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SIMULTANEOUS DETERMINATION OF PIRPROFEN AND ITS METABOLITE, THE PYRROLE DERIVATIVE, IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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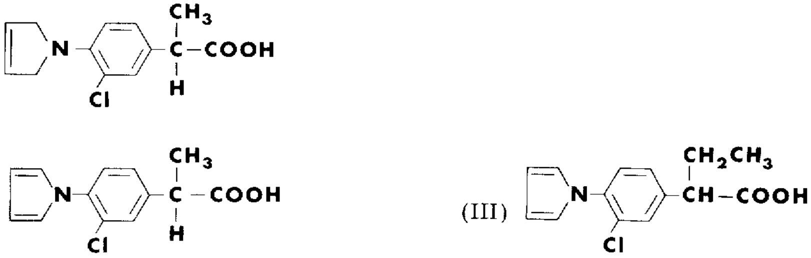
SUMMARY

A method for the simultaneous determination of pirprofen and its metabolite, the pyrrole derivative, in human plasma is described. The two compounds and the butyric acid analogue of the pyrrole derivative used as internal standard are extracted from plasma with chloroform, then back-extracted into an alkaline buffer. After addition of acid, the aqueous phase is assayed by high-performance liquid chromatography using a fixed-wavelength ultraviolet detector at 254 nm. The limit of quantitation is 0.1 μg/ml (0.396 μmol/l for pirprofen and 0.400 μmol/l for the pyrrole derivative).

INTRODUCTION

Pirprofen, 2-[3-chloro-4-(3-pyrrolin-1-yl)phenyl]propionic acid (Fig. 1) is an analgesic and anti-inflammatory agent. Most (80—90%) of the pirprofen (I) is present in plasma in the unchanged form; the remainder is in the form of the pyrrole metabolite (II) [1, 2].


(I)

(II)

(III)

Fig. 1. Chemical structures of pirprofen (I), the pyrrole metabolite (II) and the internal standard (III).

A gas chromatographic (GC) method [1] for the determination of the sum I and II has been described. Another GC method [3] allows the simultaneous determination of I and II; this method, using flash methylation, is performed without an internal standard.

This paper describes a procedure which permits the simultaneous determination of I down to 0.396 μmol/l and II down to 0.400 μmol/l in human plasma by high-performance liquid chromatography (HPLC) with a two-step extraction and with the butyric acid analogue of the pyrrole derivative as internal standard.

EXPERIMENTAL

Chemicals and reagents

Pirprofen (I) ($C_{13}H_{14}ClNO_2$; MW 252), the pyrrole metabolite (II) ($C_{13}H_{12}ClNO_2$; molecular weight, MW 250) and the butyric acid analogue of the pyrrole derivative (GP 48 921), the internal standard ($C_{14}H_{14}ClNO_2$; MW 264), were supplied by Ciba-Geigy (Basle, Switzerland). All solvents were of analytical grade: methanol (Prolabo 20 847), chloroform Uvasol (ref. 2447; E. Merck, Darmstadt F.R.G.). Distilled water was filtered on a Millipore filter 0.8 μm Type AA WP 04700. pH 13 Titrisol buffer was purchased from Merck (9893). Phosphate buffer pH 3.5 was prepared by dissolving 1.36 g of potassium dihydrogen phosphate (Merck 4873) in filtered water to make a volume of 2000 ml, and adding a few drops of phosphoric acid (Merck 573) up to pH 3.5. Acetate buffer pH 5.3 was prepared by diluting 5.72 ml of acetic acid (Merck 63) in distilled water up to a volume of 100 ml (solution A), then by dissolving 8.20 g anhydrous sodium acetate (Merck 6264) in distilled water to make a volume of 100 ml (solution B) and adding 20 ml of solution A to 80 ml of solution B.

Chromatography equipment and conditions

Chromatography is performed on a Hewlett-Packard 1081 B high-performance liquid chromatograph equipped with a fixed-wavelength (254 nm) UV detector. The chromatographic column (stainless steel tube, 25 cm × 4.7 mm I.D.) is filled with μBondapak C_{18} 10-μm by a slurry packing technique. The filling was performed with a pneumatic pump at 500 bars using methanol as pressurizing solvent. Chromatography is performed at room temperature using the degassed mobile phase methanol—pH 3.5 phosphate buffer (61:39) at a constant flow-rate of 1.5 ml/min. The top pressure is about 105 bars. The retention times of II, I and internal standard are 6.8, 7.7 and 9.2 min, respectively.

