Journal of Chromatography, 414 (1987) 131–137
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3431

DETERMINATION OF THE (+)- AND (−)-ENANTIOMERS OF PIRPROFEN IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(First received July 10th, 1986; revised manuscript received September 25th, 1986)

SUMMARY

A high-performance liquid chromatographic (HPLC) method was developed to determine the (+)- and (−)-enantiomers of pirprofen, an anti-inflammatory drug. After addition of an internal standard, the plasma sample was brought onto a glass column pre-packed with silica and eluted with dichloromethane. The extracts were derivatized with 1,1′-carbonyldiimidazole and *R*(+)-1-methylbenzylamine to form the two diastereomeric amides. The diastereoisomers were separated on a chiral column by HPLC with ultraviolet detection at 272 nm using *n*-hexane–dichloromethane (64:36, v/v) as the mobile phase. The limit of quantitation was 0.992 μmol/l (0.25 μg/ml) for each enantiomer.

INTRODUCTION

Pirprofen, 2-[3-chloro-4-(3-pyrrolin-1-yl)-phenyl]propionic acid (Rengasil®), is a non-steroidal anti-inflammatory drug with a chiral center in the α-position to the carboxyl group (Fig. 1). Therefore, it was of interest to investigate a possible stereoselective disposition of the pirprofen enantiomers after administration of the racemate.

Methods are described in the literature to quantitate the enantiomers of arylpropionic acid derivatives by formation of the diastereomeric amide [1–4] or ester [5] by reaction with an optically active reagent and separation on a silica column by high-performance liquid chromatography (HPLC). In all these methods, the diastereomeric derivative of the (−)-enantiomer of the anti-inflammatory drug was eluted first.

Wainer and Doyle [6] developed an HPLC procedure for the resolution of enantiomers of α-methylarylacetic acids after derivatization with a non-chiral reagent and separation by HPLC on a chiral stationary phase (CSP). The deriv-

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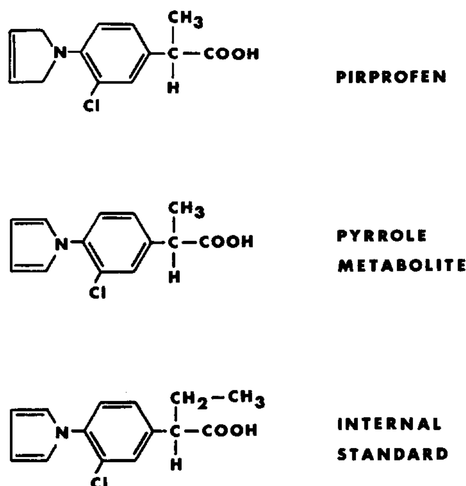

Fig. 1. Structures of pirprofen, its pyrrole metabolite and the internal standard.

ative of the (+)-enantiomer eluted before the derivative of the (−)-enantiomer. This procedure has been used by Crowther et al. [7] for the liquid chromatographic–mass spectrometric determination of optically active drugs.

This paper describes a method to determine the (+)-and (−)-enantiomers of pirprofen in human plasma after reaction with an optically active reagent and separation on a CSP-HPLC column. The limit of quantitation for any one enantiomer of pirprofen in plasma was 0.992 μmol/l (0.25 μg/ml).

EXPERIMENTAL

Chemicals and reagents

Pirprofen ($C_{13}H_{14}ClNO_2$; mol. wt. 251.71), its pyrrole metabolite ($C_{13}H_{12}ClNO_2$; mol. wt. 249.70) and the internal standard ($C_{14}H_{14}ClNO_2$; mol. wt. 263.72), the butyric acid analogue of the pyrrole derivative (Fig. 1), were supplied as racemates by Ciba-Geigy (Basle, Switzerland). The (+)- and (−)-enantiomers of pirprofen were supplied by Ciba-Geigy Pharmaceuticals (Horsham, U.K.).

