QUANTITATIVE DETERMINATION
OF PENTOXIFYLLINE IN HUMAN PLASMA

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SUMMARY

A rapid, optimized, and sensitive reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed for determination of pentoxifylline in human plasma. The analyte was extracted from the plasma with dichloromethane after addition of 0.2 mL 1 M NaOH. HPLC separation was performed on a C_{18} analytical column (250 mm × 4 mm i.d.) with acetonitrile–water, 45:55 (v/v), as mobile phase. Spectrophotometric detection was performed at 275 nm. Calibration graphs were linear from 25 to 1000 ng mL^{-1} pentoxifylline. Recovery of the drug from human plasma was 92.1%, and the detection limit was 20 ng mL^{-1}.

INTRODUCTION

Pentoxifylline, 1-(5-oxohexyl)-3,7-dimethylxanthine, is an active haemorheological drug widely used for the treatment of intermittent claudication and other circulatory disorders [1–4]. Because the drug improves perfusion in the impaired microcirculation of peripheral and cerebral vascular beds, it has also been tried as therapy for cerebrovascular disorders [5–7].

A GC procedure employing trifluoroacetyl derivatization and nitrogen-selective detection has been reported for quantification of pentoxifylline [8]. GC methods requiring extensive sample preparation and derivatization tend to be tedious. Previously described HPLC methods for analysis of pentoxifylline involve time-consuming extraction procedures [9–11], use an internal standard that is not readily available [12], or use a complex mobile phase; the methods also have a narrow range of linearity [13]. A simple and sensitive HPLC procedure has been described for determination of the concentrations of pentoxifylline and one of its major metabolites in

rat plasma, but retention times for pentoxifylline and the internal standard were too long [14,15].

It thus seems useful to develop a simple method for analysis of pentoxifylline in human plasma. The technique is based on sample preparation by liquid–liquid extraction and separation by reversed-phase high-pressure liquid chromatography. The method has been validated for linearity, precision, and accuracy and is sufficiently sensitive and rapid to be readily applicable to human pharmacokinetic studies.

EXPERIMENTAL

Reagents and Chemicals

Pentoxifylline 600 mg tablets were obtained from Heksal (Munchen, Germany). Phenacetin (internal standard) and sodium hydroxide were products of POCh (Gliwice, Poland). Acetonitrile and dichloromethane (HPLC grade) were supplied by Merck (Darmstadt, Germany). Control plasma was obtained from healthy volunteers.

Apparatus and Chromatographic Conditions

HPLC was performed with a Mini-Star K-500 solvent pump, K-2500 UV-detector, operated at 275 nm, K-3800 autosampler, and a computer system for data acquisition (Eurochrom 2000). Compounds were separated on a 250 mm × 4 mm i.d., 5 μm particle, Nucleosil-100 C_{18} reversed-phase column (Knauer, Berlin, Germany). Acetonitrile–water, 45:55 (*v/v*), was used as mobile phase at a flow-rate of 1 mL min^{-1}. Chromatograms were performed at ambient temperature.

Sample Preparation

Phenacetin (internal standard) solution in methanol (10 μg mL^{-1}, 100 μL, giving a concentration 1 μg mL^{-1} in the final sample), sodium hydroxide solution (1 M, 0.2 mL), and dichloromethane (4 mL) were added to 1 mL human plasma in a 7-mL tube. The resulting mixture was shaken mechanically for 10 min, centrifuged for 15 min at 7000g, and the upper aqueous phase was removed by vacuum aspiration. The organic layer was transferred to a conical tube and evaporated to dryness in a water bath at 45°C under a stream of argon. Finally, the residue was reconstituted in 200 μL mobile phase and 20 μL was injected into the chromatographic column. Standard samples were prepared by spiking blank plasma with

known amounts of pentoxifylline and used for construction of calibration plots.

Recovery from Human Plasma

Linear calibration graphs were obtained from 25 to 1000 ng mL^{-1} for pentoxifylline standard at concentrations of 50, 400, and 800 ng mL^{-1}. Recovery of internal standard from human plasma was determined at the concentration used in the samples. The recoveries were measured by direct comparison of peak-height ratios obtained for non-extracted standards and for plasma extracts.

