Journal of Chromatography, 380 (1986) 77—87
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam - Printed in The Netherlands

CHROMBIO. 3146

DEVELOPMENT OF CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC METHODOLOGY FOR THE SIMULTANEOUS DETERMINATION OF IBUPROFEN AND [ar-$^2H_4$]IBUPROFEN IN SERUM: DEMONSTRATION OF KINETIC EQUIVALENCE IN THE BEAGLE

DON L. THEIS*, GORDON W. HALSTEAD and KATHY A. HALM

The Upjohn Company, Control Research Laboratories, Kalamazoo, MI 49001 (U.S.A.)

(Received December 20th, 1985)

SUMMARY

A capillary gas chromatographic—mass spectrometric method for the determination of ibuprofen and tetra-deuterated ibuprofen in serum is described. Ibuprofen, [ar-$^2H_4$]-ibuprofen and the internal standard, [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen, are extracted (after acidification) from serum onto a cross-linked styrene divinyl benzene resin by an automated sample processor. After elution and evaporation of the organic phase, samples are reconstituted with solvent and analyzed without derivatization by capillary gas chromatography—mass spectrometry. This methodology was used to evaluate possible kinetic isotope effects after the coadministration of an equimolar mixture of ibuprofen and the deuterium-labeled covariant in the beagle. No significant differences in absorption or elimination were observed.

INTRODUCTION

Ibuprofen [(*RS*)-2-(4-isobutylphenyl)propionic acid] (I, Fig. 1) is a non-steroidal anti-inflammatory agent possessing analgesic and antipyretic properties. It is rapidly absorbed in humans with peak serum concentrations occurring within 1—2 h after oral dosing. Ibuprofen is metabolized by oxidation primarily on the isobutyl side-chain to form three hydroxylated metabolites and one dicarboxylated metabolite [1–3]. The major urinary metabolites are *S*(+)-2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid and *S*(+)-2-[4-(2-carboxypropyl)phenyl]propionic acid. Relatively small ($\approx$ 1 μg/ml) amounts of these are found in the circulation [3]. As with other 2-aryl-

0378-4347/86/$03.50

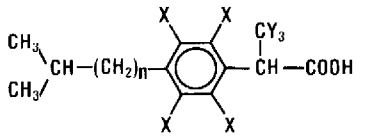

Fig. 1. Structures of ibuprofen (n = 1, X = H, Y = H), [ar-$^2H_4$]ibuprofen (n = 1, X = ^{2}H, Y = H), [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen (n = 1, X = ^{2}H, Y = ^{2}H) and 2-(4-isopropylphenyl)propionic acid (n = 0, X = H, Y = H) (II).

propionic acids [4, 5], ibuprofen has been shown to undergo chiral inversion about the 2-position of the propionic acid group from the *R*(−) to the *S*(+) enantiomer.

The relative bioavailabilities of various ibuprofen formulations have been investigated and demonstrate significant intra- and inter-subject variability [6—8]. Simultaneous coadministration of drug and a stable isotopically labeled covariant potentially eliminates this variability when assessing bioavailability. This subject has been extensively reviewed [9—14]. Primary isotope effects, in which direct cleavage of a deuterium bond takes place, and secondary effects, where the deuterium bond is not broken but still affects the rate, have been reported [14—17] for other drugs. To avoid potential metabolic isotope effects, labeled ibuprofen was synthesized with four deuterium atoms on the aromatic ring [18].

This paper focuses on the methodology required to simultaneously determine ibuprofen and [ar-$^2H_4$]ibuprofen in serum. Several liquid chromatographic assays for ibuprofen in serum have been reported [19–23]. However, since mass analysis was required for this method, a gas chromatographic (GC) method was developed. Ibuprofen has been previously determined in plasma by derivatization to the methyl ester [24—26] and the diastereomeric amide [4] followed by GC. Whitlam and Vine [27] have reported a method whereby ibuprofen and internal standard, [3,3,3-$^2H_3$]ibuprofen, were methylated prior to analysis by packed-column gas chromatography—mass spectrometry (GC—MS). Other non-derivatization methods were not used because they utilized a packed column which did not provide low carrier gas flow-rates desirable in mass spectrometry or yielded unsatisfactory results [28, 29]. For the work described here, a non-derivatization, capillary GC—MS assay was developed.

