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Short communication

Simple high-performance liquid chromatographic method for determination of pentoxifylline in human plasma

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Abstract

A simple high-performance liquid chromatographic method using ultraviolet detection was developed for the determination of pentoxifylline in human plasma. Prior to analysis, pentoxifylline and the internal standard (chloramphenicol) were extracted from plasma sample using dichloromethane. The mobile phase comprised 0.02 *M* phosphoric acid adjusted to pH 4, methanol and tetrahydrofuran (55:45:1, v/v). Analysis was run at a flow-rate of 1.4 ml/min with the detector operated at a wavelength of 273 nm. The method was specific and sensitive with a detection limit of approximately 3.0 ng/ml at a signal to noise ratio of 3:1, while the limit of quantification was 12.5 ng/ml. Mean recovery value of the extraction procedure was about 99.9%, while the within-day and between-day coefficient of variation and percent error values of the assay method were all less than 10.0%. The calibration curve was linear over a concentration range of 12.5–400.0 ng/ml.
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1. Introduction

Pentoxifylline is a haemorrheologic agent used for the treatment of peripheral arterial disease [1] and intermittent claudication [2]. Various analytical methods including thin-layer chromatography (TLC) [3], gas chromatography (GC) [4–6], and high-performance liquid chromatography (HPLC) [7–11] have been reported for its determination in biological fluids. The TLC methods may not be sufficiently selective and sensitive for routine measurement of the drug in plasma, while the GC methods were tedious, requiring extensive sample preparation and derivatization. On the other hand, some of the HPLC methods reported in the literature also required extensive sample preparation [7] and long run time approaching 18 min [8]. More recently, Mancinelli et al. [11] reported the use of a solid-phase extraction procedure in the sample preparation, but the detection limit reported was considerably high at 25 ng/ml and the accuracy and precision of the method were demonstrated with only one concentration level of 100 ng/ml. Another HPLC method was reported by Raz et al. [10] but no information was available regarding its precision and accuracy.

In this paper, we report a simple, specific, sensitive and reproducible HPLC method for the determination of pentoxifylline in human plasma using ultraviolet detection in which chloramphenicol was used as the internal standard. The structures of pentoxifylline and chloramphenicol are as shown in Figs. 1 and 2. The applicability of the method was

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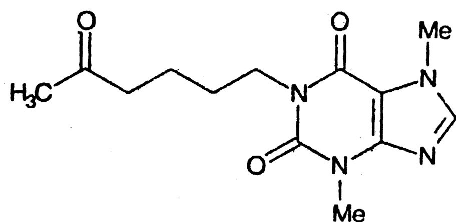

Fig. 1. Structure of pentoxifylline.

demonstrated by applying it to analyze plasma samples obtained from a bioavailability study.

2. Experimental

2.1. Materials

Phosphoric acid 85% was purchased from BDH Chemicals (Poole, UK). Pentoxifylline was obtained from Chemagis (Ramat-Hovav, Israel) and its hydroxy metabolite [1-(5-hydroxyhexyl)-3,7-dimethylxanthine] was supplied by Hoechst (Frankfurt, Germany). Chloramphenicol was obtained from United States Pharmacopeia (MD, USA). All solvents used were of HPLC grade purchased from Mallinckrodt (KY, USA).

2.2. Instrumentation

The high-performance liquid chromatography system comprised a Gilson 305 pump (Gilson, Villiers-le-Bel, France), a Rheodyne 7725I six port valve sample injector equipped with a 50 μl sample loop (Rheodyne, California, USA), a Gilson 119 UV/VIS Detector (Gilson, Villiers-le-Bel, France) and a Hitachi D-2500 Chromato-integrator (Hitachi, Tokyo, Japan). The system was operated at ambient room temperature (24°C). A KR100-5-C18 (Bioscience, KL, Malaysia) column (5 μm, 250×4.6 mm I.D.) fitted with a refillable guard column (Upchurch Scientific, Oak Habour, WA, USA) packed with Perisorb RP-18 (30-40 μm, pellicular) was used for the chromatographic separation. The mobile phase consisted of 0.02 *M* phosphoric acid adjusted to pH 4 with 10 *M* sodium hydroxide, methanol and tetrahydrofuran (55:45:1, v/v). Analysis was run at a flow-rate of 1.4 ml/min with the detector operating at a wavelength of 273 nm, sensitivity range of 0.005 AUFS and an output of 15 mV.

