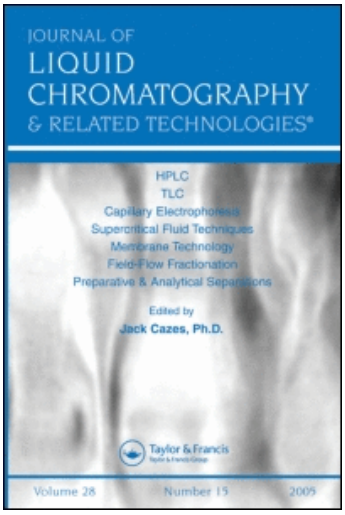

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A Rapid and Specific Method for the Determination of Tiaprofenic Acid in Human Plasma by High-Performance Liquid Chromatography

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A RAPID AND SPECIFIC METHOD FOR THE DETERMINATION OF TIAPROFENIC ACID IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

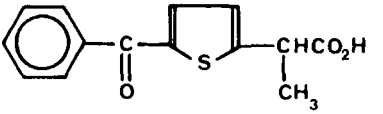
A method has been developed to assay tiaprofenic acid in human plasma. The technique is rapid, specific and sensitive, being capable of detecting 0.5 $\mu g\ ml^{-1}$ and requiring only 200 μl of plasma for a single assay.

Samples are deproteinized, extracted into chloroform and then, after evaporation of the chloroform and reconstitution in the mobile phase, are chromatographed by reverse phase liquid chromatography.

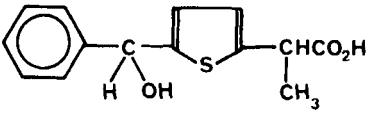
INTRODUCTION

Tiaprofenic acid, (1) 2-(5-benzoyl-2-thienyl) propionic acid (Fig. 1) is a non-steroidal anti-inflammatory and non-narcotic analgesic. Its pharmacological and therapeutic properties have been demonstrated in animals[1] and man.[2-6]

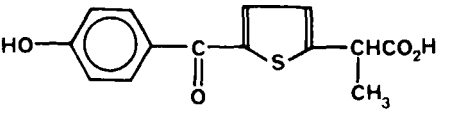
In order to study and compare the bioequivalence of tiaprofenic acid from different formulations a specific assay method was required to determine this drug in plasma.



Tiaprofenic acid I



Metabolite II



Metabolite III

FIGURE 1. Structures of Tiaprofenic Acid and its Two Major Metabolites

Previously a quantitative TLC assay has been used for pharmacokinetic studies.[7] This TLC method, whilst possessing adequate specificity and sensitivity was unsuitable for our present needs since it required relatively large samples (5 ml) and proved time consuming in operation.

The present work describes an HPLC method for tiaprofenic acid that is rapid and uses small quantities of sample. The chromatography separates tiaprofenic acid from its two major metabolites, 2-(5-α-hydroxybenzyl-2-thienyl) propionic acid (II) and 2-(5-p-hydroxybenzoyl-2-thienyl) propionic acid (III) and thus ensures specificity. The method is accurate and precise and its utility has been demonstrated in bioequivalence studies.

EXPERIMENTAL

Materials and Reagents

All reagents were of analytical grade and were used without any additional purification. Tiaprofenic acid and the two metabolites were supplied by Roussel-UCLAF (Romainville, France).

Chromatographic Apparatus

The chromatograph comprised a Magnus P4000 pump (Magnus Scientific, Cheshire, U.K.) and a Cecil 212 UV spectrophotometer (Cambridge, U.K.) fitted with an 8 µl flow cell. A Shandon 10 cm x 5 mm I.D. column fitted with a Shandon syringe injector and packed with Hypersil ODS (5 µm) (Shandon-Southern, Cheshire, U.K.) was used for chromatographic analysis. The column was slurry packed using isopropanol as suspending solvent and methanol as packing solvent.

