High-Performance Liquid Chromatographic Determination of Ketoprofen in Blood and Urine

R. FARINOTTI and G. MAHUZIER ˣ

Received April 24, 1978, from the *Laboratoire de Chimie Analytique, Centre d'Etudes Pharmaceutiques, Université de Paris-Sud, rue Jean Baptiste Clément, 92290 Chatenay Malabry, France.* Accepted for publication September 18, 1978.

Abstract □ A rapid, simple determination was developed for ketoprofen in biological fluids using high-performance liquid chromatography. The method requires selective extraction of this antirheumatic medicament and an internal standard with ether from the previously acidified plasma and urine. After evaporation of the ether, the residue is taken up by methanol and analyzed by reversed-phase liquid chromatography with detection at 254 nm. A concentration as low as 0.1 μg/ml can be determined with a 0.5-ml sample.

Keyphrases □ Ketoprofen—high-performance liquid chromatographic determination in blood and urine □ High-performance liquid chromatography—analysis, ketoprofen in blood and urine □ Anti-inflammatory agents—ketoprofen, high-performance liquid chromatographic analysis in blood and urine

Ketoprofen [2-(benzoyl-3-phenyl)propionic acid, I] is widely used in rheumatology because of its analgesic and anti-inflammatory properties. Pharmacokinetic studies of this product in humans and animals require a rapid, sensitive, and specific method. Various colorimetric, polarographic, TLC, and GLC methods have been reported. However, they generally require large test samples, and the operations are lengthy.

High-performance liquid chromatography (HPLC) eliminates these drawbacks and enables the rapid determination of plasma and urine concentrations after normal therapeutic doses.

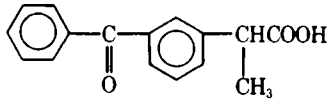
EXPERIMENTAL

Reagents—Compound I and the internal standard, 2-(benzoyl-3-phenyl)butyric acid (II), were synthesized and obtained analytically pure[1]. The ether, methanol, and other reagents were of analytical quality and were used without prior purification[2].

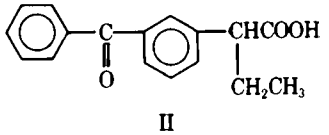
Apparatus—A high-performance liquid chromatograph[3] was equipped with a spectrophotometer[4]. The plasma and urinary extracts were introduced on a column filled with 10-μm silica particles covered by a monomolecular layer of octadecylsilane[5]. The mobile phase was methanol–distilled water (45:55 v/v). This eluent, previously disengaged by magnetic agitation, crossed the chromatographic column at ambient temperature with a flow of 1.1 ml/min at 1800 psig.

The absorption intensities of I and II were measured at 254 nm using an attenuation of 0.05 unit full scale.

Standard Solutions—All stock and standard solutions of I and II were prepared in methanol and included a 500-μg/ml ketoprofen stock solution, a 500-μg/ml internal standard stock solution, ketoprofen standard solutions (prepared by diluting the stock solution 1:5, 1:10, 3:40, 1:20, 5:200, 1:100, and 1:200), and a 25-μg/ml internal standard solution (prepared by diluting the stock solution 1:20). These solutions were stored in the dark at 4°.



I



II

Samples—Blood was collected on sodium heparinate and centrifuged to separate the erythrocytes. The plasma was frozen until assayed.

The urinary determinations were carried out on recovered fractions with no special precautions.

Plasma Determinations—One hundred-microliter aliquots of the internal standard solution, at 25 μg/ml, were transferred to 30-ml stoppered centrifuge tubes and evaporated under a nitrogen stream. Then 0.5 ml of plasma, 0.5 ml of distilled water, 100 μl of perchloric acid at 70%, and 10 ml of ether were added to the residue. After mechanical agitation for 15 min, the ether phase extract was removed with 10 ml of ether. The combined ether phases were washed by agitation for 5 min with 5 ml of 1 *N* HCl and then 5 ml of distilled water.

The organic phase was dried by passage over anhydrous sodium sulfate and evaporated to dryness in a nitrogen stream at ambient temperature. Then 100 μl of methanol was added to the residue, and 10 μl of the solution was injected into the chromatograph.

Calibration was achieved by loading the plasma of nontreated subjects with increasing quantities of I and fixed quantities of II.

One hundred microliters of each I standard solution was introduced into 30-ml centrifuge tubes, identical to those used previously, together with 100 μl of the 25-μg/ml internal standard solution. These methanol solutions were evaporated in a nitrogen stream, and then 0.5 ml of plasma from nontreated subjects was added to each tube. The tubes contained quantities of I corresponding to plasma concentrations of 0.5, 2.5, 5, 7.5, 10, and 20 μg/ml. Each loaded plasma was then treated as already described and injected into the chromatograph.

