Journal of Chromatography, 162 (1979) 122—124
Biomedical Applications

CHROMBIO. 222

Note

Determination of ketoprofen in plasma and urine by high-performance liquid chromatography

T.M. JEFFERIES*, W.O.A. THOMAS and R.T. PARFITT

School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY (Great Britain)

(Received April 17th, 1978)

The determination of the non-steroidal anti-inflammatory agent ketoprofen, 2-(3-benzoylphenyl)propionic acid, in plasma and urine is necessary in order to study its absorption and excretion in patients with rheumatoid arthritis. Previously described methods for the analysis of ketoprofen are time-consuming and lack sensitivity. They include gas—liquid chromatography (GLC) [1], thin-layer chromatography (TLC) plus GLC [2] and TLC plus UV spectrophotometry [3, 4]. We have found that high-performance liquid chromatography (HPLC) requires minimal sample preparation time and can determine a minimum quantity of 2.5 ng ketoprofen in an analytical time of 4 min.

EXPERIMENTAL

Extraction

Urine and plasma samples are acidified to pH 1 with hydrochloric acid (conc.) and stored at −11°. For total ketoprofen in urine, the ketoprofen glucuronide is hydrolysed at pH 1 at 98° for 30 min. It was found that hydrolysis is complete and the ketoprofen is stable under these conditions. The internal standard, oxyphenbutazone 500 μg, is added to 4 ml of the hydrolysed urine, and diluted to 10 ml with distilled water. For the free ketoprofen content of urine, the hydrolysis stage is omitted.

For total ketoprofen in plasma, a 2-ml sample plus 250 μg of oxyphenbutazone is made up to 5 ml with distilled water and extracted with 5 ml diethyl ether by vortex mixing for 1 min, followed by centrifugation.

* To whom correspondence should be addressed.

Chromatographic analysis

A constant pressure pump (Haskel DST-122) was used to deliver eluent to a column (50 × 4.6 mm I.D.) slurry-packed with Spherisorb-5 ODS (Particle size 5 μm; Phase Separations, Queensferry, Great Britain). An injector valve (Specac, Orpington, Great Britain) fitted with a 5-μl internal sample loop was used. Detection was at 260 nm using a variable wavelength UV monitor (CE-212, Cecil Instruments, Cambridge, Great Britain) at a range of 0.05 to 0.02 a.u. f.s. The eluent was 35% methanol in distilled water at pH 3.5 with acetic acid at a flow-rate of 2 ml/min. Ketoprofen and oxyphenbutazone gave capacity factors of 6 and 8, respectively. Calibration graphs of peak height ratio (ketoprofen to oxyphenbutazone) to ketoprofen concentration in the range 1.5 to 25 μg/ml were constructed, from 3 to 5 replicate injections of all solutions.

RESULTS AND DISCUSSION

The determination of ketoprofen has been studied when dissolved in the HPLC mobile phase, in diluted urine, in ether extract of diluted urine, in diluted plasma and in ether extract of diluted plasma (Fig. 1). All the calibrations were linear and statistically identical with correlation coefficients (r) between 0.995 and 0.999, so that experimental factors influence the analytical method chosen.

For urine samples, direct analysis of diluted urine is preferred since ether extraction is not necessary. All the components of urine are eluted before ketoprofen so that no residues accumulate on the column. The mean extraction recovery is 101.1% (n = 18) with a limit of error (at 95% degree of confidence) of ± 1.5%. It is a very rapid and simple technique capable of estimating both free drug and glucuronide levels. A rheumatoid patient on 300 mg ketoprofen daily excreted 29.1% free and 30.3% as the glucuronide over 24 h in the urine.

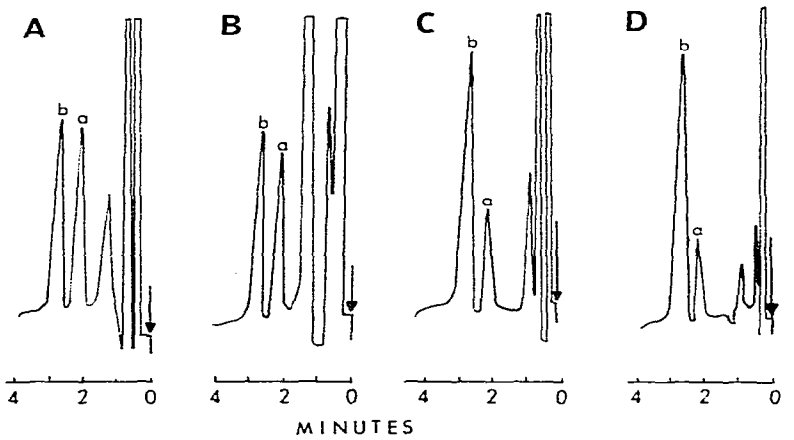

Fig. 1. HPLC of internal standard, oxyphenbutazone (a) and ketoprofen (b) contained in urine and plasma collected from rheumatoid patients. Eluent: 35% methanol, pH 3.5, flow-rate 2 ml/min. Column: 50 × 4.6 mm, Spherisorb-5 ODS. UV detection at 260 nm. A = diluted urine; B = ether extract of diluted urine; C = diluted plasma; D = ether extract of diluted plasma.

For plasma samples an ether extract is recommended in order to prevent the precipitation and accumulation of plasma proteins at the top of the column. Ether extraction increases the sample preparation time slightly, but results in a more accurate procedure, with a mean extraction recovery (n = 18) of 100.6 ± 2.1%. For both urine and plasma samples, the proposed method is accurate, rapid, produces excellent baseline stability and the columns may be used indefinitely.

ACKNOWLEDGEMENTS

We thank the Lagos State Government of Nigeria for the award of a research studentship to Mr. Thomas, Dr. J. Davies and Dr. N.D. Hall of the Royal National Hospital for Rheumatic Diseases for clinical samples of urine and plasma and May and Baker Ltd. for a sample of ketoprofen.

REFERENCES

1 P. Populaire, B. Terlain, S. Pascal, B. Decourelaere, G. Lebreton, A. Renard and J.P. Thomas, Ann. Pharm. Fr., 31 (1973) 679.
2 A.C. Kennedy, Curr. Med. Res. Op., 3 (1975) 423.
3 B. Lotti, Boll. Chim. Farm., 114 (1975) 351.
4 R. Ballerini, A. Cambi and P. Del Soldato, J. Pharm. Sci., 66 (1977) 281.