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Measurement of Plasma Ibuprofen by Gas Chromatography-Mass Spectrometry

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A gas-chromatography-mass spectrometry (GC-MS) method for the determination of plasma ibuprofen was developed. Plasma samples from cystic fibrosis (CF) patients receiving high-dose ibuprofen therapy were analyzed by GC-MS and the result compared to analysis by high-performance liquid chromatography (reference method). Analysis of ibuprofen was sensitive to at least 5 mg/L, and the method was linear to 200 mg/L. Within-run variations of plasma samples were 4.6% (131.7 ± 6.0 mg/L) and 5.4% (44.4 ± 2.4 mg/L), respectively. The between-run variation was 9.3% (45.4 ± 4.2 mg/L) and 7.4% (88.0 ± 6.5 mg/L). This method is suited for routine clinical use for the monitoring of plasma ibuprofen levels in treatment of CF. It may be particularly applicable in pediatric laboratories, which are likely to possess GC-MS capability. J. Clin. Lab. Anal. 11:336–339, 1997. © 1997 Wiley-Liss, Inc.

Key words: ibuprofen; anti-inflammatory agents; cystic fibrosis; NSAIDS; GC-MS

INTRODUCTION: MEASURING IBUPROFEN

The inflammatory response to repeated pulmonary infection (primarily with *Pseudomonas* species) contributes to the progressive loss of lung function seen in cystic fibrosis (1–6). In a recent double blind study of cystic fibrosis (CF) patients with mild lung disease, therapy with high doses of ibuprofen was shown to slow the progression of lung disease (7). Dose adjustment to achieve peak plasma levels of ibuprofen between 50 mg/L and 100 mg/L resulted in increased preservation of lung function in CF patients. It is not possible to predict the dose that would achieve the desired plasma level in an individual patient, and weight gain may require adjustments of dose (1). Hence, periodic monitoring of the peak plasma level achieved by a patient is needed as a guide to therapy.

The recent increase in the use of ibuprofen as part of the treatment of cystic fibrosis has increased the demand for the measurement of plasma concentration. Plasma ibuprofen has been accurately and precisely measured by high performance liquid chromatography (HPLC) (8). Here, we report the development of a gas chromatography-mass spectroscopy (GC-MS) method to quantitate ibuprofen in human plasma. With this technique, ibuprofen can be measured accurately with analytic instrumentation which is available in many laboratories, offering an alternative to HPLC.

MATERIALS AND METHODS

Flurbiprofen, horse serum, and benzene were from Sigma (St. Louis, MO), pyridine was from Aldrich (Milwaukee, WI), Bis (trimethylsilyl) trifluoroacetamide/10% trimethylchlorosilane (BSTFA) was from Regis Technologies (Morton Grove, IL), and diethyl ether was from Mallinkrodt (Chesterfield, MO). All other chemicals were reagent grade from standard sources.

Samples

Blood specimens (EDTA plasma), which were collected for the measurement of plasma ibuprofen by HPLC at a reference laboratory, were split for analysis by GC-MS. With informed consent, plasma samples were also obtained from volunteers for interference and linearity studies. All studies were performed in accordance with a protocol approved by the Human Studies Committee of Washington University.

Preparation of Controls and Internal Standard

Flurbiprofen internal standard (1.0 g/L) was prepared in 50% ethyl alcohol and stored at room temperature until use. High and low ibuprofen controls (40 mg/L and 90 mg/L, respectively) were prepared in equine serum, aliquotted, and stored frozen at –70°C until use.

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Assay Procedure

Specimen (0.5 mL plasma) and 50 μL of internal standard (flurbiprofen, 1 g/L) were combined and acidified to pH <1 with 12 N HCl. Ibuprofen was extracted twice with diethyl ether (2.5 mL). The ether layers were combined, evaporated to dryness under N_2, redissolved in 200 μL benzene, and again evaporated to dryness under N_2. Pyridine (25 μL) and BSTFA (25 μL) were added and the samples heated at 60°C for 15 min. Specimens so prepared were stable for 7 d at room temperature. Ibuprofen was separated from other specimen components by chromatography (injection volume 0.5 μL) on a 0.22 mm × 15 m DB-1 column (Varian 3700 gas chromatograph). Injection port temperature was 250°C, initial temperature was 100°C, and final temperature 280°C with a temperature increase of 12°C per min. Ibuprofen and flurbiprofen were detected with a Finnigan ion trap detector. Respective target and qualifier ions for ibuprofen (160, 234 m/z) and flurbiprofen (165, 301 m/z) were chosen for quantitation using Envirolink software (Technivent, Maryland Heights, MO). The retention times for ibuprofen and flurbiprofen were 17 min and 21 min, respectively.