Pharmacokinetic Study

A single 600-mg dose of pentoxifylline was administered orally to three healthy volunteers. Blood samples were taken before administration of the dose and again 0, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 h after ingestion, using heparin-Vacutainer collection tubes. The tubes were centrifuged at 1000g for 15 min and the plasma was collected and stored at −20°C until analysis.

RESULTS AND DISCUSSION

Chromatography

Symmetrical peaks were observed for pentoxifylline and the internal standard. Typical chromatograms are illustrated in Figs 1A and 1B. The retention times of pentoxifylline and the internal standard were 2.85 and 4.25 min, respectively. The overall chromatographic run time was 8.0 min.

Validation

The linearity and precision of the method were tested using spiked plasma samples. For a 1-mL sample the calibration plot was linear in the range 25–1000 ng mL^{-1} with a correlation coefficient of 0.9997 ($n = 6$). The mean regression equation was $H/H_{IS} = 0.0014\ (\pm 0.000009)C - 0.012\ (\pm 0.005)$, where H/H_{IS} is the peak height for pentoxifylline divided by the peak height for the internal standard and C the concentration of pentoxifylline. The numbers in parentheses are the standard errors. This plot was constructed from results from eight different concentrations.
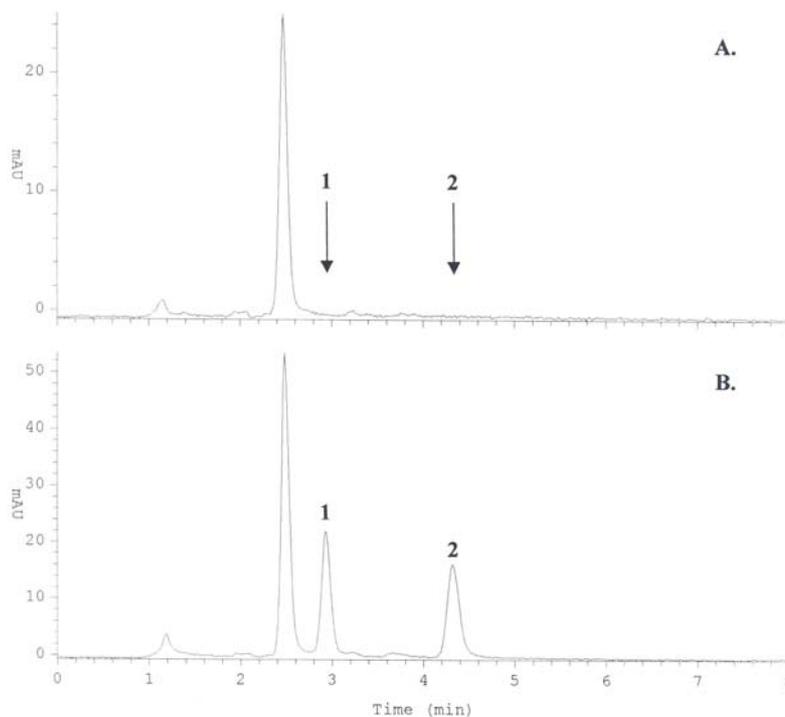
Fig. 1

Chromatograms obtained from analysis of pentoxifylline in plasma. (A) Blank plasma. (B) Plasma spiked with 1 μg mL^{-1} pentoxifylline (1) and 1 μg mL^{-1} phenacetin (2; internal standard).

The precision of the method was assessed by determination of six concentrations in six independent series of samples; the results are shown in Table I. The lower limit of quantitation, i.e. the amount for which the coefficient of variation was <10% for six replicate measurements, was 25 ng mL^{-1}.