Extraction procedure

Internal standard (100 μl, 0.379 nmol) solution prepared in mobile phase is introduced into a glass tube. Then 1 ml of plasma, 2 ml of pH 5.3 buffer and 4 ml of chloroform are added. The tube is stoppered and mechanically shaken for 15 min at about 200 rpm with an Infors shaker, then centrifuged for 10 min at 2500 *g*. The aqueous phase is discarded and the chloroform solution transferred into another tube; 0.5 ml of pH 13 buffer is added. The tube is shaken for 20 min at about 200 rpm and centrifuged for 3 min at 2500 *g*. The aqueous phase is transferred into a tube containing three drops of 0.5 *M* hydrochloric acid to obtain a pH value of about 2—4; 50 μl of this solution are injected onto the column. The extract must be stored at —20° C up to the time of injection to avoid the degradation of I to II.

Calibration

The calibration samples are prepared by adding 100 μl of the internal standard solution and 20—80 μl of suitable mobile phase solutions containing I or II, to 1 ml of plasma. The concentration of these samples ranged from 0.1 to 4 μg/ml (from 0.396 to 15.8 μmol/l for I and from 0.400 to 16.0 μmol/l for II). The calibration curves were constructed from the ln—ln plot of the peak area ratios against the plasma concentrations. Their equations were calculated by the least-squares method. Calibration samples with six single concentrations for each compound are extracted three times a week (the calibration extracts are stored at —20° C). On every analysis day the calibration samples are injected to correct for the variation of the chromatographic system (UV lamp and analytical column); concentrations of I and II are calculated using the daily curve.

RESULTS

Plasma interference and selectivity

Fig. 2 shows the chromatograms corresponding to the extract of 1 ml of plasma and to the extract of the same plasma containing 0.396 μmol/l I and

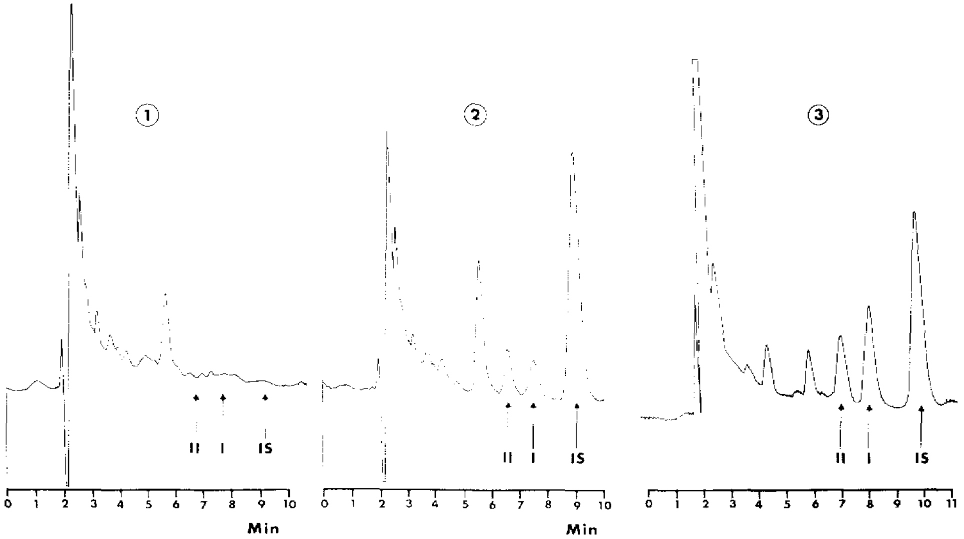


Fig. 2. Examples of chromatograms (column, μBondapak C_{18} 10-μm, 25 cm long; mobile phase, methanol—pH 3.5 phosphate buffer, 61:39). (1) Human plasma blank (1 ml of plasma). (2) Human plasma spiked with 0.396 μmol/l I, 0.40 μmol/l II, and IS (0.379 nmol). (3) Plasma (0.5 ml) of a subject 24 h after the start of perfusion of I into the intestine. Concentrations found: 3.27 μmol/l I and 0.76 μmol/l II. Peaks: I = pirprofen; II = pyrrole metabolite; IS = internal standard.