Plasma Standards

Aqueous standards were prepared daily containing 10, 20, 50, 100 and 200 µg ml^{-1} of tiaprofenic acid. 20 µl of each of these standards were diluted in Dreyer tubes (Poulten, Self and Lee, Wickford, U.K.) to 200 µl with human drug-free plasma producing plasma standards containing 1, 2, 5, 10 and 20 µg ml^{-1} of tiaprofenic acid.

Sample Preparation

200 µl aliquots of plasma were pipetted into Dreyer tubes using an Oxford pipettor. To the plasma standards and the samples, 200 µl of 6% trichloroacetic acid was added and the contents were mixed on a vortex mixer for 20 seconds. 200 µl of the internal standard solution (20 µg ml^{-1} of ortho-nitrophenyl acetic acid in chloroform) was added and the contents of the tubes were mixed on a vortex mixer for 40 seconds and the tubes were centrifuged for

2 minutes using an Eppendorf 5412 centrifuge (the 0.4 ml test tube centrifuge adaptors were slightly drilled out to accept the Dreyer tubes).[8] The upper aqueous layers were discarded allowing the lower chloroform layers (150 µl) to be transferred to a second set of tubes. The chloroform was removed by evaporation under a stream of nitrogen at room temperature and the residues were reconstituted in 50 µl of mobile phase. 10 µl injections were made onto the chromatograph by syringe.

Chromatographic Analysis

The mobile phase was 50% methanol 50% distilled water and the pH adjusted to 3.0 with glacial acetic acid. The flow rate was 1 ml min^{-1} and the detector was operated at a wavelength of 313 nm and a sensitivity of 0.04 AUFS. The chromatograms were recorded on a strip chart recorder set at a chart speed of 20 cm hr^{-1}.

Quantitation

Quantitation was achieved by measuring the peak heights and calculating the peak height ratios of the tiaprofenic acid and internal standard. The plasma concentrations of tiaprofenic acid were then estimated from calibration curves generated graphically or by linear regression analysis.

RESULTS AND DISCUSSION

Sample Preparation

Tiaprofenic acid is easily and efficiently extracted into water immiscible solvents such as chloroform when the aqueous phase is acidified. The technique of acidifying with 6% trichloroacetic acid also precipitates the plasma proteins which aids in the production of cleaner extracts for chromatographic

analysis. The cleanliness of the sample for chromatography was such that over 400 plasma samples were assayed in duplicate during a six week working period without any appreciable loss of column performance.

Specificity

The chromatography developed for tiaprofenic acid is straightforward using a microspheroidal ODS packing with a relatively simple mobile phase. No interference from the plasma was observed with this system, even as shown in Fig. 2 the blank extract was run at a fourfold higher sensitivity.

Interference from the two metabolites of tiaprofenic acid was also investigated. It was found that this chromatography system is capable of separating tiaprofenic acid and the metabolites II and III. Fig. 3 shows the separation of the metabolites and also that of metabolite II, which has negligible absorbance at 313 nm and is not detected at this wavelength. Hypersil ODS was the packing used in this study but equally good chromatography has been obtained with columns of Spherisorb ODS.

Assay Validation

The calibration curves produced from the plasma standards were linear over the range used and passed through the origin. A typical example is shown in Fig. 4.

To estimate the accuracy and precision of the method a series of plasma samples covering the range 1 to 20 $\mu g\ ml^{-1}$ of tiaprofenic acid were prepared and assayed ten times each. The results are presented in Table 1 and show that the method is accurate and has good precision over the concentration range examined.

