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Note

Determination of ibuprofen in human plasma by high-performance liquid chromatography

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Ibuprofen, D,L-2-(4-isobutylphenyl)propanoic acid, is an anti-inflammatory, antipyretic, analgesic drug widely used in the treatment of arthritis. Numerous gas—liquid chromatographic (GLC) [1—4] and gas chromatographic—mass spectrometric [5—7] assays with varying sensitivities have been reported. The most sensitive of the GLC assay techniques is the procedure reported by Kaiser and Martin [4]. Their determination is sensitive to 0.1 μg/ml ibuprofen in serum; however, their procedure is both complicated and time-consuming. Other GLC assays [1—3], which employ flame-ionization detection, offer more rapid sample preparation but have limited sensitivity and require large serum volumes. High-performance liquid chromatographic (HPLC) techniques [8—13] have been reported for the determination of ibuprofen. These methods have employed chloroform [8], hexane [9] or dichloromethane [10, 13] extractions. Detection limits have been reported as 1 μg/ml [9], 0.5 μg/ml [8, 10, 12] and 0.2 μg/ml [11, 13]. There is no reported evaluation of the extraction conditions for ibuprofen.

We have examined the extraction of ibuprofen from heparinized plasma including the solvent systems used by others [8—10, 13] and have devised a system to achieve quantitative recovery. A rapid, selective and sensitive HPLC method had been developed using ibufenac (4-isobutylphenylacetic acid) as the internal standard. The method has been used for determination of ibuprofen in plasma over a concentration range of 0.2—60 μg/ml. The detection

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limit was found to be 0.04 μg/ml corresponding with 4 ng ibuprofen injected into the column.

MATERIALS AND METHODS

Reagents and standard solutions

Ibuprofen, D,L-2-(4-isobutylphenyl)propanoic acid, generously supplied by Dr. Paul O'Connell of Upjohn (Kalamazoo, MI, U.S.A.), was prepared in a standard solution of 200 mg/l in methanol. The internal standard ibufenac, 4-isobutylphenylacetic acid (supplied by Dr. O'Connell), was also prepared in a standard solution of 200 mg/l in methanol. Isooctane, 2-propanol, toluene, chloroform and petroleum ether were purchased as analytical-grade reagents from Fisher Scientific (Cleveland, OH, U.S.A.). Acetonitrile (UV grade) was purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Methanol and acetonitrile were filtered before use; all other reagents were used without purification.

Chromatographic conditions

The liquid chromatograph comprised a Model M6000A pump, a U6K syringe loading injection valve, and an RCM-100 radial compression unit purchased from Waters Assoc. (Milford, MA, U.S.A.). Detection was accomplished by a Model LC-75 variable-wavelength detector purchased from Perkin-Elmer (Norwalk, CT, U.S.A.) and operated at a detection wavelength of 220 nm. A Linear Instruments (Irvine, CA, U.S.A.) Model 585 chart recorder was used for recording the detector output signal. Peak height and area measurements and subsequent sample concentration computations were performed by a Model 3354 laboratory automation system from Hewlett-Packard (Avondale, PA, U.S.A.). A 10 × 0.5 cm Radial-Pak C_{18} (10 μm nominal particle diameter) reversed-phase cartridge (Waters Assoc.) was used for the chromatographic separation. The chromatographic mobile phase was prepared by combining 500 ml of water, 500 ml of acetonitrile, and 1 ml of concentrated phosphoric acid (85%). The eluent was pumped at a flow-rate of 3.0 ml/min.

Sample preparation

Heparinized plasma specimen (1 ml) was combined with 0.25 ml of 1 *M* hydrochloric acid in a capped 15-ml glass test tube. To this were added 50 μl of the ibufenac internal standard solution and 5 ml of an isooctane—2-propanol mixture (85:15, v/v). The capped tubes were mixed at moderate speed for 5 min on a Rotamixer. The tubes then were centrifuged for 5 min at 1240 *g* and the organic layer transferred to a 10 × 75 mm glass tube by pipette. These tubes were placed in a 45°C water bath and the solvent evaporated under a gentle stream of air. The sample residues were reconstituted in 100 μl of methanol, the tubes vortexed, and 10 μl of the sample were injected into the liquid chromatograph.

Recovery experiments

Samples containing ibuprofen at 20 μg/ml were prepared by adding 100 μl of the 200 mg/l ibuprofen standard methanol solution to empty 15-ml glass

tubes. The methanol was evaporated at 45°C under a gentle air stream and 1 ml of heparinized drug-free plasma and 0.25 ml of 1 *M* hydrochloric acid were added to each tube. The capped tubes were placed on a Rotamixer for 5 min to allow dissolution of the drug in the acidified plasma. Extraction of each experimental sample group was performed with 5 ml of one of the following solvents: chloroform, toluene, hexane, dichloromethane, petroleum ether and isooctane—2-propanol (85:15, v/v). All tubes were mixed at moderate speed on the Rotamixer for 5 min and then centrifuged at 1240 *g* for 5 min. The organic layers were transferred by pipette to 10 × 75 mm glass tubes and the solvents evaporated at 45°C under a gentle air stream. A standard for 100% recovery was prepared by adding 100 μl (equal to 20 μg of the ibuprofen) standard solution in methanol and drying off the methanol in an identical manner to that of the extraction solvents. All extraction sample residues and the ibuprofen standard solution residues were reconstituted in 100 μl of a 200 mg/l solution of ibufenac in methanol. In this recovery analysis, ibufenac served as an external standard. Ibuprofen:ibufenac peak height and area ratios obtained with experimental specimens were compared with the ratios obtained with the quantitative recovery standard.

