ducible, (*b*) the pH can be controlled, (*c*) it is applicable to compounds unstable in solution, (*d*) samples need not be pure, and (*e*) quantitative analytical methods need not be considered since any compound can be detected by a UV or refractive index detector.

However, an important unsolved problem remains. What system would produce the universal lipophilic index such as log *P*? A study is now in progress to develop a normalized method for correcting the differences in log *k'* determined with different columns from different sources, solvent compositions, pH's, and temperatures and to establish a general method for all kinds of chemical compounds.

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Rapid GLC Determination of Ibuprofen in Serum

D. J. HOFFMAN

Abstract □ A rapid procedure for the determination of ibuprofen in human serum was developed using a single extraction with carbon tetrachloride after deproteinization with perchloric acid. The internal standard, 3-methyl-3-phenylbutyric acid, was added directly to the serum. Gas chromatograms were free of interfering peaks. Calibration curves (0–40 μg/ml) were linear with a sensitivity of 0.5 μg of ibuprofen/ml of serum. Relative standard deviations ranged from 1.1 to 25%.

Keyphrases □ Ibuprofen—GLC analysis, human serum □ GLC—analysis, ibuprofen in human serum □ Anti-inflammatory agents—ibuprofen, GLC analysis in human serum

Ibuprofen is a 2-phenylalkylcarboxylic acid derivative possessing potent anti-inflammatory, antipyretic, and analgesic properties (1–3). Recently, a GLC method for determining ibuprofen in human plasma was reported (4). The assay involved an extraction into benzene, followed by evaporation of the extract to dryness and subsequent derivatization of ibuprofen to the methyl ester. A rapid GLC method for the anticonvulsant valproic acid in serum utilized the underivatized acid and a 5% FFAP column (5). By using similar conditions, a GLC method was developed for the assay of serum ibuprofen levels. This method is rapid and precise and does not require derivative formation.

EXPERIMENTAL

Reagents—Reagent grade perchloric acid[1] and carbon tetrachloride[2] were used without further purification. Ibuprofen and 3-methyl-3-phenylbutyric acid were used as supplied[3].

Instrumentation—The GLC analyses were performed on a gas chromatograph[4] equipped with an automatic sampler[5], computing integrator[6], and flame-ionization detector. The chromatograph was fitted with a 1.83-m (6-ft) U-shaped glass column (2 mm i.d.) packed with 5% FFAP[7] on Gas Chrom W (HP), 80–100 mesh[8]. The column was conditioned before use at 255° for 15 hr with a carrier gas (nitrogen[9]) at a flow rate of 30 ml/min.

The chromatographic conditions were: carrier gas, 35 ml/min; air, 260 ml/min; hydrogen, 37 ml/min; injection port and detector temperatures, 250°; and oven temperature, 220°. The attenuation of the computing integrator was ×8, and the electrometer[10] range was 10^2. Under these conditions, the internal standard and ibuprofen had retention times of 2.9 and 4.3 min, respectively (Fig. 1).

Assay—*Preparation of Standards*—A 2-mg/ml stock solution of ibuprofen was prepared by dissolving 100 mg of ibuprofen in a 50-ml volumetric flask with 2 ml of 0.5 *N* NaOH and adjusting to 50 ml with distilled water. A working serum ibuprofen standard at 40 μg/ml was prepared by adding 1.0 ml of the stock solution to a 50-ml volumetric flask and adjusting to volume with commercial human serum[11]. Other serum standards were prepared from serial dilutions (with serum) of the 40-μg/ml serum standard.

The internal standard stock solution (2 mg/ml) was prepared by dissolving 100 mg of 3-methyl-3-phenylbutyric acid in a 50-ml volumetric flask with 2 ml of 0.5 *N* NaOH and adjusting to 50 ml with distilled water. A working internal standard solution (100 μg/ml) was prepared by diluting 5 ml of the internal standard stock solution to 100 ml with distilled water.

Extraction Procedure—To a 15-ml conical glass centrifuge tube were added 0.5 ml of the internal standard solution, 1.0 ml of serum, and 0.5 ml of 10% perchloric acid. After vigorous mixing[12], 0.4 ml of carbon tetrachloride was added, followed again by vigorous mixing for 15 sec. The

[1] Mallinckrodt reagent grade, 60%.
[2] Mallinckrodt (low sulfur) reagent grade.
[3] Supplied by A. Geisler, Abbott Laboratories.
[4] Hewlett-Packard model 7610A.
[5] Hewlett-Packard model 7671A.
[6] Hewlett-Packard model 3380A.
[7] Varian Aerograph.
[8] Applied Science Laboratories.
[9] Linde Division, Union Carbide Corp.
[10] Hewlett-Packard model 7650A.
[11] K-N Enterprises.
[12] Maxi Mix model M-16715, Thermolyne, Sybron Corp.