2.3. Sample preparation

A 500 μl aliquot of plasma sample was measured into a glass tube with a teflon lined screw cap, followed by the addition of 50 μl of 3 μg/ml chloramphenicol (internal standard) solution and 4 ml of dichloromethane extracting solvent. The mixture was vortexed for 1 min on a vortex mixer and centrifuged at 3500 rpm for 15 min. The organic layer was transferred into a reactivial and evaporated to dryness at 40°C under a gentle stream of nitrogen gas. The residue was reconstituted with 100 μl of mobile phase and 50 μl injected onto the column.

2.4. Assay validation

Samples were quantified using peak height ratio of pentoxifylline over the internal standard. Standard calibration curves were constructed by spiking drug free pooled plasma with a known amount of pentoxifylline at a concentration range of 12.5–400.0 ng/ml. These plasma standards were also used to determine the extraction recovery, within-day and between-day precision and accuracy ($n=6$) of the method. The recovery of the extraction procedure for pentoxifylline and the internal standard was calculated by comparing peak height obtained after extraction with that of aqueous drug solution of the corresponding concentration without extraction.

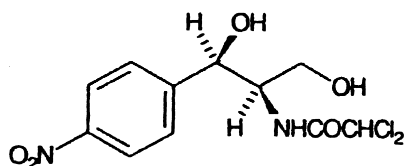

Fig. 2. Structure of chloramphenicol.

3. Results and discussion

Chromatograms obtained with blank plasma and plasma spiked with pentoxifylline and chloramphenicol are shown in Fig. 3A and B. The plasma used was pooled from 12 healthy adult male vol-

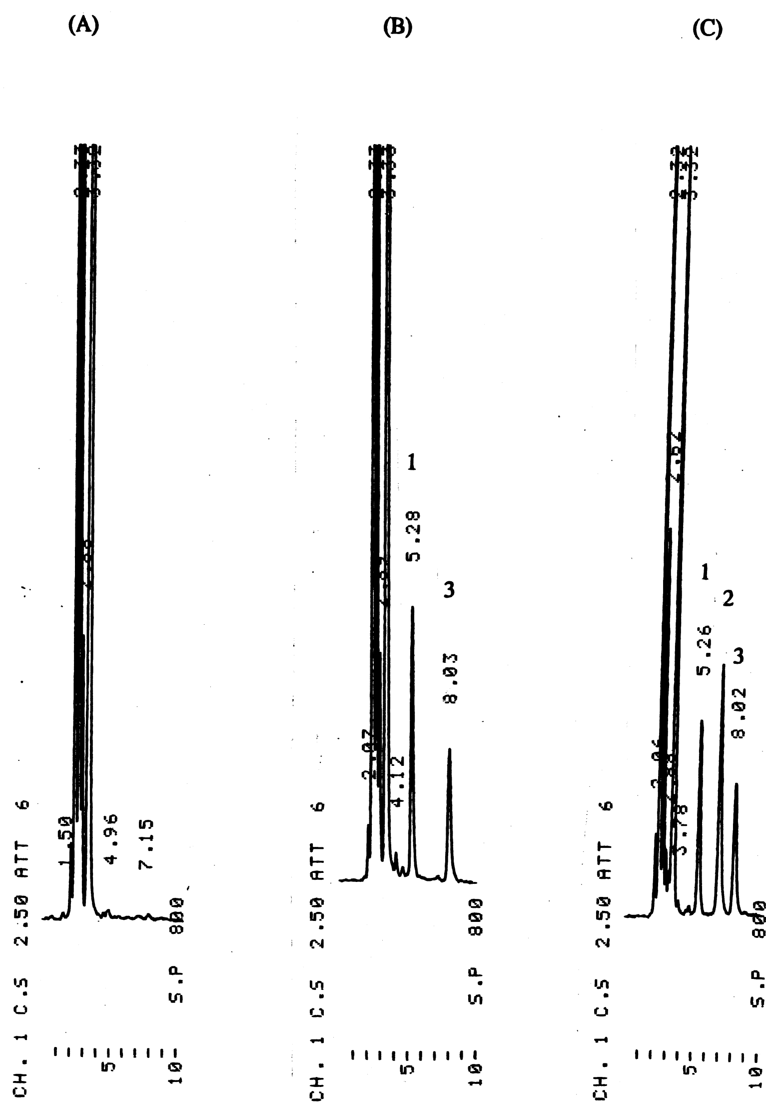

Fig. 3. Chromatograms for the analysis of pentoxifylline in plasma. (A) Blank plasma. (B) Plasma spiked with 200 ng/ml pentoxifylline and 3 μg/ml chloramphenicol. (C) A volunteer plasma containing 127.3 ng/ml pentoxifylline 40 min after oral administration of 400 mg of pentoxifylline. (Y-axis: Attenuation=0.16 AUFS/V, X-axis: Chart speed=2.5 mm/min. 1=pentoxifylline, 2=hydroxy metabolite, 3=chloramphenicol)

unteers who participated in a bioavailability study. It can be seen that the pentoxifylline and chloramphenicol peaks were well resolved and free of interference from endogenous compounds in the plasma, with retention times of 5.28 min and 8.03 min, respectively.