All solvents were of analytical grade: *n*-hexane RS for spectrophotometry (No. 446 934, Carlo-Erba), dichloromethane Uvasol for fluorimetry (No. 6064, Merck, Darmstadt, F.R.G.), chloroform Uvasol (No. 2447, Merck). The optically active reagent *R*(+)-1-methylbenzylamine (No. 9470) was purchased from Fluka (Buchs, Switzerland), the 1,1′-carbonyldiimidazole from Aldrich (Ref. No. 11553-3) and its solution (50 mg/ml of chloroform) was prepared daily. Acetate buffer pH 5.3 was prepared from two stock solutions A and B (20:80, v/v). Solution A was obtained by diluting 5.72 ml of acetic acid (No. 63, Merck) with distilled water up to a volume of 100 ml, solution B by dissolving 8.20 g anhydrous sodium acetate (No. 6264, Merck) in distilled water to make a volume of 100 ml. The extraction columns were glass columns pre-packed with silica (Extrelut 1, Ref. No. 15371, Merck).

Chromatography equipment and conditions

Chromatography was performed on a Hewlett-Packard 1084 high-performance liquid chromatograph or with a Waters M 590 pump. Both systems were connected to a variable-wavelength UV detector (Kratos 773) set at 272 nm.

A Waters U6K injector was connected to the Waters pump. A Hewlett-Packard 3388 integrator recorded the chromatograms and calculated the peak heights.

The chiral stationary phase columns used contained (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (DNBPG) covalently bound to specially treated 5-μm aminopropyl silica (J.T. Baker, Phillipsburg, NJ, U.S.A.). The diastereomeric derivatives were eluted at a flow-rate of 1.5 ml/min using a degassed mobile phase of *n*-hexane–dichloromethane (64:36, v/v).

After complete elution of the compounds of interest, the flow-rate was increased to 2.5 ml/min for 15 min to wash out the compounds with longer retention times.

Extraction procedure

An internal standard solution (5 μg in 100 μl) prepared in distilled water was introduced into a 10-ml glass tube. After addition of 0.5 ml of plasma and 0.5 ml of buffer pH 5.3, the tube was shaken for 5 s on a Vortex mixer. The sample was loaded onto a pre-packed glass column Extrelut 1 left at room temperature for 5 min before eluting the compounds of interest with 6 ml of dichloromethane into a conical glass tube. The eluate was concentrated to a final volume between 100 and 200 μl.

Derivatization procedure

The derivatization procedure was based on the method described by Van Giessen and Kaiser [8]. A 100-μl aliquot of 1,1′-carbonyldiimidazole solution was added to the concentrated eluate, the tube shaken for 10 s on a Vortex mixer and left for 5 min at room temperature. Then, one drop of concentrated acetic acid and 10 μl of *R*(+)-1-methylbenzylamine were added, the tube was shaken for 10 s on a Vortex mixer and left at room temperature for 35 min.

After addition of 1 ml of 5% ammonia solution, the tube was shaken for 10 s on a Vortex mixer and then 0.6 ml of *n*-hexane were added. After shaking for 30 s on a Vortex mixer and 2 min of centrifugation, the *n*-hexane layer was washed with 1 ml of 0.01 mol/l hydrochloric acid. The tube was briefly shaken and centrifuged. A 30-μl sample of the *n*-hexane layer was injected into the chromatograph.

Calibration curve

Pirprofen was dissolved in some drops of methanol and made up to the final volume with distilled water. The calibration samples were obtained by adding to 0.5 ml of plasma, 100 μl of the internal standard solution and a suitable volume of pirprofen solution corresponding to final concentrations in the range between 1.98 and 79.2 μmol of the racemate of pirprofen per litre of plasma. To establish calibration curves, the peak-height value of the compounds was divided by the peak-height value of the internal standard. The logarithm of the difference of the resulting ratio (H_x) and the blank value (H_0) was plotted against the logarithm of the initial concentrations (x):

