very close to −1 as predicted by Eq. 10. This finding is noteworthy since the data used for these analyses cover a wide variety of organic nonelectrolyte liquids and span over five orders of magnitude. Furthermore, the aqueous solubility and partition coefficient data were taken from the work of many independent investigators who used various experimental techniques and compounds of various degrees of purity. Moreover, the aqueous solubility data cover a temperature range of 20–40°.

Along with the variation in the solubility and partition coefficients, a slight deviation in log *PC* may be attributed to the nonideal behavior of liquid solutes. The assumption of dilute solutions and activity coefficients being unity may not be completely valid. In fact, the data include several compounds with aqueous solubilities greater than 1.0 *M*. Mutual solubility and self-association of low molecular weight solutes may contribute to the deviation observed.

If the aqueous solubilities and octanol–water partition coefficients are expressed in mole fraction units and if it is assumed that the solutes are completely miscible with octanol, then log S_o in Eq. 10 is expected to be zero. Examination of Eq. 5, which relates the mole fractional aqueous solubilities and partition coefficients, reveals that the intercept is indeed close to zero.

The significance of the intercept can be further explained as follows. If it is assumed that the solutes are miscible with octanol and that the average density of liquid solutes is ~1 g/ml, then the solubility of liquid solutes in octanol can be approximated with their densities. Upon substitution of the average density value in Eq. 10, an intercept value of 3.0 is expected. The intercept value obtained (2.54) from Eq. 4 is in reasonable agreement with the value expected from Eq. 10. The assumption of miscibility and the use of average density were verified in great detail by Roseman[1].

These results, although in qualitative agreement with those of Hansch *et al.* (9), differ significantly in the coefficient of log *PC*. The relationship between molal aqueous solubility and partition coefficients of several liquid nonelectrolytes was reported in their work (9):

$$\log S = -1.339 \log PC + 0.978 \qquad \text{(Eq. 11)}$$
$$n = 156 \quad r = 0.935 \quad s = 0.472$$

Almost all of the partition coefficients used in Eq. 11 were calculated from the group contribution approach. Only 22 of 156 partition coefficients reported were experimentally determined. The coefficient of log *PC* in Eq. 11 is significantly different from −1, the value expected from Eq. 10. The deviation may be attributed to a systematic error in the calculated partition coefficients for hydrocarbons[2]. The significance of the results reported in this study can be illustrated by the following:

1. Only experimentally determined partition coefficients and solubility values were used.
2. A greater number of experimentally measured partition coefficients are available now than were available in 1968.
3. More solubility values were used.
4. Since no calculated values were used, systematic errors in values for a series of compounds are not likely.

Furthermore, these results are supported by the equality of partition coefficients and solubility ratios observed by Roseman[1] for various drugs in several oil–water systems. This relationship is important in understanding the solubility and partitioning phenomena of liquids and will provide a basis for crystalline solids and gaseous compounds.

These equations can be helpful in assessing the reliability of reported values for aqueous solubility estimation. These equations were employed to verify the solubility or partition coefficients of a few compounds that showed a great difference between the calculated and observed values, and this study will be the subject of a separate report.

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High-Performance Liquid Chromatographic Assay for Fenoprofen in Human Plasma

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Received April 8, 1980, from the *Analytical Development Department, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.* Accepted for publication October 15, 1980.

Abstract □ A high-performance liquid chromatographic method is described for the quantitation of fenoprofen, *dl*-2-(3-phenoxyphenyl)-propionic acid, in human plasma. The proteins in plasma were precipitated by the addition of hydrochloric acid. Fenoprofen and the internal standard, *dl*-2-(4-phenoxyphenyl)valeric acid, were extracted into butyl chloride and then back-extracted into sodium hydroxide. The aqueous solution was injected onto a reversed-phase alkylphenyl column, and the compounds were eluted using a mobile phase of acetonitrile–water–acetic acid (50:50:2 v/v/v). At a flow rate of 1 ml/min, the retention times of fenoprofen and the internal standard were 8 and 12 min, respectively. The absorbance was monitored at 272 nm. The method requires 1.0 ml of plasma and is sensitive to 0.5 μg/ml. This procedure has been used for routine assay of multiple samples from bioavailability and compliance studies.

Keyphrases □ Fenoprofen—high-performance liquid chromatographic analysis, human plasma □ High-performance liquid chromatography—assay, fenoprofen, human plasma □ Anti-inflammatory agents—fenoprofen, high-performance liquid chromatographic analysis, human plasma

The pharmacological and toxicological properties of fenoprofen [*dl*-2-(3-phenoxyphenyl)propionic acid, I] have been studied extensively and were reviewed recently (1–3). Compound I was shown to be absorbed readily after oral administration. It is extensively metabolized to I glucuronide and to *dl*-2-[3-(4-hydroxyphenoxy)phenyl]propionic acid (II) glucuronide, both of which are excreted rapidly in the urine (4, 5).