The framework for the development of the sample preparation and extraction via the automated sample processor was reported by Snider et al. [20]. Buffers and some solvents were changed to enhance recovery and/or accommodate the capillary GC—MS instrumentation. After optimizing extraction and chromatographic conditions using a chemical analogue of ibuprofen, 2-(4-isopropylphenyl)propionic acid (II) with a gas chromatographic–flame ionization detection method (GC—FID), parameters were adapted to the GC—MS system. [ar-$^2H_4$,3,3,3-$^2H_3$]Ibuprofen was used as the internal standard [18]. This method was then utilized to analyze serum collected after the coadministration of an equimolar oral solution dose of ibuprofen and [ar-$^2H_4$]-ibuprofen in a beagle.

EXPERIMENTAL

Reagents and materials

[ar-$^2H_4$]Ibuprofen and [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen (internal standard) were prepared as previously described [18]. Unlabeled ibuprofen, 2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid and 2-[4-(2-carboxypropyl)-phenyl]propionic acid were available from Upjohn. Acetone, acetonitrile, tetrahydrofuran and chloroform were obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). *p*-Isopropylphenylacetic acid was purchased from Fairfield (Blythewood, SC, U.S.A.). Lithium diisopropylamide and methyl iodide were obtained from Aldrich (Milwaukee, WI, U.S.A.). All other chemicals were from standard sources. Blank dog serum was obtained from Pel-Freeze Biologicals (Rogers, AR, U.S.A.).

2-(4-Isopropylphenyl)propionic acid (II)

Under nitrogen, a solution of *p*-isopropylphenylacetic acid (5.00 g, 28.1 mmol) in 35 ml of dry tetrahydrofuran (distilled from $LiAlH_4$) was added with stirring to a −78°C solution of lithium diisopropylamide (12.02 g, 112.1 mmol) in 150 ml of dry tetrahydrofuran. After 15 min, 3.50 ml of methyl iodide (7.98 g, 56.2 mmol) were added via syringe and the temperature was maintained at −78°C for 1 h. The reaction mixture was poured onto 500 ml of crushed ice, acidified to ca. pH 2 with hydrochloric acid and then extracted with three 150-ml portions of ether. The combined extracts were dried over magnesium sulfate and the solvent removed in vacuo. The residue was purified by recrystallization from methanol–water. Mass spectrum: *m/z* (relative intensity) 192 (44), 177 (62), 147 (100), 131 (31), 119 (26), 117 (21), 105 (34), 91 (36), 77 (17), 43 (26).

Sample preparation

After clotting, venous blood was centrifuged and the serum separated. The serum was frozen if further preparation was delayed by more than 8 h. Serum extractions were performed by a DuPont PREP I automated sample processor (DuPont, Wilmington, DE, U.S.A.). Solid-phase extraction columns containing cross-linked styrene divinyl benzene macroreticular resin (Type W) were used to extract drug and internal standard from the serum. Prior to use, the cartridges were washed twice using program 14 (Software Revision D as described in ref. 30) with acetone (25 ml) and water (25 ml) in reservoirs 1 and 2, respectively. For each sample, 1.0 ml of serum, 1.0 ml of 0.05 *M* trichloroacetic acid and 0.1 ml of internal standard solution [470 nmol of [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen per ml of acetonitrile—water (40:60)] were added to each cartridge. An internal standard solution containing ca. 470 nmol of II per ml was used for the GC—FID assay. Calibration standards were prepared by pipeting 1 ml of blank serum, 1.0 ml of 0.05 *M* trichloroacetic acid, 0.1 ml of internal standard solution and 0.1 ml of a standard spiking solution. The latter solutions were prepared by making appropriate dilutions of ibuprofen and [ar-$^2H_4$]ibuprofen in acetonitrile—water (40:60) over the expected concentration range of samples. After gentle mixing, the cartridges were placed into the sample processor, which contained water (25 ml) and acetone (25 ml)

in reservoirs 1 and 2, respectively. The automated extraction, wash, elution and drying sequences were accomplished using program 15 at a temperature of 50°C. This process requires ca. 31 min. The extracted samples in aluminum cups were reconstituted in ca. 1 ml of chloroform, mixed by vortexing and transferred to a glass vial by a glass pipet.

