Journal of Chromatography, 377 (1986) 410—414
Biomedical Applications
Elsevier Science Publishers B.V , Amsterdam — Printed in The Netherlands

CHROMBIO. 3022

Note

Rapid high-performance liquid chromatographic determination of ibuprofen in human plasma

M. LALANDE*, D.L. WILSON and I.J. McGILVERAY

Bureau of Drug Research, Health Protection Branch, Ottawa K1A 0L2 (Canada)

(First received August 16th, 1985; revised manuscript received November 25th, 1985)

Ibuprofen, D,L-[2-(4-isobutylphenyl)propanoic acid], is a non-steroidal, anti-inflammatory agent used for the treatment of arthritis. It also possesses antipyretic and analgesic properties. Various gas chromatographic procedures for the analysis of ibuprofen in human plasma have been described [1—7]. Since these methods require extraction and, except for one [7], derivatisation, they are subject to greater error and are tedious for multiple clinical assays. High-performance liquid chromatography (HPLC) has also been applied, but again, most of the methods require extraction [8—19]. However, two groups described simple protein precipitation [20,21] prior to chromatography, although one [20] used rabbit plasma and the other [21] reported a retention time of 14 min for ibuprofen. Moreover, neither of these methods specify the accuracy and variability of their systems for ibuprofen.

It was therefore considered appropriate to develop a sensitive, selective and rapid assay of ibuprofen in human plasma that could be used in multi-subject pharmacokinetic studies with automated instrumentation.

EXPERIMENTAL

Reagents and standard solutions

Ibuprofen, kindly supplied by Upjohn (Kalamazoo, MI, U.S.A.) was prepared as a 1 mg/ml solution in methanol. The internal standard, phenylbutazone, supplied by Ciba-Geigy (Mississauga, Canada) was also prepared as a 1 mg/ml methanolic solution. Acetonitrile and methanol, both of HPLC grade, were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.). Phosphoric acid (85%), AnalaR grade, was obtained from BDH (Poole, U.K.) and was diluted in doubly glass-distilled, deionised water to obtain a 0.05% solution. This solution and the methanol were filtered (0.2 μm) before use.

Instrumentation

An HPLC pump, Series IV, from Perkin-Elmer (Norwalk, CT, U.S.A.) was combined with a Perkin-Elmer UV detector LC 85B with the wavelength set at 220 nm. Injection of the samples was by means of a Perkin-Elmer ISS-100 autosampler. Output, recorded as peak-area integrations, was obtained with a Model 3600 data station (Perkin-Elmer) incorporating a disk storage system. The mobile phase, methanol–0.05% phosphoric acid (70:30, v/v) was run at room temperature with a flow-rate of 2 ml/min through an ODS reversed-phase, 5 μm particle size, 150 × 4 mm I.D., Ultrasphere column (Altex, Berkeley, CA, U.S.A.). The chromatograms were obtained from the Perkin-Elmer 660 printer.

Sample preparation

Blood was collected by venipuncture or by a butterfly inserted in a suitable forearm vein in a 10-ml Vacutainer® containing either EDTA or heparin as anticoagulant. After centrifugation (1000 *g*), an aliquot (500 μl) of the plasma was acidified with one drop of phosphoric acid (85%). After brief mixing, 1 ml of acetonitrile (containing the internal standard at a concentration of 10 μg/ml) was added to denature the proteins. The tubes were then vortexed for 15 s before centrifugation at 5000 *g* for 15 min at 4°C. The supernatant was diluted with water to 50% (v/v) and approx. 300 μl were transferred to the vials of the autosampler from which 100 μl were injected into the liquid chromatograph.

Calibration procedures

Blank human plasma was spiked with ibuprofen over the range 1.0–40 μg/ml. Plasma prepared in this way was subjected to the above procedure. From the chromatograms obtained, the ibuprofen/phenylbutazone peak-area ratio was calculated and a calibration curve was constructed by plotting the concentration of ibuprofen against the respective peak-area ratios. Unknown samples were quantified by reference to this standard curve.

Recovery experiments

Samples containing ibuprofen at 3, 15 and 30 μg/ml, as well as phenylbutazone at 10 μg/ml, were prepared in empty culture tubes and treated as described above. A standard for 100% recovery was prepared by diluting a known amount of the two compounds in mobile phase. The percentage recovery was determined by comparing the areas of the peaks obtained from experimental specimens with the areas obtained from the recovery standards.

RESULTS AND DISCUSSIONS

Fig. 1 shows typical chromatograms of (A) a drug-free plasma containing no internal standard, (B) a drug-free plasma spiked with 10 μg/ml ibuprofen and internal standard and (C) a patient sample which was taken 3 h after oral ingestion of 300 mg of ibuprofen. The calculated concentration in this sample is 15.5 μg/ml. The retention times of phenylbutazone and ibuprofen are 2.2 and 3.8 min, respectively.

