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Note

Determination of ibuprofen by high-performance liquid chromatography

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Ibuprofen is a non-steroidal, anti-inflammatory, antipyretic and analgesic drug [1]. It is used predominantly in the treatment of adult and juvenile rheumatoid arthritis [2, 3], relief of pain from dysmenorrhea [4] and for the treatment of fever [5]. Due to the growing popularity and increased use of ibuprofen, the need for routine therapeutic monitoring of this drug has also increased.

Previously published methods for analysis of ibuprofen have included gas chromatography [6—9] and high-performance liquid chromatography (HPLC) [10—13]. The gas chromatographic assays require a minimum of 1 ml of serum, and are probably not well suited for routine ibuprofen monitoring in the laboratory. HPLC methods appear to offer the most useful methodologies for routine clinical determination of ibuprofen. The purpose of this report is to describe a new HPLC procedure using 50 μl of sample for the routine quantitation of ibuprofen, which makes it well suited for pediatric patients.

MATERIALS AND METHODS

Chromatography

Analysis was performed on a Perkin-Elmer Series II HPLC instrument equipped with an LC75 UV-VIS variable-wavelength detector interfaced with a Sigma 10 data system (Perkin-Elmer, Norwalk, CT, U.S.A.). The data system provided a read-out of the digitally integrated area under the peaks, determined the retention times, and calculated response factors for ibuprofen and the internal standard. All assays were performed using a 3-μm Rainin Microsorb 10 cm × 4.6 mm C_{18} reversed-phase column (Rainin Instrument, Woburn, MA,

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U.S.A.) maintained at 50°C. The flow-rate was 1.5 ml/min and the effluent was monitored at 220 nm.

Reagents

Ibuprofen analytical standard was supplied by Boots Pharmaceuticals (Shreveport, LA, U.S.A.). 5-Ethyl-5-*p*-tolylbarbituric acid (ETBA), used as the internal standard, was obtained from Applied Science (State College, PA, U.S.A.). All other chemicals and organic solvents were HPLC or reagent grade.

The mobile phase consisted of 35% acetonitrile in 0.1 *M* sodium acetate. The pH was adjusted to 6.4 with a few drops of glacial acetic acid. This solution was freshly prepared and degassed under vacuum just prior to use. A 1.0 *M* sodium acetate (pH 4.6) solution was also prepared in a similar manner.

The stock standard of ibuprofen (1.0 g/l) was prepared by dissolving an appropriate amount of ibuprofen in methanol. Working standards were prepared fresh in drug-free sera from the stock standard to yield concentrations from 1.0—100 mg/l. The internal standard was added to the extraction solvent to yield a final concentration of 1.0 mg/l.

Procedure

A 50-μl aliquot of standard, control, or patient serum was placed in a 1.5-ml Eppendorf centrifuge tube. To each tube, 100 μl of 1.0 *M* sodium acetate (pH 4.6) was added and mixed. This was followed by addition of 1.0 ml of the extraction solvent (ethyl acetate) containing ETBA. The tubes were vortexed vigorously for at least 1 min and then centrifuged for 3 min at 21,000 *g* in a Brinkman table-top microcentrifuge. The upper organic phase was transferred to a clean glass tube (75 × 10 mm) and evaporated to dryness at 40°C under nitrogen. The dried sample residue was reconstituted with 50 μl methanol, 10 μl of which was injected onto the column.

Within-run precision was evaluated by assaying a prepared ibuprofen serum pool and day-to-day precision was evaluated by analyzing samples on consecutive days. Stability studies were also conducted using a pool of serum to which known quantities of ibuprofen were added. Aliquots of these samples were frozen at −10°C and analyzed over a period of 18 weeks.

RESULTS AND DISCUSSION

Table I shows the results of within-run and day-to-day precision. Table II shows that there was no appreciable change in the concentration of the drug under these conditions. The accuracy of the method was further validated by analysis of five blind check samples supplied by Boots Pharmaceuticals. The concentrations ranged from 0.0 to 60 mg/l ibuprofen as tabulated in Table III. The correlation coefficient between known and measured concentrations was 0.999.