RESULTS AND DISCUSSION

The results of the extraction efficiency and recovery experiments are shown in Table I. The isooctane—isopropanol (85:15, v/v) extraction solvent gave complete recovery of the drug from the heparinized plasma samples. Recovery of ibuprofen from plasma with the other organic extraction solvents generally was less than 70%. Chloroform (the solvent employed by Pitre and Grandi [8]) gave recoveries of 65.0 ± 3.0%. Although we used hydrochloric acid rather than phosphoric acid to acidify the plasma, hexane (used by Shimek et al. [9]) gave less than 50% recovery. Dichloromethane (used by Kearns and Wilson [10] and Lockwood and Wagner [13]) gave recoveries of 68.6 ± 7.9%.

TABLE I

EXTRACTION OF IBUPROFEN FROM PLASMA

The fraction of ibuprofen extracted from plasma into the named solvents was evaluated as described in the text using an external standard method. The data are expressed as mean ± S.D. for six individual samples.

Solvent	Percentage ibuprofen recovered
Hexane	48.2 ± 1.4
Petroleum ether	55.3 ± 1.2
Chloroform	65.0 ± 3.0
Dichloromethane	68.6 ± 7.9
Toluene	69.5 ± 2.7
Isooctane—2-propanol	102.6 ± 4.7

A chromatogram of a processed drug-free plasma specimen without added internal standard is shown in Fig. 1. The chromatogram of a processed plasma sample containing 1 μg/ml ibuprofen and added internal standard is shown in

Fig. 2. The detection limit for ibuprofen in plasma (at a signal-to-noise ratio greater than 5:1) was found to be 0.04 μg/ml, corresponding with 4 ng ibuprofen injected into the chromatograph. There was complete separation of the chromatographic peaks of the internal standard and ibuprofen without interference from endogenous compounds in plasma samples from normal volunteers and uremic patients. The separation time is 3.3 min but a chromatographic peak of an endogenous sample constituent occurs at 4.3 min. Subsequent injections can be made at intervals of 6 min. Digoxin, clonidine, quinidine, procainamide, theophylline and propranolol were shown not to interfere with the assay. There is good stability of the instrument signal baseline at maximum detector sensitivity. Accuracy and precision of ten replicate

