing large numbers of samples. Gas—liquid chromatographic conditions for the separation of pentoxifylline have been reported [1] but have only been applied qualitatively. Similarly, although a high-performance liquid chromatographic (HPLC) procedure for the analysis of pentoxifylline and its metabolite (II) in plasma has been used in bioavailability studies [3], the method requires a large sample volume, multiple solvent extraction of plasma is necessary and few analytical details or precision data were reported.

As part of a clinical trial in progress at this centre it was necessary to monitor plasma concentrations of pentoxifylline and its metabolite (II). In recent years, the convenience and versatility of HPLC has led to its acceptance as one of the most useful techniques available for the analysis of drugs in biological fluids [4]. Thus, an HPLC method suitable for the co-determination of pentoxifylline and its metabolite (II) in plasma has been developed which is simpler and more rapid than previously reported procedures. In addition, the method is sensitive, reproducible and readily applicable to pharmacokinetic studies.

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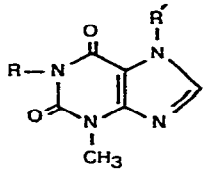

	R	R'
(I)	$-CH_2-(CH_2)_3-CO-CH_3$	$-CH_3$
(II)	$-CH_2-(CH_2)_3-CH(OH)-CH_3$	$-CH_3$
(III)	$-CH_2-(CH_2)_3-CO-CH_3$	$-CH_2CH_3$

Fig. 1. Structures of pentoxifylline (I), metabolite (II) and internal standard (III).

EXPERIMENTAL

Reagents and standards

Pure samples of pentoxifylline, its metabolite (II) and the internal standard [III; 7-ethyl-3-methyl-1-(5-oxohexyl)-xanthine] were supplied by Hoechst Roussel Pharmaceuticals (Melbourne, Australia). Other reagents and solvents were of analytical grade.

A stock solution was prepared by dissolving 20 mg of both pentoxifylline and its metabolite (II) in 1 l of distilled water. Standards were then prepared by diluting the appropriate volume of stock solution with drug-free plasma or distilled water to give final concentrations of pentoxifylline and metabolite (II) of 1000, 500, 250, 100 and 50 μg/l. The internal standard was prepared by dissolving 4 mg of the compound (III) in 1 l of distilled water. All solutions were stable for at least three months when stored at 4°C.

Chromatography

The high-performance liquid chromatograph used (Waters Assoc., Milford, MA, U.S.A.) consisted of a Model 45 solvent delivery system, a Model U6K universal injector, and a Model 450 ultraviolet absorbance detector operating at 275 nm. The instrument was fitted with a 30 cm × 3.9 mm I.D. reversed-phase μBondapak C_{18} column (10 μm, Waters Assoc.) and operated at ambient temperature. The mobile phase was methanol—phosphate buffer, 10 m*M*, pH 7.0 (38:62) used at a flow-rate of 2.0 ml/min.

Sample preparation

To 1 ml of plasma or aqueous standard in a 15-ml glass culture tube were added 0.1 ml of internal standard solution and 10 ml of dichloromethane. The solution was vortex-mixed for 1 min and then centrifuged at 1500 *g* for 3 min. After aspiration of the aqueous layer the organic phase was transferred to a conical-tipped glass tube and evaporated to dryness under vacuum at 40°C. The residue was redissolved in 0.1 ml of the mobile phase and injected into the chromatograph.

Unknown concentrations were determined by comparison of the pentoxifylline, metabolite (II)/internal standard peak height ratios with those of the calibration curves.

RESULTS AND DISCUSSION

Representative chromatograms of extracts obtained following this procedure are shown in Fig. 2. Sharp, symmetrical peaks with retention times of 5.0, 6.5 and 7.6 min are obtained for pentoxifylline, metabolite (II) and internal standard respectively. Fig. 2A shows the chromatogram of the extract of an aqueous standard containing 250 μg/l of both pentoxifylline and metabolite (II). Similarly, Fig. 2B shows the chromatogram of an extract of plasma, taken 3 h after a dose from a patient on chronic pentoxifylline treatment (400 mg, 12-hourly), containing 180 and 200 μg/l of pentoxifylline and metabolite (II) respectively. Drug-free plasma gave no interfering peaks under the chromatography conditions described (Fig. 2C).