Table I—Precision and Sensitivity of Ibuprofen Assay in Serum

Ibuprofen Concentration, μg/ml	Peak Area Ratio[a] (Ibuprofen/Internal Standard)	*RSD*, %
0	0	—
0.5	0.016 ± 0.004	25.0
2.5	0.068 ± 0.005	7.4
5.0	0.135 ± 0.005	3.7
10.0	0.273 ± 0.003	1.1
20.0	0.563 ± 0.015	2.7
30.0	0.858 ± 0.031	3.6
40.0	1.18 ± 0.024	2.0

[a] Mean ± *SD* of four determinations at each concentration.

sample was centrifuged for 10 min at 2500 rpm, and the clear upper aqueous layer was discarded by aspiration. The lower carbon tetrachloride layer was transferred to a microvial[13] using a disposable pipet[14]. After the microvial was capped, a 5-μl aliquot was injected into the GLC using an automatic sampler.

Calculations—A calibration curve was constructed by plotting peak area ratios (ibuprofen/internal standard) against the serum ibuprofen concentration. All calibration curves were linear and passed through the origin. Unknowns were automatically calculated by a computing integrator, programmed with the slope of a least-squares calibration curve. A least-squares regression line from the values in Table I yielded parameters of 0.0293, −0.0085, and 0.999 for the slope, *y*-intercept, and correlation coefficient, respectively.

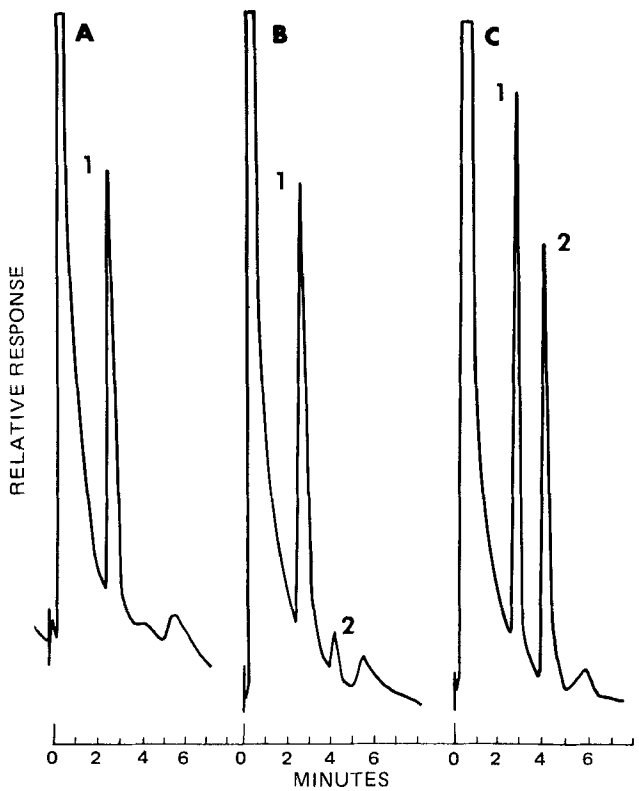

Figure 1—*GLC tracings of extracted serum samples. Key: A, serum blank with internal standard; B, serum containing ibuprofen at 5 μg/ml; C, serum containing ibuprofen at 40 μg/ml; 1, internal standard; and 2, ibuprofen.*

[13] Hewlett-Packard 100-μl vials.
[14] Pasteur transfer pipets, Curtin Matheson Scientific.

Table II—Accuracy of Ibuprofen GLC Assay

Ibuprofen Concentration, μg/ml		
Actual[a]	Calculated	Percent Difference
0	0	0
20.0	19.7	−1.5
5.0	4.6	−8.0
0.5	0.6	+20.0
10.0	10.2	+2.0
2.5	2.8	+12.0
30.0	31.4	+4.7
40.0	39.1	−2.2

[a] Serum spiked with ibuprofen at the indicated concentrations.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of an extract from 1 ml of serum spiked with ibuprofen. Peak shapes were good with only a small amount of tailing. Ibuprofen and the internal standard were well resolved from coextractives.

The precision of the assay is shown in Table I. Relative standard deviations ranged from 1.1 to 25.0%. Assay sensitivity was 0.5 μg/ml for a 1-ml sample volume, the lowest concentration of ibuprofen in serum that was accurately detected and integrated by the instrumentation used. The accuracy of this assay was estimated by analyzing eight spiked serum unknowns under blind conditions. These results (Table II) show excellent accuracy at 5 μg/ml and above. Satisfactory accuracy (12–20% error) was achieved at low ibuprofen concentrations (2.5 and 0.5 μg/ml).

Ibuprofen in serum can be assayed by this method in less than one-half the time required by a derivatization method (4). By using those recovery data as an estimate of assay precision, a relative standard deviation of 7.0% was calculated for standards between 2 and 80 μg/ml. The average relative standard deviation of this method is 3.4% (excluding the 0.5-μg/ml concentration). Both methods have a sensitivity of 0.5 μg of ibuprofen/ml.

This assay provides the precision and accuracy needed for pharmacokinetic and bioavailability studies in humans where serum ibuprofen concentrations range from 0.5 to 30 μg/ml.

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