Fig. 1 shows typical chromatograms of: (A) drug-free serum containing the internal standard; (B) drug-free serum reconstituted with 20 mg/l of ibuprofen and the internal standard; and (C) a patient sample which was taken 3 h after oral ingestion of 400 mg ibuprofen. The determined concentration in this

TABLE I

PRECISION OF SERUM IBUPROFEN ANALYSIS

	Within-run	Day-to-day
Amount added (mg/l)	20.0	50.0
Amount obtained		
Mean (mg/l)	20.1	49.7
S.D.	0.6	1.5
C.V. (%)	3.0	3.0
Number of analyses	10.0	13.0

TABLE II

STABILITY OF IBUPROFEN

Samples were stored frozen at −10° C.

Day	Value (mg/l)	Day	Value (mg/l)
1	9.2	60	9.7
4	9 5	95	10.5
5	10.0	98	10.1
8	10.6	122	9.9
11	11 1	126	10.0
Mean (mg/l)	10.1		
S D.	0.6		

TABLE III

DETERMINATION OF IBUPROFEN BLIND-CHECK SAMPLES

Sample	Values obtained (mg/l)	Actual concentration (mg/l)
A	9.7	10.0
B	40.5	40.0
C	Not detected	0.0
D	20.2	20.0
E	59.9	60.0

$r = 0.999$

sample was 19.1 mg/l. The retention times for internal standard and ibuprofen were 3.0 and 4.0 min, respectively. The concentration of ibuprofen, calculated from the integrated area under the peaks, was linearly related to the internal standard area over the concentration range from 1.0 to 100 mg/l. The mean recovery of ibuprofen from serum samples was 95%.

The optimal wavelength (220 nm) for detection of ibuprofen was employed which increased sensitivity, selectivity, and decreased sample size requirements. Previously reported HPLC procedures require at least 0.5 ml of serum volume for analysis, ten times the amount required for this assay. In addition, the internal standard is incorporated in the extraction solvent allowing one pre-chromatography step (extraction and drying) for sample preparation thereby minimizing dilution and manipulation errors.

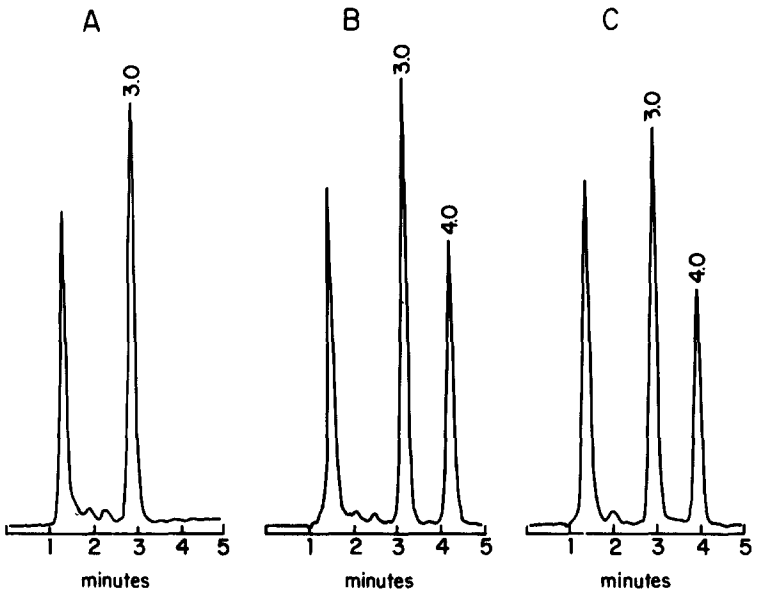

Fig. 1. (A) Drug-free serum containing the internal standard; (B) drug-free serum reconstituted with 20 μg/ml ibuprofen and the internal standard; and (C) a patient sample which was taken 3 h after an oral dose of 400 mg ibuprofen. Retention times: internal standard = 3.0 min; ibuprofen = 4.0 min.

The assay is sensitive to 1 mg/l and linear to 100 mg/l. This encompasses the range of therapeutic concentrations, reported to be from 1.0 to 42 mg/l [7]. The sample size of 50 μl makes it an assay ideally suited for pediatric patients. Gentamycin, tobramicin, chloramphenicol, salicylates, and acetaminophen did not interfere with the assay. The method is readily adaptable for routine therapeutic monitoring in those laboratories equipped with HPLC systems.

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