The reproducibility of the method was also examined. A set of 42 plasma samples at seven concentrations ranging from 0 to

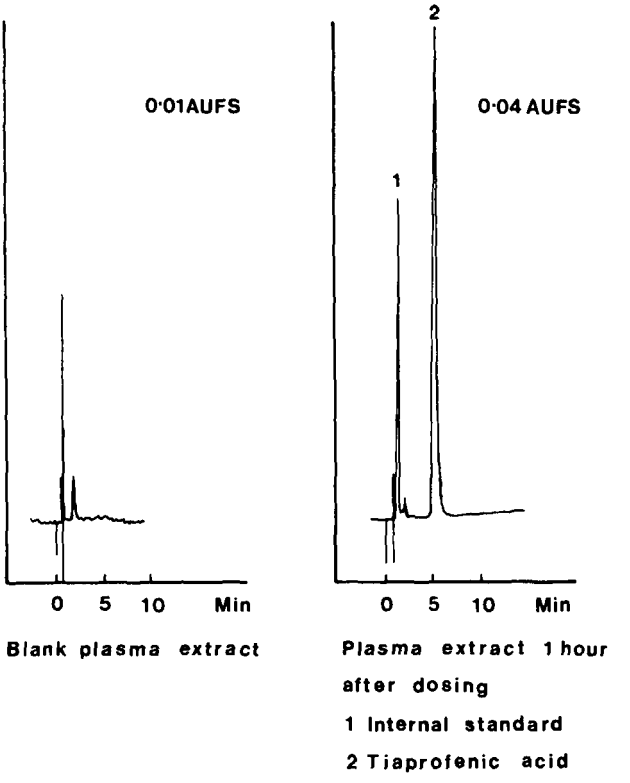


FIGURE 2. Chromatograms of a Blank Plasma Extract and of a Plasma Sample taken from a Volunteer 1 hour after a 200 mg oral dose of Tiaprofenic Acid

25 μg ml^{-1} of tiaprofenic acid were prepared by others, randomized and coded numerically. The samples were assayed over a period of four days and a summary of the results is shown in Table 2.

The recoveries and the coefficients of variation, which are comparable to those of Table 1, confirm the accuracy and reproducibility of the method and indicate the absence of operator or assay bias since the mean recovery values are all close to theory and the zero level samples were determined as zero.

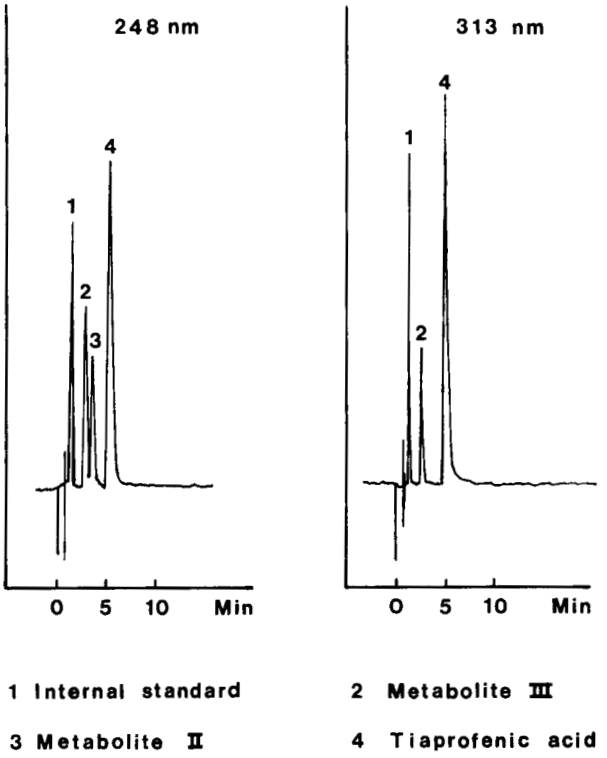


FIGURE 3. Chromatograms of Tiaprofenic Acid and its Major Metabolites

TABLE 1

Recovery of Tiaprofenic Acid

Amount Added $\mu g\ ml^{-1}$	Mean Amount Found $\mu g\ ml^{-1}$	Recovery %	Coefficient of Variation %
1.10	1.14	103.6	3.31
5.59	5.95	106.4	5.80
14.31	14.10	98.5	4.84
20.53	21.47	104.6	3.43