In vivo testing

An oral solution containing 0.967 mmol of ibuprofen and 0.952 mmol of [ar-$^2H_4$]ibuprofen was prepared by first dissolving the drug in ca. 3.5 ml of 0.2 *M* sodium hydroxide with sonication. Sufficient 0.2 *M* monobasic potassium phosphate (ca. 5 ml) was added to adjust the pH to approximately 7.2. The mixture was then diluted to ca. 20 ml with water to yield a 0.05 *M* solution. The resulting solution was administered to a pure-bred beagle via gastric intubation. Blood samples were drawn from the antebrachial section of the cephalic vein in the foreleg. The samples were collected in a red stoppered tube which has no anticoagulant (Vacutainer, Becton-Dickinson, Rutherford, NJ, U.S.A.).

Capillary gas chromatography—flame-ionization detection (GC—FID)

The GC—FID system used was a Hewlett-Packard 5880A Series gas chromatograph. The fused-silica column (Hewlett-Packard; 12 m × 0.2 mm I.D.) was internally coated with cross-linked methyl silicone stationary phase; film thickness (df) = 0.33 μm. The injection mode was splitless with a solenoid activation-gas bypass of 0.25 min. The carrier gas (1 ml/min) and make-up gas (25 ml/min) were helium, while the detector support gases were hydrogen (25 ml/min) and air (350 ml/min). Injector and detector temperatures were 250 and 300°C, respectively. The column temperature was programmed from an initial temperature of 100°C (0.5 min) to 250°C (20°C/min) and held isothermally for 10 min. Since the flame-ionization detector is not mass differentiating, II was used as an internal standard. Peak-area ratios of ibuprofen and internal standard were plotted versus ibuprofen concentration and the resulting line used as a calibration curve.

Gas chromatographic—mass spectrometric conditions

A Hewlett-Packard Model 5970B mass-selective detector equipped with an HP 5890 gas chromatograph was used for the analysis of samples. Electron-impact ionization was utilized with an electron energy of 70 eV. Scan experiments were made over a mass range of *m/z* 40—300 with a scan rate of 1.6 scans/s. Selected-ion monitoring (SIM) was performed at *m/z* 206, 210 and 213 for the ibuprofen, [ar-$^2H_4$]ibuprofen and [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen (internal standard) parent ions, respectively. GC conditions were the same as those described in the GC—FID experimental section. An automated liquid sampler (HP 7672A) and software (HP 59972A) allowed continuous data collection. The column was run directly into the MSD source via a transfer line, which was held at 280°C. The electron multiplier was set at 1600 V. Peak areas were determined from the SIM output for each ion. Calibration curves were generated by plotting the peak-area ratios ibuprofen/[ar-$^2H_4$, 3.3.3-$^2H_3$]-ibuprofen and [ar-$^2H_4$]ibuprofen/[ar-$^2H_4$, 3.3.3-$^2H_3$]ibuprofen versus concentrations. The instrument was previously calibrated with perfluorotributylamine.

RESULTS AND DISCUSSION

Since the majority of the metabolism of ibuprofen occurs at the isobutyl side-chain, ibuprofen was synthesized with four deuterium atoms on the aromatic ring to avoid metabolic isotope effects. The increase in mass to *m/z* 210 for the parent ion allows sufficient resolution from the unlabeled