0.400 μmol/l II. There is no interference of the plasma components with I, II and internal standard peaks.

Six anti-inflammatory drugs (phenylbutazone, indomethacin, ketoprofen, piroxicam, diclofenac and oxyphenbutazone) were injected under the same chromatographic conditions. Only phenylbutazone has a retention time near that of I and can prevent its determination.

Reproducibility and accuracy

Various spiked plasma samples were prepared and analysed several times. Table I shows the within-day reproducibility of I over the concentration range 0.396—15.8 μmol/l. The mean overall recovery was 101.3% with a coefficient of variation (C.V.) (relative standard deviation S.D./mean · 100) of 5.1% (n = 23). Table II shows the within-day reproducibility of II over the concentration range 0.4—4 μmol/l. The mean overall recovery was 102.4% with a C.V. of 5.8% (n = 23). Samples spiked with I and II over the concentration range 0.4—8 μmol/l were also analysed. The mean overall recoveries were 97.8% (C.V. = 5.9%) for I (n = 22) and 100.9% (C.V. = 8%) for II (n = 22). A good reproducibility was obtained for the concentration 0.4 μmol/l; C.V. = 5.2% for I and 8.4% for II in samples spiked with I plus II. Tables III and IV show the reproducibility of standard curves. A least-squares ln—ln regression line was generated from the data points of six standard curves and each concentration added to plasma was calculated from this regression line.

TABLE I

PRECISION AND RECOVERY OF THE DETERMINATION OF PIRPROFEN IN SPIKED HUMAN PLASMA

Amount added (μmol/l)	n	Amount found (mean ± S.D.) (μmol/l)	Mean recovery (%)	C.V. (%)
0.396	5	0.399 ± 0.034	100.8	8.5
3.96	6	4.08 ± 0.13	103.1	3.4
7.93	6	8.06 ± 0.39	101.6	4.9
15.8	6	15.7 ± 0.5	99.3	3.3

TABLE II

PRECISION AND RECOVERY OF THE DETERMINATION OF THE PYRROLE METABOLITE IN SPIKED HUMAN PLASMA

Amount added (μmol/l)	n	Amount found (mean ± S.D.) (μmol/l)	Mean recovery (%)	C.V. (%)
0.4*	6	0.42 ± 0.04	104.4	8.4
0.8	6	0.84 ± 0.04	105.1	5.6
1.6	5	1.58 ± 0.05	99.2	3.6
4	6	4.01 ± 0.07	100.3	1.7

*These samples are spiked with 0.4 μmol/l II and 0.396 μmol/l I.

TABLE III

DAY-TO-DAY REPRODUCIBILITY OF SIX CALIBRATION CURVES USED TO DETERMINE PIRPROFEN IN PLASMA

A ln—ln regression line was generated from the 34 experimental points. Each experimental point was read off this regression line and the recovery was calculated.

Amount added (μmol/l)	Recovery (%)						Mean recovery (%, ± S.D.)
0.396	97.4	96.7	92.9	97.4	106.8	106.8	99.6 ± 5.7
0.792	95.8	100.5	93.4	99.5	105.1		98.8 ± 4.0
1.58	100	102.5	102.5	97.4	103.8	102.5	101.4 ± 2.3
3.96	98.5	95.7	98.5	101.5	105.3	101.5	100.1 ± 3.3
7.92	99.2	93.3	101.6	99.7	101.6		99.1 ± 3.4
15.8	103.5	99.3	95.8	96.8	99.3	103.8	99.7 ± 3.3

TABLE IV

DAY-TO-DAY REPRODUCIBILITY OF SIX CALIBRATION CURVES USED TO DETERMINE THE PYRROLE METABOLITE IN PLASMA

A ln—ln regression line was generated from the 31 experimental points. Each experimental point was read off this regression line and the recovery was calculated.