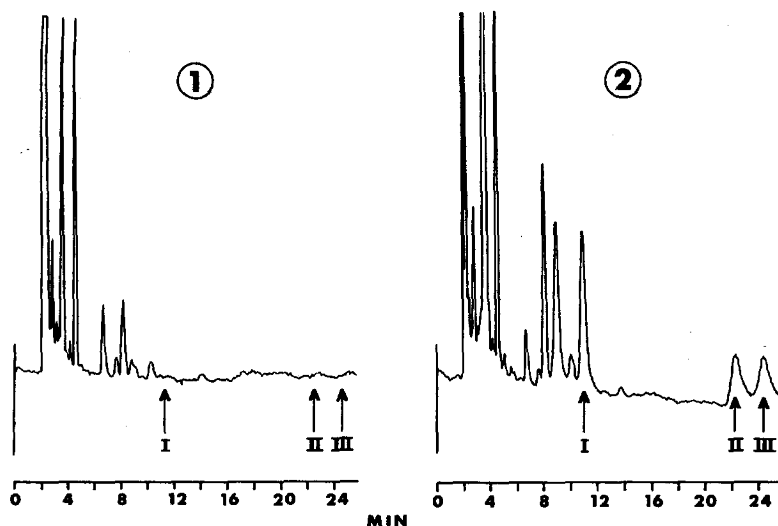

Fig. 2. Typical chromatograms of (1) extract of a blank human plasma sample (0.5 ml) and (2) extract of a human plasma sample spiked with 0.5 μg of pirprofen racemate and 5 μg of internal standard racemate. Peaks: I = derivative of one enantiomer of the internal standard; II = derivative of the (+)-enantiomer of pirprofen; III = derivative of the (−)-enantiomer of pirprofen.

$$\log (H_x - H_0) = \log a + b \log x$$

in normal coordinates:

$$H_x = H_0 + ax^b$$

For $b=1$, one obtains $H_x = H_0 + ax$, which is the equation of a straight line. For instance, the parameters of a calibration curve for the (−)-enantiomer of pirprofen were: slope = 1.0419, intercept = −1.944, blank = 0.0132; the correlation coefficient was 0.9998. The calibration curve differs from a straight line. For all the calibration curves, the intercept H_0 was very close to zero.

A calibration curve was established every week with freshly prepared solutions of pirprofen and internal standard.

RESULTS AND DISCUSSION

Plasma interference

Fig. 2 shows a chromatogram corresponding to the extract of 0.5 ml of human blank plasma. There is no interference of the reaction products or of the plasma components with the peaks of the diastereomeric derivatives of the enantiomers of pirprofen and of the internal standard. The same plasma was spiked with 0.5 μg of the racemate of pirprofen and 5 μg of the racemate of the internal standard. Of the diastereomeric derivatives of the enantiomers of pirprofen, the derivative formed by reaction with the (+)-enantiomer is eluted first.

The diastereomeric derivatives of the pyrrole metabolite interfered with peaks in the blank plasma extract and, therefore, were not quantitated.

TABLE I

WITHIN-DAY PRECISION AND ACCURACY OF THE DETERMINATION OF (+)- AND (−)-ENANTIOMER OF PIRPROFEN IN SPIKED HUMAN PLASMA

Concentration of each enantiomer added (μmol/l)	n	Accuracy (%)*	
		(+)-Enantiomer of pirprofen	(−)-Enantiomer of pirprofen
0.992	5	104.5 (3.7)	94.2 (4.3)
1.984	6	100.2 (1.3)	105.5 (5.7)
3.174	6	99.9 (9.3)	95.9 (3.5)
5.952	6	94.8 (3.4)	104.2 (9.2)
21.824	6	104.2 (2.6)	106.3 (7.6)
Mean		100.6 (5.8)	101.6 (8.0)

*Values in parentheses are coefficients of variation (%).

Within-day precision and accuracy

Six plasma samples spiked with five different concentrations of pirprofen were repeatedly analysed on the same day. The results are shown in Table I.

In the concentration range 0.992–21.824 μmol/l, the mean accuracy [(concentration found/concentration added)·100%] was 100.6% (coefficient of variation, C.V. 5.8%) for the (+)-enantiomer of pirprofen and 101.6% (C.V. 8.0%) for the (−)-enantiomer of pirprofen. The limit of quantitation was 0.992 μmol/l (0.250 ng/ml) for any one enantiomer of pirprofen.

Day-to-day precision and accuracy

Plasma samples containing pirprofen at five different concentrations were analysed in duplicate on five days. The concentrations of each of the enantiomers ranged from 0.992 to 21.824 μmol/l. The mean accuracy was 99.9% (C.V. 6.7%)

TABLE II

DAY-TO-DAY PRECISION AND ACCURACY OF THE DETERMINATION OF (+)- AND (−)-ENANTIOMER OF PIRPROFEN IN SPIKED HUMAN PLASMA

Concentration of each enantiomer added (μmol/l)	n	Accuracy (%)*	
		(+)-Enantiomer of pirprofen	(−)-Enantiomer of pirprofen
0.992	5	104.9 (8.1)	108.9 (13.5)
2.380	5	97.6 (5.2)	97.5 (7.4)
4.760	5	99.8 (4.9)	98.5 (4.2)
9.920	5	95.6 (6.4)	96.3 (4.2)
21.824	5	101.3 (6.9)	100.8 (5.2)
Mean		99.9 (6.7)	100.4 (8.7)

*Values in parentheses are coefficients of variation (%).