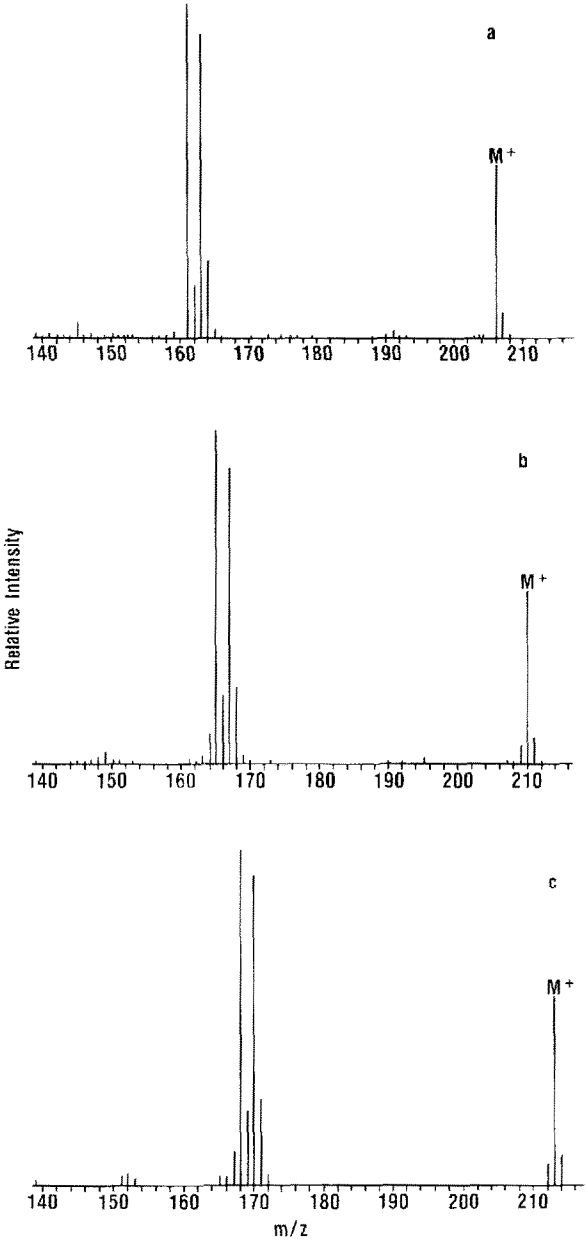

Fig. 2. Electron-impact mass spectra of ibuprofen (a), [ar-$^2H_4$]ibuprofen (b) and [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen (c). Conditions as described in Experimental.

ibuprofen at *m/z* 206, as shown in Fig. 2. Similarly, ibuprofen incorporated with seven deuterium atoms was chosen as the internal standard, such that no significant overlap occurs when the parent ion at *m/z* 213 is monitored in the presence of the other ibuprofen covariants. Other advantages of a deuterated internal standard include similar solubility properties, nearly isographic chromatographic elution and analogous fragmentation patterns to the analytes. The solid-phase extraction onto a column containing the styrene divinyl benzene was automated for unattended operation and proved more convenient than conventional two-phase solvent methods.

Prior to use, columns were first washed with both water and acetone to remove potentially interfering substances. After addition of sample and internal standard, buffer (pH $\approx$ 1.2) was added to ensure that ibuprofen, with a pK_a of ca. 5.2, was in the unionized state. After adsorption onto the column, samples were washed with water and the solutes quantitatively eluted with acetone into aluminum recovery cups. The solvent was then evaporated with air at 50°C. Fig. 3 shows a SIM chromatogram of a sample preparation using blank serum processed by this method. No interferences at the monitored masses were observed. Other solvents and buffer systems were investigated; however, no significant improvements were realized.

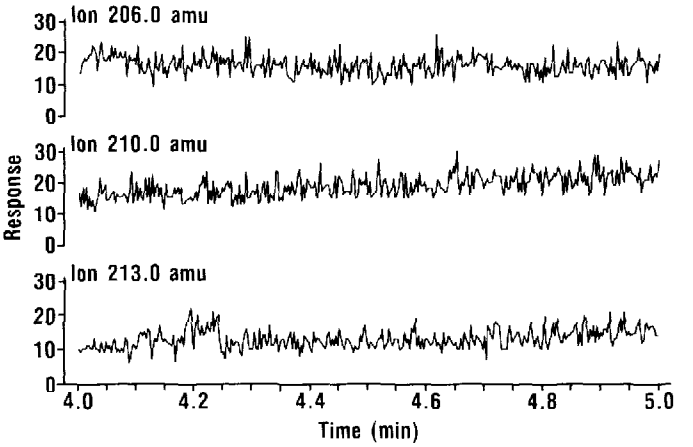

Fig. 3. SIM chromatograms of a sample preparation of blank dog serum showing no significant interferences at the ibuprofen elution time window ($\approx$ 4.7 min). Conditions as described in Experimental.