Table I shows that the intra-day variation of the assay is good. Six indepen-

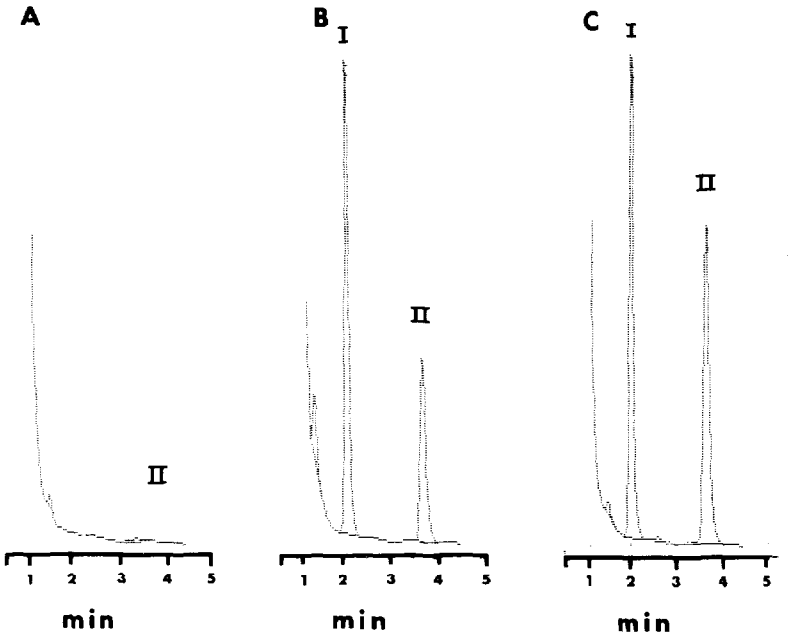

Fig. 1. (A) Drug-free plasma containing no internal standard; (B) drug-free plasma spiked with 10 μg/ml ibuprofen and internal standard; (C) a patient plasma sample which was taken 3 h after an oral dose of 300 mg ibuprofen. The concentration of ibuprofen was established at 15.5 μg/ml. Peaks: I = internal standard; II = ibuprofen.

TABLE I

INTRA-DAY VARIABILITY FOR IBUPROFEN OBTAINED FROM INDEPENDENTLY PREPARED SAMPLES ANALYSED ON THE SAME DAY

$n=6$.

Nominal concentration (μg/ml)	Mean determined concentration (μg/ml)	Within-day precision (%)
0.5	0.43	3.5
10.0	10.4	5.3
20.0	20.9	0.8

TABLE II

INTER-DAY VARIABILITY FOR IBUPROFEN AS DETERMINED BY THE ANALYSIS OF QUALITY CONTROLS

$n=10$.

Nominal concentration (μg/ml)	Mean determined concentration (μg/ml)	Coefficient of variation (%)
6.0	6.0	8.6
19.0	19.2	3.5
30.0	30.0	7.0

dently prepared samples were run at each of three different concentrations of ibuprofen. The highest coefficient of variation was less than 6%. The inter-day variation was checked by the analysis of spiked plasma samples derived from the same pools and run on ten consecutive days. The results are shown in Table II. As expected over such a long period of time, slightly larger coefficients of variation are observed but they remain acceptable.

The linearity of this assay was established by running a series of standard curves over the concentration range 1—40 μg/ml. The mean values of intercept and slopes were −0.019 and 19.02, respectively. The coefficient of variation of the slope is 9.3% while the coefficient of determination (r^2) was higher than 0.998. The mean recovery of ibuprofen from plasma samples was 110±5% at the three concentrations studied and the limit of reliable quantitation was set at 1.0 μg/ml, which encompasses the range of clinical concentrations reported to be 1.0—40 μg/ml [9]. Salicylic acid, aspirin, naproxen, ketoprofen and sulindac do not interfere with the assay but indomethacin does. Flurbiprofen could be used as internal standard since its retention time is 2.7 min but this compound is less readily available than phenylbutazone. The methanolic solution of the latter was checked for stability over one month according to the method of Fabré et al. [22] and no significant difference was found.

The application of this procedure is demonstrated (Fig. 2) with the plasma ibuprofen concentration–time profiles obtained from two male volunteers each receiving one 300-mg ibuprofen tablet. In addition, no difference in ibuprofen concentration was found between samples collected at the same time with heparin or EDTA as anticoagulant in the vacutainers. There was also no difference when simultaneous heparin lock collection was compared with vacutainers. Finally, we have observed that this assay was valid from column to column even if they came from different companies (i.e. Beckman or Supelco).