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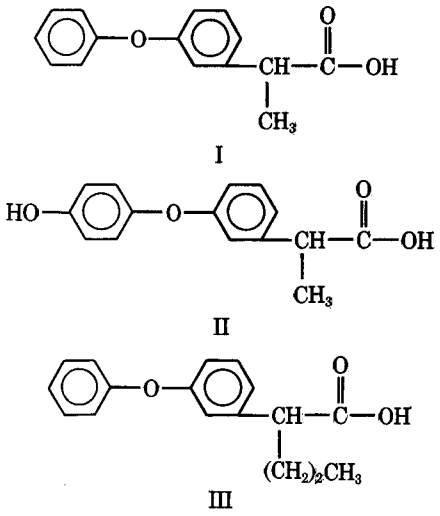

I

II

III

As I and other anti-inflammatory agents of the arylalkanoic acid family were discovered, methodology for assaying these compounds in plasma was developed (6–12). Most of the methods employ GLC of the derivatized drug. Although these methods are reported to be accurate and reproducible, they require extensive sample cleanup prior to derivatization of the extracted compounds. Liquid chromatographic methods were reported recently for the analysis of naproxen in plasma and urine (13) and of ketoprofen in biological fluids (14, 15).

This paper reports a simple, sensitive, and reproducible high-performance liquid chromatographic (HPLC) plasma assay of I that was used in bioavailability studies.

EXPERIMENTAL

Chemicals and Reagents—Fenoprofen sodium [sodium *dl*-2-(3-phenoxyphenyl)propionate dihydrate], *dl*-2-[3-(4-hydroxyphenoxy)-phenyl]propionic acid (II), and *dl*-2-(4-phenoxyphenyl)valeric acid (III) as the internal standard were synthesized[1]. All organic solvents were distilled in glass[2]. Sodium hydroxide, hydrochloric acid, and acetic acid were analytical grade. Plasma was separated from fresh heparinized blood of normal human volunteers. The plasma was pooled and stored at 4° in glass containers and used within 1 month.

Instrumentation—The chromatograph consisted of a pump[3], an autosample injector[4] modified with a pneumatic-actuated injection valve fitted with a 50-μl sample loop[5], a microparticulate reversed-phase column[6] (30 cm × 3.9 mm), a variable-wavelength detector[7], and a potentiometric recorder[8].

The HPLC system was operated at ambient temperature with acetonitrile–distilled water–acetic acid (50:50:2 v/v/v) as the mobile phase at a flow rate of 1.0 ml/min. Prior to use, the mobile phase was degassed by refluxing for 10 min. The detector was set at 272 nm and a sensitivity of 0.02 aufs.

Procedure—A calibration curve was prepared by pipetting 1.0 ml of fresh human plasma into five individual centrifuge tubes followed by the addition of 0, 50, 150, 300, and 600 μl of an aqueous solution of 100 μg of I/ml. The tubes represented 0, 5, 15, 30, and 60 μg of I/ml of plasma, respectively. To each tube was added 0.5 ml of III (60 μg/ml) in 0.01 *N* NaOH.

The standards were acidified with 1 ml of 1.0 *N* HCl and vortexed, and I and III were extracted into 10 ml of butyl chloride by use of a rotating mixer[9] set at 20 rpm for 10 min. The rotating speed should be low enough so that emulsification does not occur. The organic phase was separated by centrifugation at 2000 rpm for 5 min and then transferred to another centrifuge tube containing 1.0 ml of 0.1 *N* NaOH. The phases were mixed at 40 rpm for 5 min and separated by centrifugation, and the butyl chloride was discarded. The aqueous phase (0.7 ml) was placed into an autoinjector sample vial[10], and 50 μl of the solution was injected onto the column. One milliliter of the unknown plasma samples was assayed for I in a similar manner.

The calibration curve was constructed by plotting the peak height

[1] Eli Lilly and Co., Indianapolis, Ind.
[2] Burdick & Jackson Laboratories, Muskegon, Mich.
[3] Model 6000A, Waters Associates, Milford, Mass.
[4] Model 725, Micromeritics Instrument Corp., Norcross, Ga.
[5] Model 70-10A, Rheodyne, Berkeley, Calif.
[6] μBondapak alkylphenyl, Waters Associates, Milford, Mass.
[7] Model 450, Waters Associates, Milford, Mass.
[8] Model 355, Linear Instruments Corp., Irvine, Calif.
[9] Roto-Torque, Cole Parmer, Chicago, Ill.