When extraction was first carried out using chloroform, several extra peaks were produced in the chromatograms of both blank and spiked plasma. In comparison, dichloromethane gave cleaner chromatograms with a lower number of peaks. A mean recovery of 99.9% was obtained for pentoxifylline and 79.5% for chloramphenicol.

The extraction recovery, within-day and between-day accuracy and precision values are presented in Table 1. The coefficient of variation (C.V.) values of

Table 1
Extraction recovery, within-day and between-day precision and accuracy ($n=6$)

Concentration (ng/ml)	Recovery		Within-day		Between-day	
	Mean (%)	C.V. (%)	Precision (C.V. %)	Accuracy (% error)	Precision (C.V. %)	Accuracy (% error)
12.5	99.3	2.1	7.2	6.8	9.9	4.5
25.0	98.8	3.0	5.2	7.8	7.8	5.2
50.0	100.4	3.9	1.8	2.3	3.0	2.2
100.0	100.2	3.0	2.0	0.6	2.2	0.4
200.0	99.7	1.3	3.5	0.8	1.6	0.4
400.0	100.7	6.0	0.9	1.1	3.1	1.0

both the within-day and between-day precision were all less than 10.0%, while those of accuracy with percent error values of less than 8.0%. The standard calibration curve ($n=6$) was found to be linear over the concentration range used. A slope of 148.55 with an intercept of −1.48, and a correlation coefficient of 0.9999 were obtained. The present method has a detection limit of 3.0 ng/ml at a signal to noise ratio of 3:1, being more sensitive than that reported by Chivers et al. [9], Lambert et al. [8] and Mancinelli et al. [11]. The sensitivity could be further improved by using either a larger volume sample loop or plasma sample. The limit of quantification was set at 12.5 ng/ml, being the lowest concentration used in constructing the standard curve.

The tetrahydrofuran content in the mobile phase was found to be critical in the separation of pentoxifylline from its major hydroxy metabolite. The hydroxy metabolite and chloramphenicol peaks overlapped when the content of tetrahydrofuran in the mobile phase was at 0.3% v/v but became well separated when increased to 1% v/v. On the other hand, when the content of tetrahydrofuran was more than 1.5% v/v, the pentoxifylline peak was found overlapping with an adjacent endogenous compound peak in the plasma. The optimal concentration of tetrahydrofuran for resolving the peaks was found to be at 1% v/v. The present method afforded good selectivity, in which structurally related compounds, such as caffeine, theobromine and theophylline commonly found in food and beverages, do not interfere with the assay. All these compounds were eluted in less than 4 min.

The present method was applied to analyze plasma samples of 12 healthy adult male volunteers from a comparative bioavailability study of two different pentoxifylline controlled release tablet preparations, namely, Trental and Pentoxifylline 400, the latter being a generic preparation. In Fig. 3C is shown a chromatogram obtained from a volunteer 40 min after dosing with 400 mg of pentoxifylline, while Fig. 4 shows the plasma concentration–time profiles of two volunteers who participated in the bioavailability study. In all cases, the last concentration quantifiable was less than 12.0% of the peak concentration. This would enable the pharmacokinetic parameters such as the elimination rate constant (k_e) and total area under the plasma concentration–time curve ($AUC_{0-\infty}$) to be estimated with a reasonable level of accuracy.

In conclusion, the present HPLC method was simple, specific, sensitive and suitable to be used for determination of plasma pentoxifylline in pharmacokinetic/bioavailability studies.

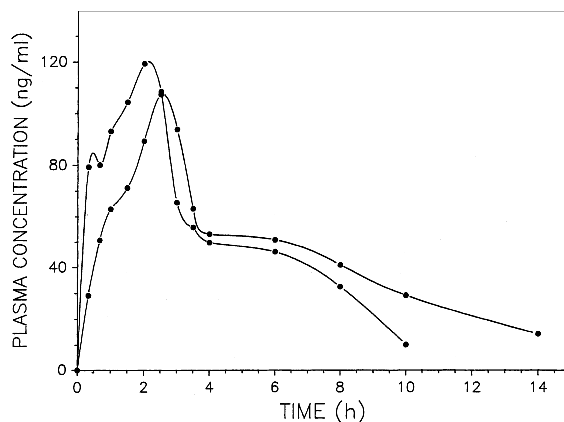

Fig. 4. Plasma concentration versus time profiles from two volunteers following the oral administration of 400 mg of pentoxifylline.

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