GC was chosen as the chromatographic system for the separation of ibuprofen from other substances, since it is compatible with MS. Capillary fused-silica columns were desirable because of their low flow-rates, resolution and relatively few active sites. The latter was especially important when trying to chromatograph an underivatized carboxylic acid such as ibuprofen. The cross-linked methyl silicone stationary phase afforded good selectivity, peak shape and, unlike other more polar phases, low column bleed. Chromatographic conditions were first optimized on a gas chromatograph equipped with a flame-ionization detector. Fig. 4 shows a chromatogram of serum sample spiked at the nmol/ml level (1 μg of ibuprofen = 4.85 nmol of ibuprofen). Compound II was used as the internal standard since separation of the deuterated ibuprofen

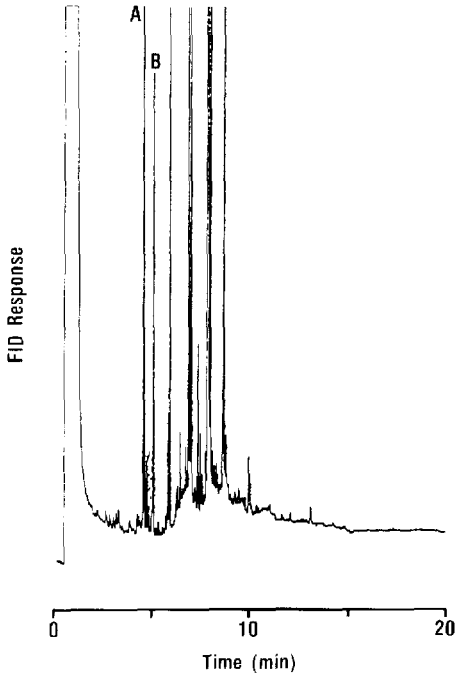

Fig. 4. GC—FID profile of a sample preparation of serum containing 52 nmol of II (A) and 2.4 nmol of ibuprofen (B). Conditions as described in Experimental.

analogues was not achieved. As observed in earlier studies with other compounds [31, 32], the deuterated analogues eluted earlier than the unlabeled ones. The relative retention times of II, 2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid and 2-[4-(2-carboxypropyl)phenyl]propionic acid to ibuprofen (4.7 min) were 0.90, 1.2 and 1.4, respectively. This method was linear over the range tested from 0.485 to 485 nmol/ml (0.1—100 μg/ml). The resulting line was described by the equation $y = 0.107x - 0.00086$, with a correlation coefficient of 1.000, as determined by linear regression. While the major purposes of developing this system were to optimize the extraction system and develop a GC—MS procedure, the GC—FID system could be used as an assay for ibuprofen in a conventional non-isotopic study and is more efficient than many existing assays since derivatization is unnecessary.

The GC—MS system utilized the same sample preparation and chromatographic system as the GC—FID method, with the exception of the internal standard. To increase the sensitivity and selectivity of the method, the mass-selective detector was used in the SIM mode over the time window of 3.5—6.5 min. The major ions produced were at *m/z* 161, 165 and 168 for ibuprofen, [ar-$^2H_4$]ibuprofen and [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen, respectively, owing to the loss of the carboxylic acid group. However, monitoring these ions was not useful for quantitation purposes since other fragmentation caused overlap in that mass range. Therefore, the major ions monitored were the parent ions at *m/z* 206, 210 and 213. Calibration curves consisted of blank serum spiked with varying amounts of ibuprofen and [ar-$^2H_4$]ibuprofen. Fig. 5 shows a SIM chromatogram of a standard preparation spiked with 48 nmol/ml of these