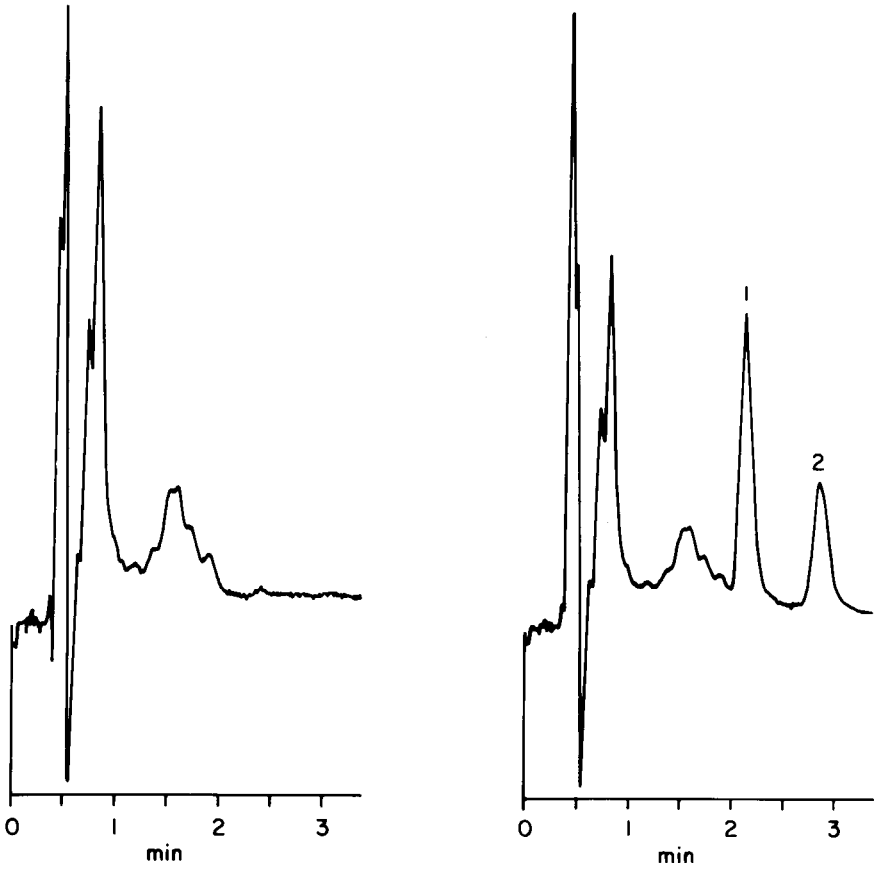

Fig. 1. Chromatogram obtained upon extraction of a drug-free plasma specimen without added internal standard according to the outlined experimental procedure. The column was a 10 × 0.5 cm I.D. radially compressed cartridge of Radial-Pak C_{18} (10 μm nominal particle diameter). The chromatographic eluent was aqueous 20 m*M* phosphoric acid—acetonitrile (50:50) and was pumped at a flow-rate of 3.0 ml/min. The absorbance detector was operated at 220 nm. The full scale of the ordinate is 0.04 absorbance units.

Fig. 2. Chromatogram obtained upon preparation of a plasma containing 1 μg/ml ibuprofen and internal standard according to the described method. The chromatographic conditions were as described in Fig. 1. The internal standard ibufenac (1) was eluted at 2.3 min; ibuprofen (2) was eluted at 3.0 min.

injections of a single prepared serum specimen containing 2 μg/ml ibuprofen was 2.04 ± 0.05 μg/ml.

A series of standard curves of ibuprofen:internal standard peak height ratios were established over sample ibuprofen concentration ranges of 0.2—60 μg/ml. A low-concentration standard curve (0.2—2 μg/ml) of ibuprofen:internal standard peak height ratios versus sample ibuprofen concentration was linear (r^2 = 0.999) with an intercept value of about 5% of the peak height ratio obtained for the standard solution containing 0.2 μg/ml. A 5—60 μg/ml standard curve was linear (r^2 = 0.999) and passed through the origin. A plasma sample (containing 0.5 μg/ml ibuprofen) determined as an experimental sample in six replicate trials against the low-concentration standard curve was 0.52 ± 0.01 μg/ml.

The assay procedure was used in pharmacokinetic studies with normal volunteers and uremic patients on dosage regimens of 400 mg ibuprofen twice daily. Fig. 3 shows an ibuprofen plasma concentration—time curve for a normal volunteer following a morning 400-mg oral dose of ibuprofen. Full results of these studies will be reported elsewhere.

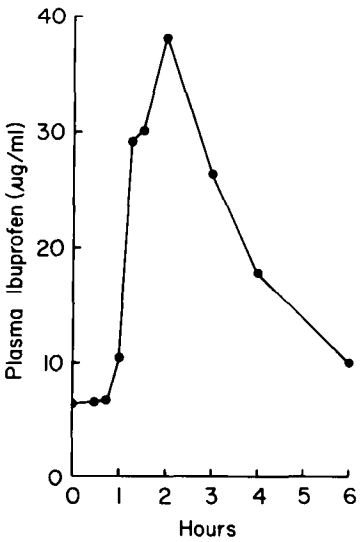

Fig. 3. Plasma ibuprofen concentration—time curve following oral ingestion of 400 mg ibuprofen. A normal male volunteer took 400 mg ibuprofen at 12-h intervals for five days. On the fifth day, a zero-time plasma sample was obtained before the morning dose of ibuprofen.

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REFERENCES

1 D.G. Kaiser and G.J. Vangiessen, J. Pharm. Sci., 63 (1974) 219.
2 D.J. Hoffman, J. Pharm. Sci., 66 (1977) 749.
3 L.P. Hackett and L.J. Dusci, Clin. Chim. Acta, 87 (1978) 301.
4 D.G. Kaiser and R.S. Martin, J. Pharm. Sci., 67 (1978) 627.
5 C.J.W. Brooks and M.T. Gilbert, J. Chromatogr., 99 (1974) 541.
6 J.E. Pettersen, G.A. Ulsaker and E. Jellum, J. Chromatogr., 145 (1978) 413.
7 J.B. Whitlam and J.H. Vine, J. Chromatogr., 181 (1980) 463.
8 D. Pitrè and M. Grandi, J. Chromatogr., 170 (1979) 278.
9 J.L. Shimek, N.G.S. Rao and S.K. Wahba Khalil, J. Pharm. Sci., 70 (1981) 514.
10 G.L. Kearns and J.T. Wilson, J. Chromatogr., 226 (1981) 183.
11 A. Ali, S. Kazmi and F.M. Plakogiannis, J. Pharm. Sci., 70 (1981) 944.
12 B.G. Snider, L.J. Beaubien, D.J. Sears and P.D. Rahn, J. Pharm. Sci., 70 (1981) 1347
13 G.F. Lockwood and J.G. Wagner, J. Chromatogr., 232 (1982) 335.