The plasma was screened for interference at the retention times of pentoxifylline and phenacetin. The representative chromatograms obtained from a processed plasma blank shown in Figs 1A and 1B show that no endogenous compounds occur at the retention times of pentoxifylline and internal standard. Recovery of pentoxifylline from plasma matrix was 92.1%, irrespective of concentration in the range 25 to 1000 ng mL^{-1}. Pentoxifylline was shown to be stable in plasma maintained at −20°C for up to two months. Stability was also established through two freeze–thaw cycles for spiked plasma samples.

Table I

Within-day precision and accuracy

Substance	Nominal concentration (ng mL^{-1})												
Pentoxi-fylline	25	50	75	100	200	300	400	500	600	700	800	900	1000
Phenacetin	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Series	Back-calculated concentration (ng mL^{-1})												
1	27.14	57.86	80.00	101.43	215.71	308.57	422.86	515.71	622.85	708.57	822.85	915.71	1037.14
2	26.43	57.14	87.14	108.57	230.00	315.71	430.00	508.57	615.71	715.71	830.00	922.86	1044.28
3	24.29	52.14	80.00	94.29	222.86	301.43	415.71	501.43	608.57	694.28	808.57	908.57	1037.14
4	23.57	47.14	87.14	87.14	208.57	294.28	408.57	494.29	608.57	701.43	808.57	894.29	1022.86
5	22.14	46.43	72.76	87.14	201.43	301.43	401.43	494.29	601.43	687.14	801.43	901.43	1015.71
6	25.00	48.57	72.86	101.43	201.43	287.14	401.43	487.14	622.86	694.29	815.71	908.57	1022.86
AVG	24.76	51.55	80.00	96.67	213.33	301.43	413.33	500.24	613.33	700.24	814.52	908.57	1030.00
SD	1.84	5.02	6.39	8.65	11.66	10.10	11.66	109.51	8.65	10.51	10.51	10.10	11.06
RSD (%)	7.43	9.74	7.99	8.95	5.46	3.35	2.82	2.10	1.41	1.50	1.29	1.11	1.07

AVG, SD, and RSD denote mean value, standard deviation, and relative standard deviation, respectively ($n = 6$)

Day-to-day precision was also determined over a period of two months by taking plasma samples containing 50 ng mL^{-1}, 400 ng mL^{-1}, and 800 ng mL^{-1} pentoxifylline and processing them daily. Low coefficients of variation were observed – 13.85% at the lowest concentration, 4.64% at the middle concentration, and 1.23% at the highest concentration; the results obtained are presented in Table II.

Table II

Between-day precision and accuracy

Day	Calibration plot data				Back-calculated concentration (ng mL^{-1})		
	a	b	r	s	50	400	800
A	0.0014 (0.00004)	−0.014 (0.022)	0.9995	0.023	56.43	381.43	786.71
B	0.0015 (0.00005)	−0.029 (0.025)	0.9995	0.025	57.33	385.00	806.00
C	0.0014 (0.00004)	−0.007 (0.023)	0.9994	0.023	43.57	419.28	805.00
D	0.0013 (0.00004)	0.024 (0.022)	0.9995	0.023	43.08	401.54	804.61
E	0.0014 (0.00004)	0.003 (0.023)	0.9995	0.023	44.28	426.43	819.28
F	0.0015 (0.00003)	−0.046 (0.016)	0.9998	0.017	54.67	390.67	804.00
AVG					49.89	400.72	804.27
SD					6.91	18.58	10.37
RSD (%)					13.85	4.64	1.23

Numbers in parentheses denote standard deviations
a is the slope, b the intercept, r the correlation coefficient, and s the standard error for the calibration plot regression equation
AVG, SD, and RSD denote mean value, standard deviation, and relative standard deviation, respectively ($n = 6$)