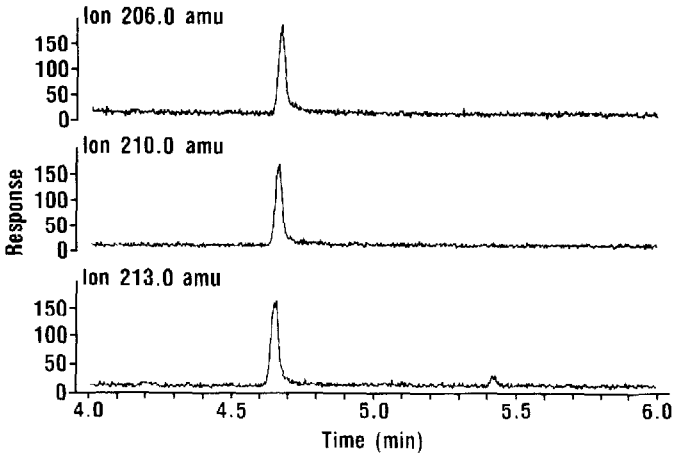

Fig. 5. SIM chromatograms of a sample preparation of a serum standard containing ca. 50 nmol/ml ibuprofen, [ar-$^2H_4$]ibuprofen and [ar-$^2H_4$,3,3,3-$^2H_3$]ibuprofen. Conditions as described in Experimental.

ibuprofen compounds. Linear regression analysis of a typical curve over the range of 2.4—240 nmol/ml yielded equations of $y = 0.1091x + 0.0359$ for ibuprofen and $y = 0.09856x + 0.0554$ for [ar-$^2H_4$]ibuprofen. Correlation coefficients of 0.999 and 0.997 were obtained, respectively. Precision of the method was assessed by determining the ibuprofen/[ar-$^2H_4$, 3,3,3-$^2H_3$]ibuprofen and [ar-$^2H_4$]ibuprofen/[ar-$^2H_4$, 3,3,3-$^2H_3$]ibuprofen area ratios of six samples processed from the same pool of serum spiked at the 48 nmol/ml level. The relative standard deviations (R.S.D.) for the unlabeled and labeled ibuprofen determinations were 9.6 and 8.3% ($n = 6$), respectively. Replicate sample injections of the same smaple yielded R.S.D. values of 3.6 and 1.4% ($n = 3$), respectively. Due to the number of samples often present in a bioavailability study and the requirement that a calibration curve be generated daily, auto-

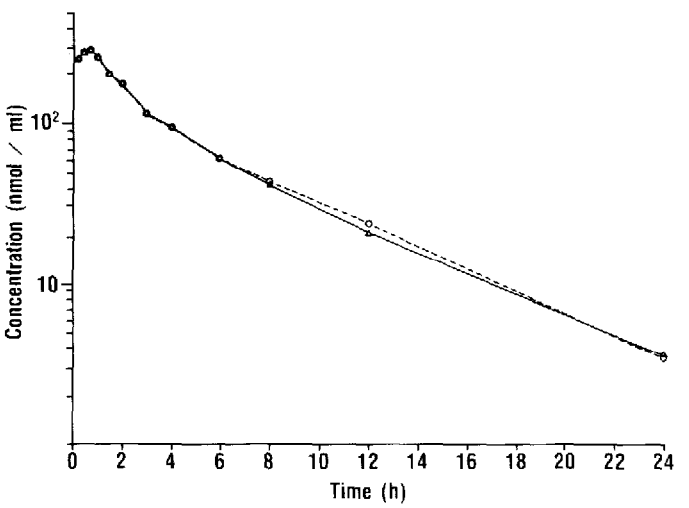

Fig. 6. Serum concentration versus time curves for ibuprofen (△) and [ar-$^2H_4$]ibuprofen (○) following an oral solution dose of ca. 0.96 mmol of each.

mated sample injection was also applied. The software provided with the mass-selective detector allowed continuous unattended data collection and storage. This system also allowed syringe wash before or after each sample, to reduce carry-over.

Application of the method was demonstrated by evaluating the bioequivalence of ibuprofen and [ar-$^2H_4$]ibuprofen in a beagle. An oral aqueous solution containing ca. 0.96 mmol of ibuprofen and [ar-$^2H_4$]ibuprofen was administered via gastric intubation. Venous samples were collected for 24 h.