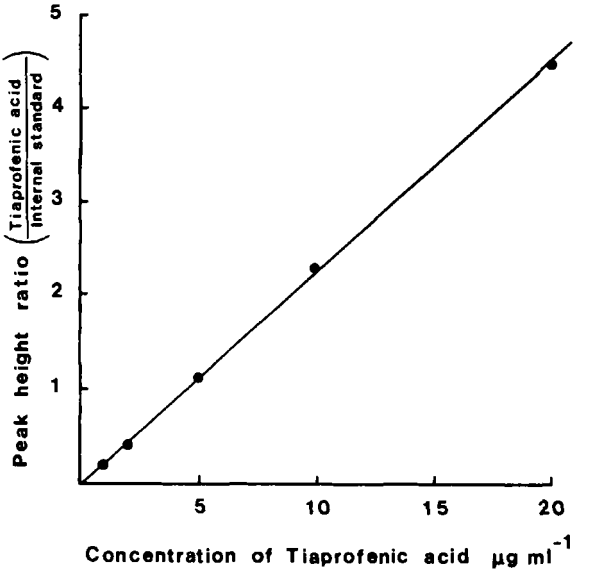


FIGURE 4. Typical Calibration Curve of Plasma to which Standard Solutions of Tiaprofenic Acid were added

TABLE 2

Recovery and Reproducibility of Tiaprofenic Acid

	Amount Added µg ml^{-1}	Mean Amount Found µg ml^{-1}	Recovery %	Coefficient of Variation %
A	0	0		
B	1.0	1.03	103.0	5.0
C	1.98	2.02	102.0	7.30
D	5.8	5.92	102.1	7.04
E	11.8	11.97	101.4	6.13
F	19.0	20.03	105.4	5.83
G	25.0	25.47	101.9	10.56

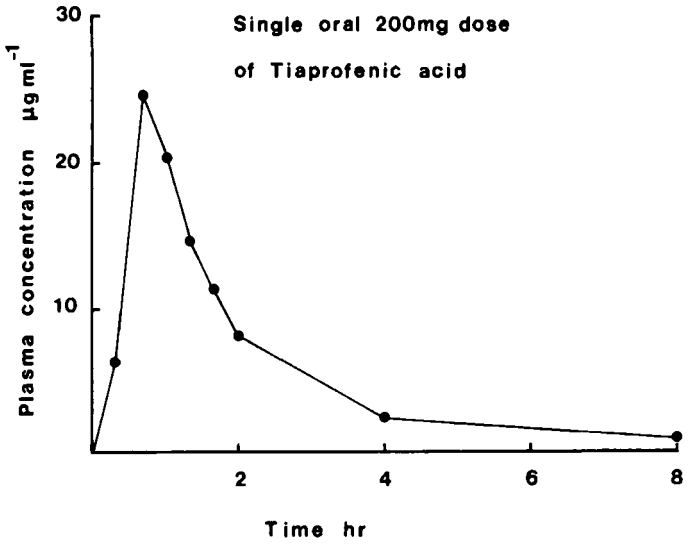


FIGURE 5. Plasma Concentration of Tiaprofenic Acid against Time

The method has successfully be applied to the analysis of plasma samples from bioequivalence studies and a typical plasma concentration against time curve from a volunteer after a 200 mg oral dose of tiaprofenic acid is shown in Fig. 5. The full results of this bioequivalence study will be published elsewhere.

Conclusion

The method developed to assay tiaprofenic acid in plasma is specific, accurate and precise. It is a rapid micro method requiring only 200 µl of plasma for a single assay. Recently it has been possible to automate the HPLC procedure by a minor modification to the final step of the sample preparation, and by using a Magnus M7100 Auto Sampler (Magnus Scientific, Cheshire, U.K.).

ACKNOLEDGMENTS

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