Urinary Determination—Two hundred-microliter aliquots of the internal standard stock solution (500-μg/ml solution) were transferred to similar 30-ml centrifuge tubes together with 1 ml of the urine to be determined, 100 μl of 10 *N* NaOH, and 10 ml of ether. After a contact period of 10 min and mechanical agitation for 10 min, the ether phase was separated and elminated. The aqueous phase was acidified with 1 ml of 3 *N* HCl and then, as with the plasma, ether extraction was performed. The residues of the ether extracts were taken up by 1 ml of methanol, and 5 μl of this methanol solution was injected into the chromatograph.

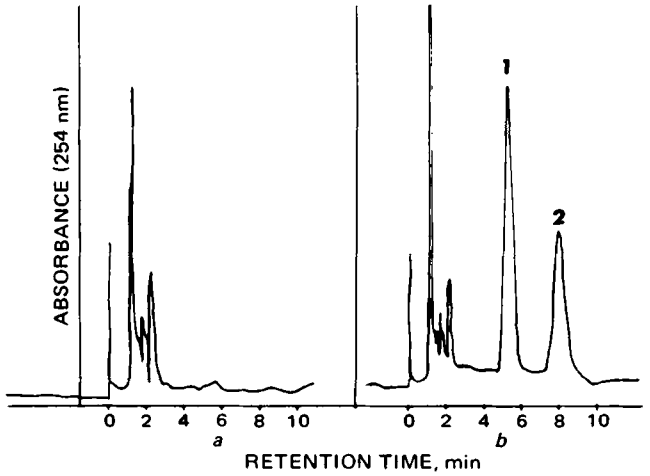


Figure 1—*Chromatogram of human plasma. Key:* a, *control plasma;* b, *plasma of a patient who received 100 mg of ketoprofen orally (2 hr after administration); 1, ketoprofen; and 2, internal standard.*

[1] Laboratories Specia, Paris, France.
[2] Prolabo Normapur.
[3] Model 6000 A pump and U6K injector, Waters Associates.
[4] Varian Varichrom.
[5] μBondapack C_{18} column (4 mm i.d. × 30 cm), Waters Associates.

0022-3549/79/0400-0484$01.00/0

Table I—Values of Plasma and Urinary Calibration Curve Points

Biological Medium	Concentration, μg/ml	Peak Height Ratios between I and II	Equation
Plasma	0.5	0.125 ± 0.01	$y = 0.366x + 0.022$
	1	0.33 ± 0.02	
	2.5	0.935 ± 0.03	
	5	1.98 ± 0.04	
	7.5	2.94 ± 0.046	
	10	3.61 ± 0.055	
	15	5.43 ± 0.07	
Urine	10	0.155 ± 0.003	$y = 0.019x - 0.07$
	50	0.91 ± 0.07	
	100	1.73 ± 0.02	
	150	2.67 ± 0.02	
	200	3.80 ± 0.03	

For calibration, 20, 100, 200, and 400 μl of the 500-μg/ml I stock solution were introduced into 30-ml centrifuge tubes. These methanol solutions were then evaporated in a nitrogen stream. To each tube were added 1 ml of urine from a nontreated subject and 200 μl of the internal standard stock solution. The rest of the procedure was as described for plasma.

Calculations—The peak height ratios between the ketoprofen standard and those of the internal standard were plotted as the ordinate, and the corresponding ketoprofen concentrations were plotted on the abscissa. The unknown plasma or urine concentrations were calculated from the calibration curves thus obtained.

RESULTS

Separation—Separation of the ketoprofen and the internal standard was satisfactory since retention times were 5.3 and 8 min, respectively (Figs. 1*b* and 2*b*), under the experimental conditions proposed. Moreover, none of the normal constituents of either plasma or urine appeared on the chromatograms.

Extraction Yield—Extraction yields of I were determined by adding known quantities of I to 10 reference plasma samples. Extractions were performed as already described, and the internal standard was added to the extracts obtained. The average extraction yield was 84.1 ± 5.2%.

Linearity—Peak height ratio values of I and II as a function of the concentration of I are given in Table I. Good linearity was observed both for plasma and urine.

Accuracy and Precision—The accuracy of the method was checked by carrying samples at three concentration levels in replicates of five through the entire method; the detected concentration was calculated from a pooled standard curve. The accuracy is indicated by the mean error between the detected and theoretical values (Table II). The mean errors were −0.054, +0.050, and +0.27 μg/ml for the 1.00-, 2.50-, and 5.00-μg/ml samples, respectively. Their corresponding relative errors were −5.4, +2, and +5.4%.

