Journal of Chromatography, 424 (1988) 201–204
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3949

Note

Determination of furprofen in human plasma by solid-phase extraction and reversed-phase high-performance liquid chromatography

GIANCARLO PALUMBO and GIAN CARLO PANTALEONI

Dipartimento di Medicina Interna e Sanità Pubblica, Cattedra di Farmacologia, Università degli Studi dell'Aquila, Via S. Sisto, I-67100 L'Aquila (Italy)

and

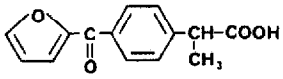
GIUSEPPE CARLUCCI*

Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università degli Studi dell'Aquila, Via Assergi, 4, I-67100 L'Aquila (Italy)

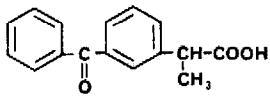
(First received June 5th, 1987; revised manuscript received September 2nd, 1987)

Furprofen, 2-[4-phenyl-(2′-furoyl)]propionic acid (Fig. 1), is an analgesic, antipyretic non-steroidal anti-inflammatory drug (NSAID). The arylpropionic acids are a more recently developed class of NSAIDs with an assumed mode of action inhibiting prostaglandin synthesis, as found with other NSAIDs [1–3]. Methods for the determination of many other NSAIDs have been described (e.g., refs. 4–6).

The interest in this group of compounds has prompted us to develop a selective



FURPROFEN



KETOPROFEN

Fig. 1. Structures of furprofen and the internal standard, ketoprofen.

0378-4347/88/$03.50

and sensitive high-performance liquid chromatographic (HPLC) method for the therapeutic monitoring of furprofen. The straightforward, high-recovery sample preparation procedure makes use of Bond-Elut disposable extraction columns to adsorb the drug from the biological matrix.

EXPERIMENTAL

Materials and reagents

Furprofen was supplied by Istituto Farmacoterapico Italiano (Pomezia, Italy) and ketoprofen by Sigma (St. Louis, MO, U.S.A.). HPLC-grade methanol was obtained from Farmitalia Carlo Erba (Milan, Italy). All other reagents were of analytical grade. Bond-Elut C_{18} columns (1 ml capacity) and the Vac-Elut manifold (Analytichem, Harbor City, CA, U.S.A.) were purchased from Analytical Service (Bologna, Italy). HPLC-grade water was obtained by double distillation in glass and purification through a Milli Q water purification system (Millipore, Bedford, MA, U.S.A.). Water was filtered through an HA 0.45-μm filter, and methanol was filtered through an FA 0.5-μm filter (Millipore). The mobile phase was prepared daily. The stock standard of furprofen (1.0 mg/ml) was prepared by dissolving an appropriate amount of furprofen in methanol. Eight reference samples containing 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 4.0 μg/ml furprofen were prepared by diluting this stock solution with pooled human sera free from NSAIDs. These reference samples were used to assess accuracy and intra-run and inter-run precision.

Chromatography

The HPLC system (Waters Assoc., Milford, MA, U.S.A.) consisted of an M 6000 A pump, a U6K injector, a Model 440 fixed-wavelength UV detector and a 740 Data Module integrator. For simultaneous determination in plasma of furprofen and ketoprofen, the internal standard, an Ultrasphere-Octyl column (25 cm $\times$ 4.6 mm I.D., 5 μm particle size, Beckman Instruments, Fullerton, CA, U.S.A.) connected to a Supelguard LC-8 5-μm (2 cm $\times$ 4.6 mm I.D.) precolumn (Supelco, Bellefonte, PA, U.S.A.) was used. The mobile phase was methanol–water (80:20, v/v) and the flow-rate was 1.2 ml/min. The separation was carried out at room temperature (20–22°C). The eluate was monitored at 280 nm at an attenuation of 0.001 a.u.f.s.

Extraction procedure

Furprofen was extracted using a Bond-Elut column containing ODS-modified silica (C_{18} column). The Bond-Elut columns were placed in a luer that fitted the top of the Vac-Elut cover, which may be loaded with up to ten columns. A vacuum of 250–500 Torr was applied to the manifold to carry out the various stages of the extraction.

A 100-μl volume of internal standard was placed in each column, then 1 ml of plasma was passed through the activated C_{18} Bond-Elut column. The vacuum was connected and each column was washed with two column volumes of distilled water followed by 100 μl of methanol.

The vacuum was disconnected, and a rack containing appropriate glass tubes was set in place to collect the eluate. Methanol (200 μl) was added to each column, then the vacuum was connected and the eluate collected. A 200-μl volume of methanol was added, and the eluates were collected again and combined with the previous eluates.

The methanol was evaporated to dryness in a water-bath at 45 °C under a gentle stream of nitrogen. The residue was taken up with 100 μl of methanol–water (80:20, v/v), and 25-μl aliquots were used for chemical analysis.

RESULTS AND DISCUSSION

Three chromatograms obtained from human plasma containing furprofen are given in Fig. 2. The peaks corresponding to internal standard and furprofen were well resolved. The retention times for furprofen and the internal standard were 3.02 and 3.95 min, respectively.