Amount added (μmol/l)	Recovery (%)						Mean recovery (%, ± S.D.)
0.4	100	92.5	84.2	107.5	92.5	115	98.6 ± 11.2
0.8	111.2	102.5	91.2	111.2	111.2		105.4 ± 8.8
1.6	96.8	92.5	92.5	103.1	91.8		95.3 ± 4.7
4	105	97.5	97.5	94.5	108.7	95	99.7 ± 5.7
8	97.5	91.2	102.5	98.7	112.5		100.5 ± 7.8
16	104.3	100	94	99.8			99.4 ± 5.1

Stability

The stability of I has been studied [1]. Pirprofen is oxidized to the pyrrole derivative during evaporation. In the present procedure, no evaporation is performed. It is important to avoid the degradation of I to II by storing the extracts at −20°C until injection.

If directly introduced into gas chromatograph—mass spectrometer, the pyrrole derivative content of pirprofen was about 3% from powder or chloroform solution. About 25% of I from a chloroform solution is oxidized to II upon storage at room temperature for 24 h. Antioxidants have been tried to prevent oxidation of I but neither ascorbic acid nor sodium metabisulphite afforded protection. The best protection seemed to be storage at −20°C: 4.2% (S.D. = 0.9, n = 10) of II was found when extracts of I were kept 3—4 h at 4°C until injection; 9.4% (S.D. = 3.4, n = 12) of II was found when extracts of I were kept 24 h at 4°C; 2.7% (S.D. = 0.2, n = 6) and 2.9% (S.D. = 0.3, n = 6) of II was found when extracts of I were kept at −20°C for 3—4 h and 24 h, respectively. The oxidation of I to II was not important in the plasma; the

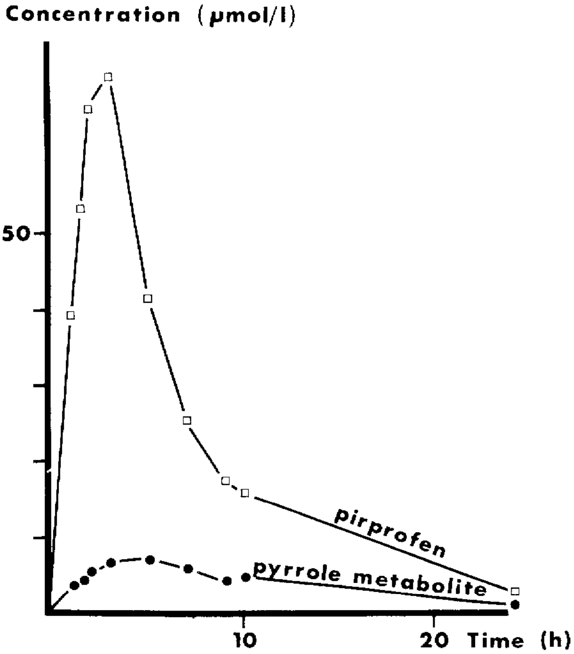


Fig. 3. Mean concentrations (n = 2) of I and II in plasma after perfusion of 187.5 mg of I into the intestine.

mean value of II was 3.5% (S.D. = 0.96, n = 11) when plasma spiked with I was kept for 24 h at 37°C.

Application

The method was applied to the determination of plasma I and II concentrations reached in two subjects after a perfusion of 187.5 mg of I into the intestine (Fig. 3). The mean maximum concentration of the pyrrole metabolite was ca. 15% of that of pirprofen.

CONCLUSION

The described procedure permits the simultaneous determination of I down to 0.396 μmol/l and II down to 0.400 μmol/l with convenient specificity, precision and accuracy.

REFERENCES

1 P.H. Degen, A. Schweizer and A. Sioufi, J. Chromatogr., 290 (1984) 33.
2 H.P. Egger, F. Bartlett, H.P. Yuan and J. Karliner, Drug Metab. Dispos., 10 (1982) 529.
3 R.C. Luders, M.B. Maggio-Cavaliere, H.P. Egger, O.B. Gum, O. Resnik, F. Bartlett, M.J. Gaito, A. Soo and C. Li, Clin. Pharmacol. Ther., 21 (1977) 721.