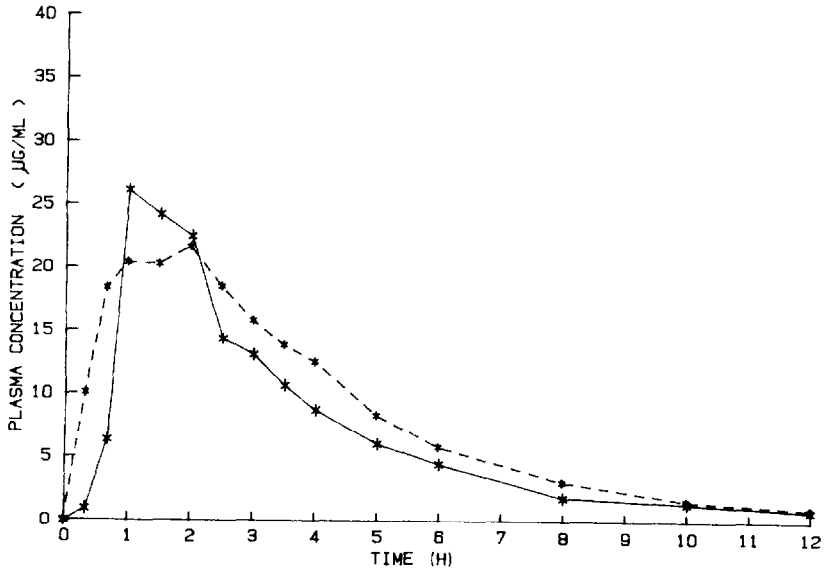

Fig. 2. Plasma ibuprofen concentration—time curves following oral ingestion of one 300-mg ibuprofen tablet in two subjects.

In conclusion, this HPLC assay is rapid, selective and of adequate sensitivity to be readily applied for routine therapeutic monitoring in those laboratories equipped with conventional HPLC systems.

ACKNOWLEDGEMENT

The authors are grateful to Mrs. Nicole Beaudoin for her skilful technical assistance.

REFERENCES

1 J.B. Whitlam and J.H. Vine, J. Chromatogr., 181 (1980) 463.
2 D.G. Kaiser and R.S. Martin, J. Pharm. Sci., 12 (1977) 749.
3 K.K. Midha, J.K. Cooper, J.W. Hubbard and I.J. McGilveray, Can. J. Pharm. Sci., 12 (1977) 29.
4 D.J. Hoffman, J. Pharm. Sci., 66 (1977) 749.
5 G.J. Vangaissen and D.G. Kaiser, J. Pharm. Sci., 64 (1975) 798.
6 D.G. Kaiser and G.J. Vangaissen, J. Pharm. Sci., 63 (1974) 219.
7 L. Heikkinen, J. Chromatogr., 307 (1984) 206.
8 M.K. Aravind, J.N. Miceli and R.E. Kauffman, J. Chromatogr., 308 (1984) 350.
9 E.J.D. Lee, K.M. Williams, G.G. Graham, R.O. Day and G.D. Champion, J. Pharm. Sci., 73 (1984) 1542.
10 K.S. Albert, A. Raabe, M. Garry, E.J. Antal and W.R. Gillespie, J. Pharm. Sci., 73 (1984) 1487.
11 J.H.G. Jonkman, R. Schoemaker, A.H. Holtkamp and J. Hempenius, Pharm. Weekbl., 6 (1984) 254.
12 H. Litowitz, L. Olanoff and C.L. Hoppel, J. Chromatogr., 311 (1984) 443.
13 D.J. Greenblatt, R.M. Arendt and A. Locniskar, Arzneim.-Forsch., 33 (1983) 1671.
14 G.J. Lockwood and J.G. Wagner, J. Chromatogr., 232 (1982) 335.
15 B.G. Snider, L.J. Beaubien, D.J. Sears and P.D. Rahn, J. Pharm. Sci., 70 (1981) 1347.
16 G.L. Kearns and J.T. Wilson, J. Chromatogr., 226 (1981) 183.
17 J.L. Shimek, N.G.S. Rao and S.K. Wahba Khalil, J. Pharm. Sci., 70 (1981) 514.
18 R.A. Upton, J.N. Buskin, T.W. Guentert, R.L. Williams and S. Riegelman, J. Chromatogr., 190 (1980) 119.
19 D. Pitrè and M. Grandi, J. Chromatogr., 170 (1979) 278.
20 A. Ali, S. Kazmi and F.M. Plakagiannis, J. Pharm. Sci., 70 (1981) 944.
21 F. Nielsen-Kudsk, Acta Pharmacol. Toxicol., 47 (1980) 267.
22 H. Fabré, N. Hussam-Eddine and B. Mandrou, J. Pharm. Sci., 73 (1984) 1706.