It is apparent from the chromatograms obtained from human plasma spiked with pentoxifylline and phenacetin that no interference occurred in the region of elution of the compounds investigated (Fig. 2).
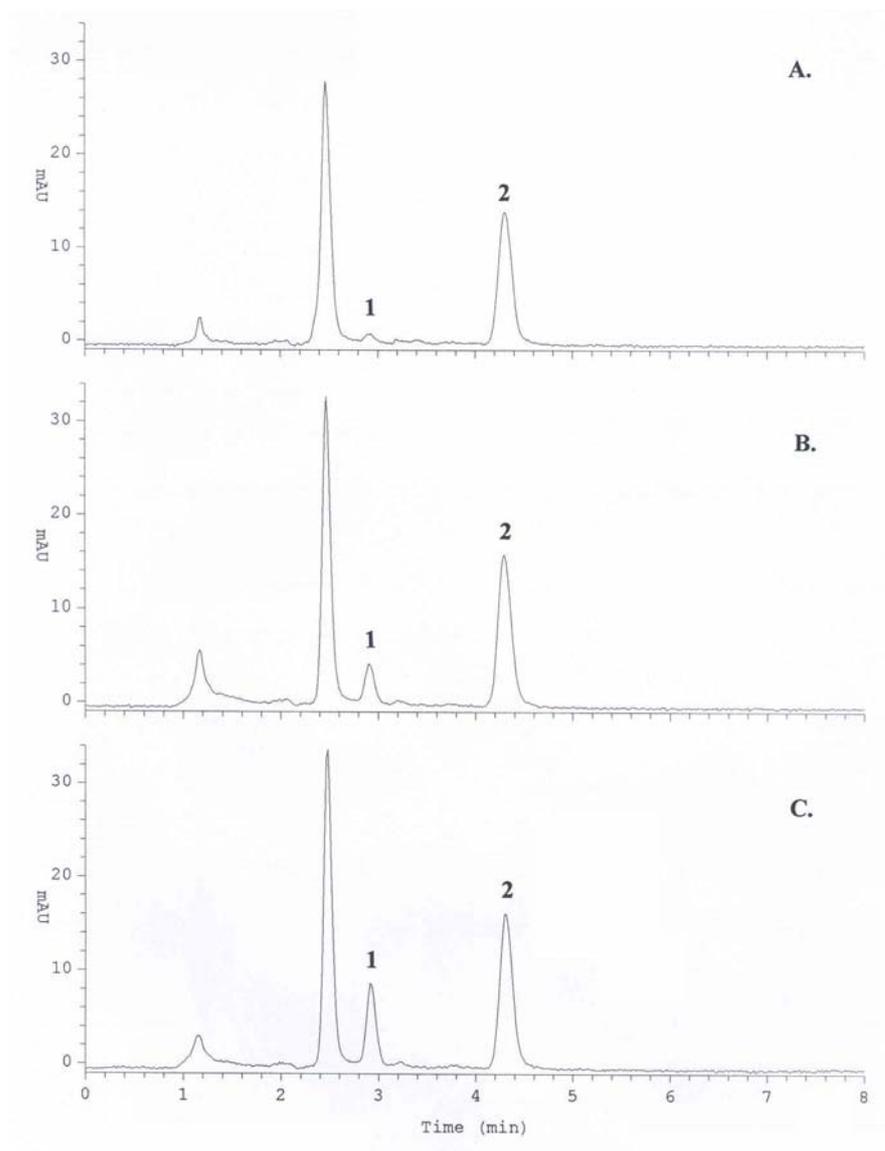

Fig. 2

Chromatograms obtained from human plasma spiked with 50 ng mL^{-1} (A), 200 ng mL^{-1} (B), and 400 ng mL^{-1} (C) pentoxifylline (1) and 1 μg mL^{-1} phenacetin (2; internal standard)