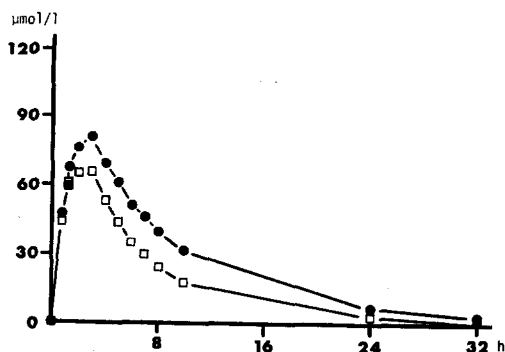

Fig. 3. Mean curve of the concentrations of (+)- or (−)-enantiomer of pirprofen obtained after administration of a single oral dose of 400 mg of pirprofen racemate to eleven healthy volunteers. (●) (+)-Enantiomer of pirprofen; (□) (−)-enantiomer of pirprofen.

for the (+)-enantiomer of pirprofen and 100.4% (C.V. 8.7%) for the (−)-enantiomer of pirprofen. More detailed results are given in Table II.

Stability

In a previous paper [9], we discussed the stability of pirprofen and the formation of the pyrrole derivative during sample manipulation.

It was confirmed that pirprofen did not oxidize to its pyrrole derivative during the assay by determining the amount of pyrrole derivative formed during manipulation of plasma samples spiked with 79.2 μmol of the racemate of pirprofen per litre:

(i) five times out of eight the transformation of pirprofen to its pyrrole derivative was less than 1%; (ii) once, it was 3%; (iii) twice, it was 4%.

Chiral derivatization and chiral column

In the literature, the determination of the enantiomers of the anti-inflammatory non-steroidal drugs (AINSD) was reported either with a chiral derivatization or on a chiral column. The methodology and the analytical results were similar for both these methods and for the method described above combining chiral derivatization and the use of a chiral column. This method was necessary to separate the enantiomers of pirprofen from the enantiomers of its pyrrole metabolite. The three methods with chiral derivatization [2], with a chiral column [10] or with both chiral derivatization and a chiral column allowed the determination of the enantiomers of AINSD after administration of the racemate.

Application

This method was applied to the plasma samples of eleven subjects who received a single oral dose of 400 mg of pirprofen as racemate. Mean plasma concentrations for the (+)- and (−)-enantiomer of pirprofen are shown in Fig. 3.

The mean concentrations of the (+)-enantiomer of pirprofen were higher than those of the (−)-enantiomer. Ku and Wasvary [11] have shown that the (+)-enantiomer of pirprofen showed a six-fold higher ability to inhibit the conversion

of arachidonic acid into prostaglandin E_2 by sheep seminal vesicle prostaglandin synthase in vitro. This is in good agreement with the results reported for other arylpropionic acids. Higher concentrations of the (+)-enantiomer of arylpropionic acid derivatives have been reported for naproxen in rabbits by Goto et al. [2] and for carprofen and ibuprofen in humans by Stoltenborg et al. [1] and Lee et al. [5], respectively. The specific inversion of the (−)- to the (+)-enantiomer has been reported for naproxen in rabbits by Goto et al. [2] and for ibuprofen in humans by Stoltenborg et al. [1].

This specific inversion results in an apparently slower elimination of the (+)-enantiomer following administration of the racemate [1].

For ibuprofen in vitro studies demonstrated that the (+)-enantiomer was responsible for the anti-inflammatory activity [5].

CONCLUSION

The HPLC assay described for simultaneous analysis of the (+)- and (−)-enantiomer of pirprofen in plasma using the butyric acid analogue of the pyrrole derivative as an internal standard permits the quantitation of plama levels of the enantiomers as they emerge after a single oral dose of 400 mg of the racemate of pirprofen.

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