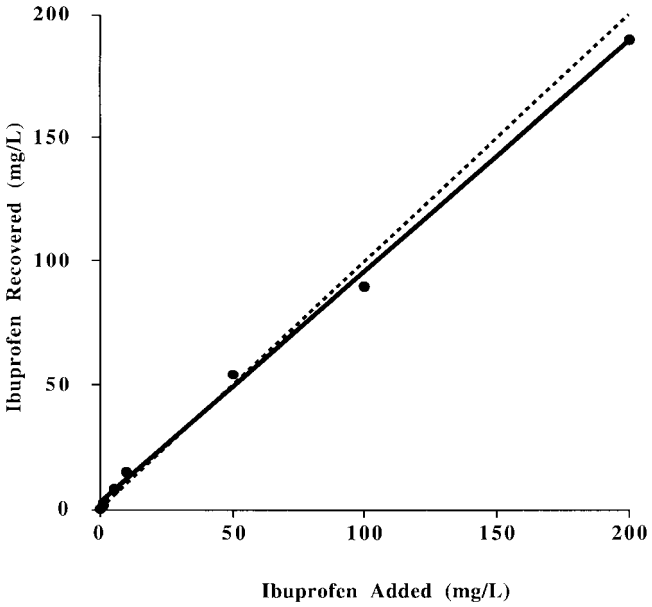

Fig. 2. Linearity/recovery was determined by analysis of a plasma pool that had been spiked with ibuprofen in concentrations from 0 to 200 mg/L. Solid line, line of best fit; dashed line, line of identity.

RESULTS

Ibuprofen and flurbiprofen (internal standard) were well separated and readily detected as trimethylsilyl derivatives on routine gas chromatographic analysis (Fig. 1). The mean recoveries of ibuprofen and flurbiprofen extracted from plasma were 86.5 ± 10.1% and 94.3 ± 11.5%, respectively (P = 0.001). Linearity was determined by analysis of a plasma pool to which ibuprofen in concentrations from 0–200 mg/L had been added. The assay was linear throughout this range (Fig. 2). The apparent recovery below 10 mg/L was >100% after correction for recovery of the internal standard (flurbiprofen).

The ability of the method to measure low concentrations of ibuprofen was investigated by repeated analysis of a sample without ibuprofen and with 5 mg/L ibuprofen added. The mean ± 1 SD results were 0.0 ± 0.1 mg/L for a sample without ibuprofen and 7.7 ± 0.9 mg/L for a sample with 5 mg/L added ibuprofen. Plasma containing ibuprofen was stable at –20°C for at least 3 mo, and derivatized samples were stable for 1 wk (data not shown).

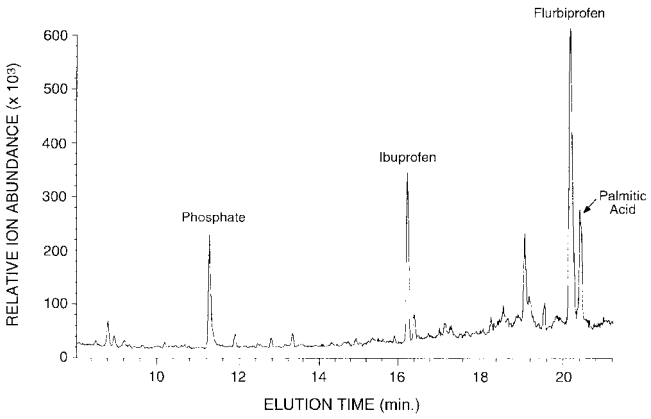

Fig. 1. Chromatogram of analysis of plasma from a patient receiving ibuprofen, prepared as described in the text. Ibuprofen quantification: 44 mg/L.

The GC/MS method was compared to an established HPLC method by paired analysis of 40 samples from cystic fibrosis patients undergoing ibuprofen kinetic studies. Least-squares regression found good correlation of the two assays [y = 1.02 x + 4.0 mg/L (r = 0.9702, Sy*x = 10.3 mg/L)] (Fig. 3).

Within-run and between-run precision was determined from analysis of quality control media. Within-run coefficients of variation (same day analysis of plasma samples containing 131.7 ± 6.0 mg/L and 44.4 ± 2.4 mg/L ibuprofen) were 4.6% and 5.4%, respectively (n = 6). Between-run coefficients of variation (n = 36) were 9.3% (45.4 ± 4.2 mg/L) and 7.4% (88.0 ± 6.5 mg/L), respectively.

Addition of hemolysate to a plasma containing 33.0 mg/L ibuprofen produced no interference at levels of added hemolysate that resulted in up to 10 g/L added hemoglobin (Table 1).