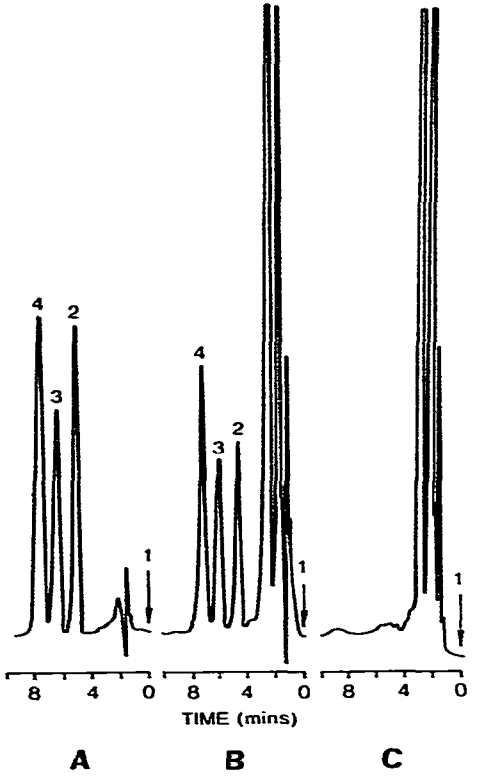

Fig. 2. Chromatograms of plasma or water extracts. (A) Aqueous standard containing 250 μg/l of both pentoxifylline and metabolite (II), 0.04 a.u.f.s.; (B) plasma sample containing 180 μg/l of pentoxifylline and 200 μg/l of metabolite (II), 0.04 a.u.f.s.; (C) blank plasma, 0.02 a.u.f.s. Peaks: 1, injection; 2, pentoxifylline; 3, metabolite (II); 4, internal standard.

Mean recoveries, calculated by comparing the peak height for extracted compound with that of an equal amount injected directly into the chromatograph, for samples containing 50—1000 μg/l of pentoxifylline and its metabolite (II) were 85.8 ± 2.9% and 75.8 ± 1.5% respectively. The recovery efficiency was essentially identical for samples extracted from equal volumes

of plasma or water thereby enabling the use of aqueous standards.

Calibration curves for pentoxifylline and its metabolite (II) were linear in the range 50—1000 μg/l and passed through the origin (Fig. 3). A total of twenty calibration curves were prepared from plasma or water over a period of approximately two months. The average coefficients of variation of the normalised peak height ratios were 5.8 ± 2.5% and 5.7 ± 1.4% for pentoxifylline and metabolite (II) respectively.

With photometric detection at 275 nm, the approximate absorption maximum for pentoxifylline and its metabolite (II) in the mobile phase, as little as 20 μg/l of both compounds may be quantitated. It should also be noted that detection at 280 nm may be used with little apparent loss of sensitivity. During several months of operation no other drugs, metabolites or endogenous plasma constituents have been found to interfere with the determination of both compounds. In addition, it has been shown that caffeine, salicylate and paracetamol do not co-chromatograph with either pentoxifylline, metabolite (II) or internal standard.

In summary, an HPLC procedure for the estimation of pentoxifylline and metabolite (II) in plasma has been developed. The method offers significant advantages in terms of rapidity, simplicity, reproducibility, and specificity over previously published procedures.

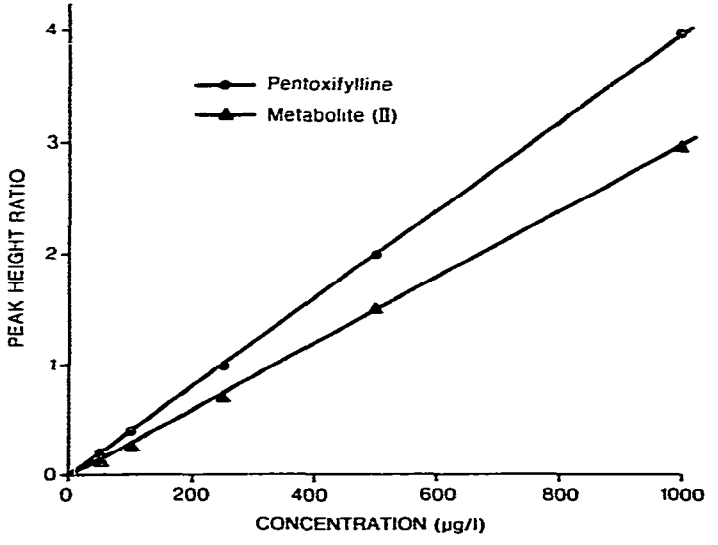

Fig. 3. Typical calibration curve for pentoxifylline and metabolite (II).

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