The analytical recovery of furprofen from plasma was determined by comparing the peak-area ratio (furprofen to internal standard) obtained by analysing

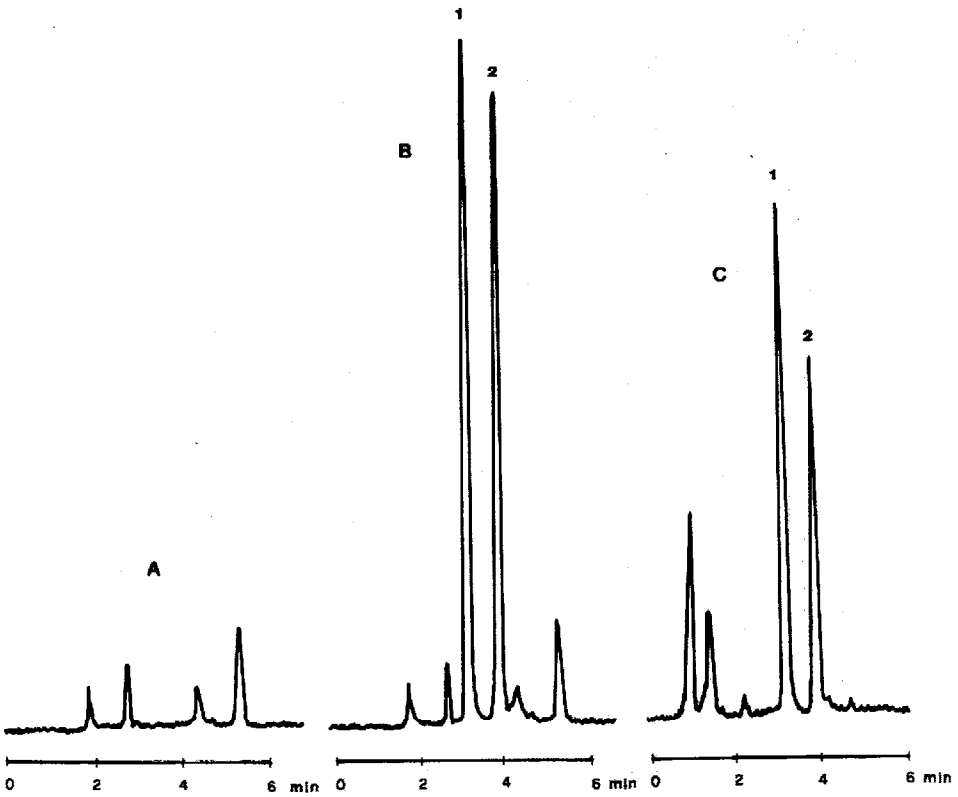


Fig. 2. Chromatograms from human plasma extracts. (A) Blank plasma; (B) blank plasma spiked with 1 μg/ml furprofen (1) and 0.5 μg/ml internal standard (2); (C) plasma sample collected 2 h after a dose of 200 mg of furprofen. Vertical axis: UV detector response (280 nm); horizontal axis: retention time (min).

TABLE 1

FURPROFEN HPLC ASSAY PRECISION

C.V. = coefficient of variation.

Concentration added (μg/ml)	Intra-run		Inter-run	
	Mean concentration found (μg/ml)	C.V. (%)	Mean concentration found (μg/ml)	C.V. (%)
0.10	0.11	6.5	0.12	20.4
0.25	0.25	5.0	0.24	10.7
0.75	0.76	4.8	0.78	7.4
1.00	1.02	4.4	1.00	3.9
1.50	1.54	3.5	1.48	4.7
2.00	2.06	2.4	1.97	3.3
4.00	4.08	2.1	4.10	3.3

extracted, spiked plasma specimens to the peak-area ratio obtained by direct injection of methanolic solutions of furprofen and internal standard, containing amounts of furprofen equal to those in the spiked plasma specimens. The mean recovery of furprofen from plasma samples was 97%.

A standard curve was constructed by calculating the peak-area ratios (furprofen to internal standard) for a series of calibration standards. The concentration of furprofen ranged from 0.1 to 4.0 μg/ml. Concentration and peak-area ratios were linear over this range. The correlation coefficient was 0.997 on ten data points, the slope 0.854 and the intercept 5.7.

The limit of detection for furprofen under the conditions described above is at least 0.1 μg/ml.

To define intra-run and inter-run precision for serum specimens with the HPLC method, we analysed each of the eight reference samples ten times in a single run and ten times in separate runs (Table I). The coefficients of variation (C.V.) ranged from 2.1 to 6.5% for intra-run precision and from 3.3 to 20.4% for inter-run precision.

The solid-phase extraction procedure described here is simple, does not require large amounts of organic solvents and is very rapid (ten samples can be processed in ca. 12 min). The recovered residue was found to be essentially free of sample matrix and showed very low background. The method is designed for pharmacokinetic studies in adults, children and laboratory animals. Such studies are currently in progress in our laboratories.

REFERENCES

1 R. Walt, B. Katschinski, R. Logan, J. Ashley and R. Langman, Lancet, i (1986) 489.
2 B.M. Goudie, G.F. Birnie and G. Watkinson, Lancet, i (1982) 959.
3 R.N. Brogden, R.C. Heel, T.M. Speight and G.S. Avery, Drugs, 18 (1979) 417.
4 M.K. Aravind, J.N. Miceli and R.E. Kauffman, J. Chromatogr., 308 (1984) 350.
5 V.A. Johnson and J.T. Wilson, J. Chromatogr., 382 (1986) 367.
6 J.H.G. Jonkman, R. Schoenmaker, A.H. Holtkamp and J. Hempenius, J. Pharm. Biomed. Anal., 3 (1985) 433.