TABLE I

RESULTS OF ORAL VERSUS ORAL STUDY OF IBUPROFEN IN A BEAGLE

Dose: 0.967 mmol of ibuprofen and 0.952 mmol of [ar-$^2H_4$]ibuprofen.

Time (h)	Concentration (nmol/ml)		Ibuprofen/[ar-$^2H_4$]ibuprofen ratio
	Ibuprofen	[ar-$^2H_4$]Ibuprofen	
0	N.D.*	N.D.*	—
0.23	256	258	0.992
0.50	282	272	1.04
0.75	281	289	0.972
1.0	263	263	1.00
1.5	205	206	0.995
2.0	176	180	0.978
3.0	117	116	1.01
4.0	96.1	96.7	0.994
6.0	61.6	61.0	1.01
8.0	42.9	43.6	0.984
12.0	21.0	23.8	0.882
24.0	3.61	3.47	1.04
Mean			0.991 ± 0.040

*N.D. = Not detected.

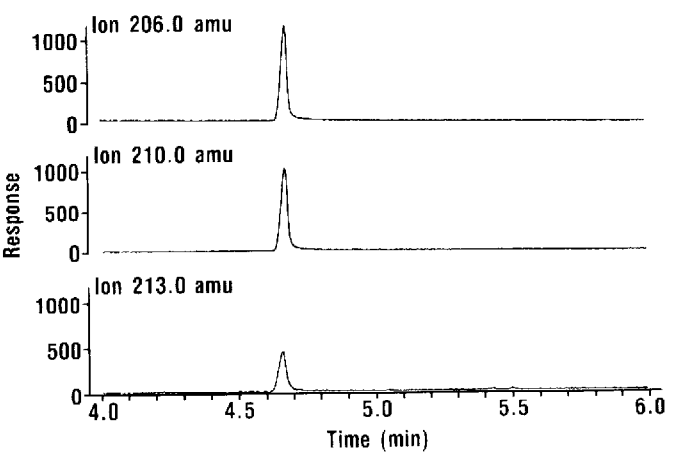

Fig. 7. SIM chromatograms of a sample preparation of the 4-h point of the oral solution (ibuprofen) versus oral solution ([ar-$^2H_4$]ibuprofen) study. Conditions as described in Experimental.

Visual examination of the results, shown in Fig. 6 and Table I, indicates bioequivalence of unlabeled and labeled drugs. According to Wagner [33], if differences between serum concentrations do not differ significantly for all time points, they are considered bioequivalent. The estimated peak serum concentrations of ca. 282 and 289 nmol/ml for ibuprofen and [ar-$^2H_4$]ibuprofen, respectively, occurred within the first hour after dosing. Submicrogram per ml (< 5 nmol/ml) levels were detectable at 24 h. The ratios of ibuprofen and [ar-$^2H_4$]ibuprofen concentrations are given in Table I. The ratios remain essentially constant throughout the time course of the study. The standard deviation is < 5% of the mean of the ratios, indicating the absence of a significant isotope effect [34]. A typical SIM chromatogram of the sample preparation for the 4-h time point is shown in Fig. 7.

CONCLUSIONS

The methodology described in this report allows the determination of ibuprofen and [ar-$^2H_4$]ibuprofen simultaneously in serum. [ar-$^2H_4$,3,3,3-$^2H_3$]-Ibuprofen is added as internal standard prior to sample preparation to minimize variation and enhance accuracy. The sample preparation is automated and samples need not be derivatized. The mass-selective detector is sensitive, selective and automated. Using this technology it was shown that the [ar-$^2H_4$]-ibuprofen was bioequivalent to the unlabeled ibuprofen in a beagle. This technique is suitable for use in the comparison of the bioavailability of various dosage forms of ibuprofen in animals and humans.

ACKNOWLEDGEMENTS

The authors would like to acknowledge F.W. Crow, W.K. Duholke and R.H. Robins for their assistance.