The specificity of the assay for ibuprofen was determined by analyzing plasma samples containing therapeutic levels of three related compounds, naproxen, fenoprofen and ketoprofen (all related in structure to ibuprofen) obtained after ingestion by a volunteer. The retention times for ibuprofen and flurbiprofen were 17.1 and 21.0 min, respectively. The retention times for naproxen, fenoprofen, and ketoprofen were 21.8, 12.1 and 12.7 min, respectively. The molecular (302 and 314) and M-15 ions (287 and 299) for naproxen and

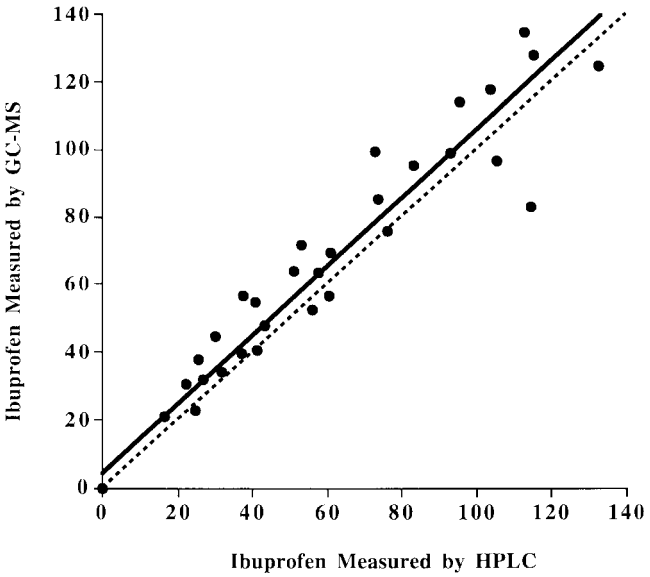

Fig. 3. Correlation of GC-MS ibuprofen measurement with HPLC. Plasma specimens (n = 40) were analyzed by both methods; the solid line is the least-squares regression (GC-MS = 1.02 HPLC + 4.0 mg/L; Sy·x = 10.3 mg/L), dashed line is line of identity.

fenoprofen, respectively, were detected. The molecular ion (326), but not the M-15 ion (311), was detected for ketoprofen. There was no interference in the selective ion monitoring of ibuprofen by naproxen, fenoprofen, and ketoprofen as none of these compounds produced ions with the mass to charge ratio of the four ions monitored for ibuprofen and flurbiprofen (160 and 234, and 165 and 301, respectively), and all of these compounds were sufficiently well separated from ibuprofen and flurbiprofen.

DISCUSSION

The GC-MS method described here is sensitive, precise, and correlates well with HPLC. The method was linear over the range of clinical interest and was free from interference by related compounds and by hemolysis. This method was developed to provide an alternative technique for the accurate measurement of ibuprofen in plasma using analytic instrumentation available in many pediatric clinical chemistry laboratories.

Prior methods of analysis of ibuprofen have included HPLC, GC, and GC-MS [8–15]. Previously described HPLC and GC methods claim linearity to maximums ranging from 40–100 mg/L (8,11–14). Ibuprofen therapy in CF patients is guided by a kinetic study of plasma levels that aims for peak levels between 50 mg/L and 100 mg/L (1,7). Peak plasma levels of some samples will be > 100 mg/L. The method described here is linear to 200 mg/L and thus will avoid dilution and repeat analysis for those specimens. The GC-MS method requires 26 min per sample for analysis. This time is longer but not unacceptable compared to an estimated 13 min per sample analyzed by HPLC (8,12).

TABLE 1. Interference From Hemoglobin

Hemoglobin (G/L)	Ibuprofen (mg/L)
0.00	32.91
0.31	32.01
0.62	37.41
1.25	36.21
2.50	31.72
5.00	34.75
10.00	32.01

A previously published GC-MS method required two centrifugation steps and the timed, sequential addition of reagents to form first a mixed anhydride and next, an amine (10). Both this and another GC-MS method (9) used deuterated ibuprofen that was prepared on site. The method detailed here requires no synthesis of reagents and offers a simple, rapid extraction and derivatization protocol.

Several different internal standards have been used in the measurement of ibuprofen. Ibufenac (8,15) is not commercially available and is no longer supplied by the manufacturer for research. Other internal standards include naphthalene [14], 5-ethyl-5-p-tolylbarbituric acid (11), flurbiprofen (12), and deuterated ibuprofen (9,10). Use of flurbiprofen as an internal standard in the method presented here has the advantage of close chemical relationship to ibuprofen and ready commercial availability. The statistically significant difference in recovery between ibuprofen and flurbiprofen internal standard was too small to yield a clinically significant error in ibuprofen measurement.

GC-MS methods for ibuprofen determination have been published for analysis in human serum and synovial fluid (9) and for quantitation of enantiomeres of ibuprofen in human plasma and synovial fluid (10). In the first study, ultrafiltration was used to measure free serum ibuprofen (9). In the second study, only the enantiomers of ibuprofen in synovial fluid and plasma were determined (10). The method described here is the first aimed at measuring total plasma ibuprofen in the range and circumstances that occur in the kinetic studies of ibuprofen in CF therapy. We conclude from this study that GC-MS offers a useful alternative to HPLC for the routine determination of plasma ibuprofen levels, particularly for those laboratories that possess GC/MS analytic capacity, but lack HPLC capability.

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