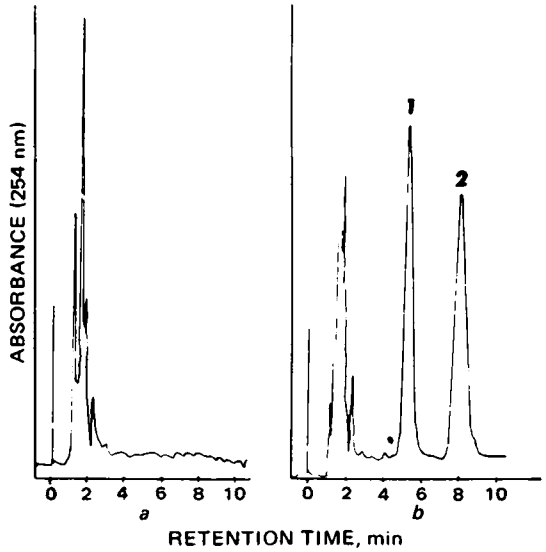


Figure 2—*Chromatogram of human urine. Key:* a, *control urine;* b, *urine of a patient who received 100 mg of ketoprofen orally (24-hr collected urines); 1, ketoprofen; and 2, internal standard.*

Table II—Accuracy and Precision of I Analysis in Plasma by HPLC

I Added to Human Plasma, μg/ml	I Detected μg/ml		Difference, μg/ml	
1		0.92		−0.08
		1.00		0.00
		0.95		−0.05
		1.00		0.00
		0.86		−0.14
	Mean ± *SD*	0.95 ± 0.06	Mean error	−0.054
	RSD	6.2%	Relative error	−5.4%
2.5		2.47		−0.03
		2.50		0.00
		2.50		0.00
		2.52		+0.02
		2.70		+0.20
	Mean ± *SD*	2.54 ± 0.09	Mean error	+0.050
	RSD	3.6%	Relative error	+2.0%
5		5.35		+0.35
		5.20		+0.20
		5.20		+0.20
		5.25		+0.25
		5.35		+0.35
	Mean ± *SD*	5.25 ± 0.07	Mean error	+0.27
	RSD	1.4%	Relative error	+5.4%

The precision of the method was examined similarly by comparing the results between these five replicate samples at each concentration level. The mean detected concentrations were 0.95, 2.54, and 5.25 μg/ml for samples of 1.00, 2.50, and 5.00 μg/ml, respectively. The standard deviations were 0.06, 0.09, and 0.07 μg/ml, respectively, and the corresponding relative standard deviations were 6.2, 3.6, and 1.4%.

DISCUSSION

The presence of two benzene rings in I allows chromatography of this molecule on a nonpolar column and its detection at 254 nm, its absorption maximum. The sensitivity achieved is sufficient for the plasma and urinary contents encountered. However, some substances present in biological media, and equally extractable in an acid medium, could show up if the extraction is not sufficiently selective.

Extraction conditions were chosen after various tests concerned with agitation time and the type and volume of solvents. Of the solvents tested (chloroform, methylene chloride, ethyl acetate, and ether), ether gave the best results. A good baseline was obtained if washing was carried out with 1 *N* HCl and distilled water as previously suggested (1).

No improvement was achieved by purification tests, either by passage through an alkaline medium followed by further extraction or by passage over Amberlite XAD2 resin.

The great affinity of I for plasma proteins (over 90%) (2) required their denaturation, and perchloric acid afforded better yields than hydrochloric acid.

Since most I is eliminated in the urine as the glucuronide, hydrolysis is necessary. In accordance with previous results (3), the addition of 10 *N* NaOH to the urine specimen gave better results after 10 min than enzymatic hydrolysis with *Helix pomatia* juice after 24 hr.

Urine contents, about 10 times those of plasma contents, require the use of more concentrated standards.

The method proposed is simple, sensitive, and rapid for the determinations of I in biological fluids during kinetic studies. It avoids the prior formation of methyl derivatives, a drawback with GLC.

REFERENCES

(1) P. Populaire, B. Terlain, S. Pascal, B. Decouvelaere, G. Lebreton, A. Renard, and J. P. Thomas, *Ann. Pharm. Fr.*, **31**, 679 (1973).

(2) R. Ballerini, A. Cambi, and P. Del Soldato, *J. Pharm. Sci.*, **66**, 282 (1977).

(3) P. Populaire, B. Terlain, S. Pascal, B. Decouvelaere, A. Renard, and J. P. Thomas, *Ann. Pharm. Fr.*, **31**, 735 (1973).

ACKNOWLEDGMENTS

The authors thank Laboratories Specia who supplied the ketoprofen and the internal standard.