Fig. 3 shows the plasma concentration–time profiles obtained for the three volunteers who participated in the bioavailability study. For all the volunteers the last concentration quantifiable was less than 10% of the maximum concentration.
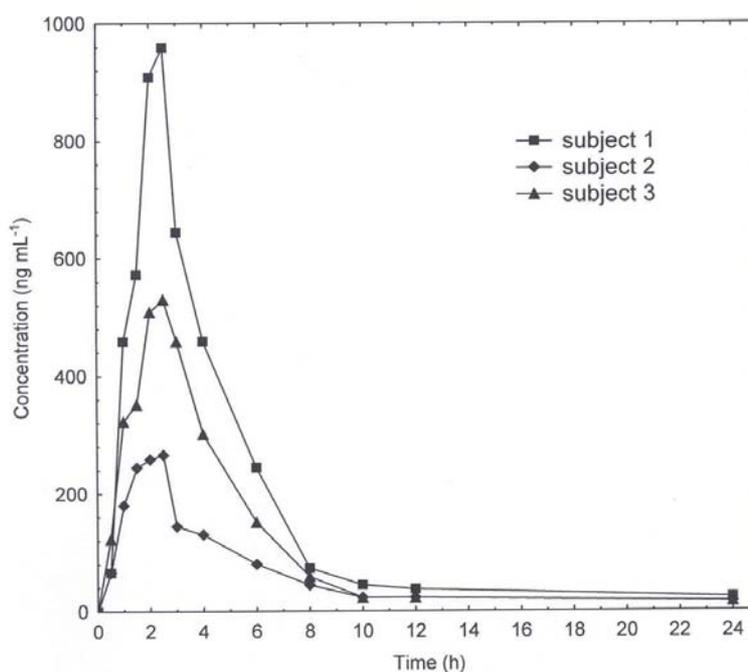

Fig. 3

Profiles of plasma concentration against time for three volunteers after oral administration of 600 mg pentoxifylline

Large inter-subject variability is apparent from the chromatograms presented in Fig. 4, obtained from plasma taken from the three volunteers 2.5 h after administration of 600 mg pentoxifylline. The amounts of pentoxifylline measured were 265.71 ng mL^{-1}, 530.0 ng mL^{-1} and 958.5 ng mL^{-1}.

The HPLC method developed and validated in this study is accurate and sensitive and enables complete recovery of pentoxifylline and internal standard from human plasma samples. The method uses a simple extraction procedure for the recovery of pentoxifylline from human plasma and furnished extracts in which both analytes were stable at 5°C, enabling automation of the method for large-scale studies. The method described was specific for assay of pentoxifylline and enabled reduction of the limit
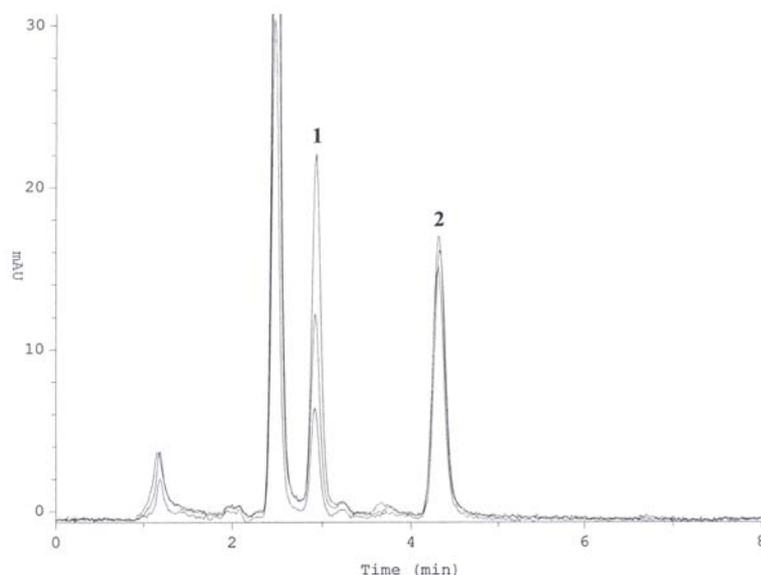
Fig. 4

Chromatograms obtained from human plasma taken from three volunteers 2.5 h after oral administration of 600 mg pentoxifylline (1) and spiked with 1 μg mL^{-1} phenacetin (2; internal standard)

of quantitation to 25 ng mL^{-1}. We conclude that this sensitive method for HPLC analysis of pentoxifylline is suitable for pharmacokinetic studies in humans after ingestion of a single dose of drug and may be recommended for monitoring this compound in patients. The method is simpler and more rapid than any other described in the literature, because it eliminates cleaning and derivatization of samples, thus reducing background noise as much as possible and protecting the equipment from excess derivatization reagent. Two other advantages are that methods involving derivatization usually suffer from low recovery of the derivatization products and are too complex and time-consuming for routine clinical analysis [8].