REFERENCES

1 S.S. Adams, E.E. Cliffe, B. Lessel and J.S. Nicholson, J. Pharm. Sci., 56 (1967) 1686.
2 S.S. Adams, R.G. Bough, E.E. Cliffe, B. Lessel and R.F.N. Mills, Toxicol. Appl. Pharmacol., 15 (1969) 310.
3 R.F.N. Mills, S.S. Adams, E.E. Cliffe, W. Dickinson and J.S. Nicholson, Xenobiotica, 3 (1973) 589.
4 C.J.W. Brooks and M.T. Gilbert, J. Chromatogr., 99 (1974) 541.
5 A.J. Hutt and J. Caldwell, J. Pharm. Pharmacol., 35 (1983) 693.
6 J.A. Stead, M. Freeman, E.G. John, G.T. Ward and B. Whiting, Int. J. Pharm., 14 (1983) 59.
7 G.F. Lockwood, K.S. Albert, W.R. Gillespie, G.G. Bole, T.M. Harkcom, G.J. Szpunar and J.G. Wagner, Clin. Pharmacol. Ther., 34 (1983) 97.
8 J.G. Wagner, K.S. Albert, G.J. Szpunar and G.F. Lockwood, J. Pharmacokinet. Biopharm., 12 (1984) 381.
9 C.T. Gregg, Eur. J. Clin. Pharmacol., 7 (1974) 315.
10 T.A. Baillie, Pharmacol. Rev., 33 (1981) 81.
11 W.A. Garland and M.L. Powell, J. Chromatogr. Sci., 19 (1981) 392.
12 M. Eichelbaum, G.E. von Unruh and A. Somogyi, Clin. Pharmacokinet., 7 (1982) 490.
13 J. Vink, Mass Spectrom. Rev., 1 (1982) 349.
14 N.J. Haskins, Biomed. Mass Spectrom., 9 (1982) 269.

15 M.I. Blake, H.L. Crespi and J.J. Katz, J. Pharm. Sci., 64 (1975) 367.
16 S.D. Nelson, L.R. Pohl and W.F. Trager, J. Med. Chem., 18 (1975) 1062.
17 C.F. Gelijkens, A. Van Peer, H. Lenoir, A. Knaeps, R. Woestenborghs and J. Heykants, Biomed. Mass Spectrom., 12 (1985) 38.
18 V.J. Capponi, G.W. Halstead and D.L. Theis, J. Labelled Compd. Radiopharm., 23 (1986) 187.
19 G.L. Kearns and J.T. Wilson, J. Chromatogr., 226 (1981) 183.
20 B.G. Snider, L.J. Beaubien, D.J. Sears and P.D. Rahn, J. Pharm. Sci., 70 (1981) 1347.
21 J.L. Shimek, N.G.S. Rao and S.K. Wahba Khalil, J. Pharm. Sci., 70 (1981) 514.
22 K.S. Albert, A. Raabe, M. Garry, E.J. Antal and W.R. Gillespie, J. Pharm. Sci., 73 (1984) 1487.
23 E.J. Lee, K.M. Williams, G.G. Graham, R.O. Day and G.D. Champion, J. Pharm. Sci., 73 (1984) 1542.
24 L. Heikkinen, Acta Pharm. Fenn., 92 (1983) 275.
25 L. Heikkinen, Acta Pharm. Fenn., 93 (1984) 97.
26 D.G. Kaiser and G.J. VanGiessen, J. Pharm. Sci., 63 (1974) 219.
27 J.B. Whitlam and J.H. Vine, J. Chromatogr., 181 (1980) 463.
28 D.J. Hoffman, J. Pharm. Sci., 66 (1977) 749.
29 L. Heikkinen, J. Chromatogr., 307 (1984) 206.
30 PREPI Instrument Manual and Protocols, DuPont, Wilmington, DE, 1981.
31 D.J. Hoffman and W.R. Porter, J. Chromatogr., 276 (1983) 301.
32 D.L. Theis, G.W. Halstead, V.J. Capponi, B.L. Roach and R.H. Robins, J. Chromatogr., 375 (1986) 299.
33 J.G. Wagner, Fundamentals of Clinical Pharmacokinetics, Drug Intelligence Publications, Hamilton, IL, 1975, p. 346.
34 R.L. Wolen and C.M. Gruber, Jr., in K.S. Albert (Editor), Drug Absorption and Disposition: Statistical Considerations, American Pharmaceutical Association, Washington, DC, 1980, p. 69.