The proposed sample-preparation procedure, entailing single liquid–liquid extraction only, developed for this HPLC method was optimized to afford the most reliable, effective, and rapid approach for routine analysis and to eliminate time-consuming purification steps, including expensive solid-phase extraction used previously [9–11].

Phenacetin was chosen as internal standard because it is routinely available, unlike 7-ethyl-3-methyl-1-(5-oxohexyl)xanthine used by Chivers et al. [12].

In this method the retention times of pentoxifylline and internal standard, 2.85 min and 4.25 min, respectively, are shorter than for other methods (for example 5.28 min and 8.03 min for active substance and chloramphenicol as internal standard [13]). This results in a shorter total analysis time for single sample – 8 min in our method compared with 10 min [13]. Short assay time is very important in analysis of series of many samples. In other HPLC methods reported by Luke et al. [14] and by Garnier-Moiroux [15] retention times for pentoxifylline 7.9 and 5.3 min, respectively, are again too long.

CONCLUSIONS

This HPLC method was found to be selective, sensitive, and robust for the measurement of pentoxifylline in plasma. The assay is also quick and requires relatively simple sample preparation. A large number of samples can be processed daily. This assay enables quantitation of levels of pentoxifylline in plasma for at least 24 h after a single 600-mg oral dose of pentoxifylline. To increase sensitivity it is possible to increase the sample volume. The procedure described is suitable for pharmacokinetic studies and for drug monitoring in hospitals after ingestion of pentoxifylline.

REFERENCES

[1]   H.R. Dettelbach and D.M. Aviado, J. Clin. Pharmacol.,
      25, 8 (1985)
[2]   M. Abramowicz, Med. Lett. Drugs Ther., **26**, 103 (1984)
[3]   B. Beermann, R. Ings, J. Mansby, J. Chamberlain,
      and A. McDonald, Clin. Pharmacol. Ther., **37**, 25 (1985)
[4]   D.M. Aviado and J.M. Porter, Pharmacotherapy, **4**, 297 (1984)
[5]   J.L. Ambrus, J.M. Anain, S.M. Anain, P.M. Anain, S. Stadler,
      P. Mitchell, J.A. Brobst, B.L. Cobert, and J.P. Savitsky,
      Clin. Pharmacol. Ther., **48**, 50 (1990)
[6]   R. Muller and F. Lehrach, Curr. Med. Res. Opin., **7**, 253 (1981)
[7]   C.D. Nicholson, Psychopharmacology, **102**, 147 (1990)
[8]   R.V. Smith, E.S. Waller, J.T. Doluisio, M.T. Bauza, S.K. Puri,
      I. Ho, and H.B. Lassman, J. Pharm. Sci., **75**, 1 (1986)

[9] A. Mancinelli, S. Pace, A. Marzo, and E. Arrigoni Martelli, J. Chromatogr., **575**, 101 (1992)

[10] G. Musch, T. Hamoir, and D.L. Massart, J. Chromatogr., **495**, 215 (1989)

[11] J. Szymura-Oleksiak, A. Ślósarczyk, A. Cios, B. Mycek, Z. Paszkiewicz, S. Szklarczyk, and D. Stankiewicz, Ceramics Int., **27**, 762 (2001)

[12] D.A. Chivers, D.J. Birkett, and J.O. Miners, J. Chromatogr., **225**, 261 (1981)

[13] J.W. Wong, K.H. Yuen, and K.K. Peh, J. Chromatogr. B, **716**, 387 (1998)

[14] D.R. Luke and M.L. Rocci, J. Chromatogr., **374**, 191 (1986)

[15] A. Garnier-Moiroux, J.M. Poirier, and G. Cheymol, J. Chromatogr., **416**, 183 (1987)