Table I—Efficiency of the Extraction of Fenoprofen from Plasma

Fenoprofen Added, μg/ml	Peak Heights, mm (*SD*) Absolute Standards[a]	Extracted Standards[b]	Extraction Efficiency, %
5	9.5 (0.0)	8.5 (0.3)[c]	89.5
15	28.8 (0.4)	27.5 (0.0)	95.5
30	57.8 (0.4)	55.6 (0.4)	96.2
60	119.0 (2.1)	113.3 (0.5)	95.2

[a] Two replicates. [b] Four replicates. [c] Corrected to reflect 75% recovery of butyl chloride.

Table II—Reproducibility of Standard Curves of Fenoprofen in Human Plasma on 3 Successive Days[a]

Fenoprofen Added, μg/ml	Peak Height Ratio (*SD*) of Fenoprofen to Internal Standard	Fenoprofen (*SD*) Found, μg/ml
5	0.401 (0.024)	5.36 (0.48)
15	1.063 (0.044)	14.70 (0.11)
30	2.131 (0.064)	29.78 (0.85)
60	4.286 (0.110)	60.15 (0.39)

[a] Linear regression equation: $y = 0.07132\ (0.00224)x + 0.02779\ (0.04308)$; $r = 0.99970\ (0.00028)$.

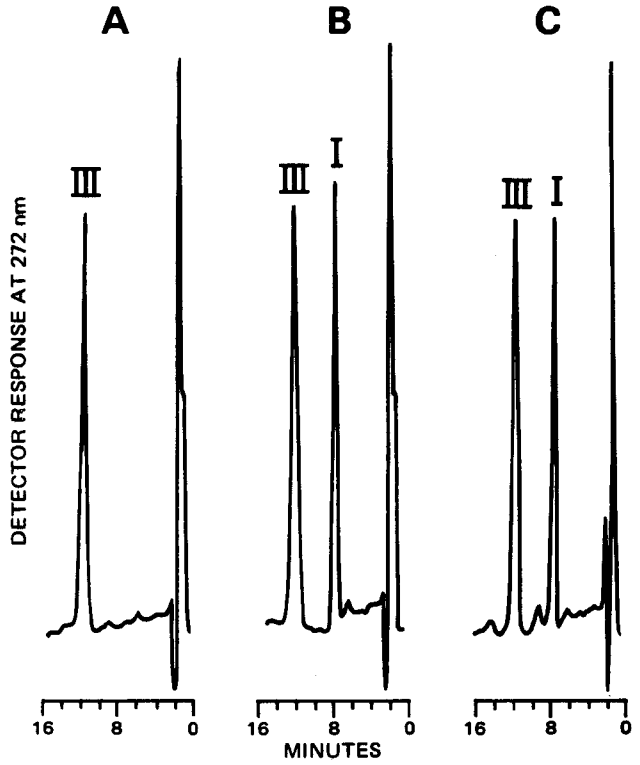

Figure 1—*Chromatograms of the extracts of a plasma sample at time zero spiked with 30 μg of III/ml (A), human plasma spiked with 15 μg of fenoprofen (I) and 30 μg of III/ml (B), and a plasma sample taken 0.5 hr after administration of 300 mg of I and spiked with 30 μg of III/ml (C).*

[10] Catalog models 08035-A and 41221013, Brockway Glass Co., Indianapolis, Ind.

Table III—Precision of the HPLC Assay for Fenoprofen in Human Plasma

Day	*In Vivo* Pools of Fenoprofen (n = 5) 1	2	3	4
Day 1				
$\bar{X}$, μg/ml	5.66	4.28	11.90	22.82
RSD, %	2.30	1.87	1.34	1.10
Day 2				
$\bar{X}$, μg/ml	4.24	3.28	10.96	22.38
RSD, %	3.54	3.96	2.65	1.16
Day 3				
$\bar{X}$, μg/ml	5.14	3.80	11.80	22.32
RSD, %	5.06	4.47	3.14	1.70
Overall precision				
Between-day *RSD*, %	14.2	13.1	4.3	1.06
Within-day *RSD*, %	3.8	3.5	2.5	1.33
Total *RSD*, %	14.7	13.6	5.0	1.70

ratios of I to III *versus* the concentration of I per milliliter of plasma. The concentration of I in the unknown samples was obtained by determining the peak height ratios of the samples and reading from the calibration curve.

Bioavailability Study (9)—The utility of this method for determining the concentration of I in plasma was demonstrated by assaying plasma samples from three subjects administered a single 300-mg oral dose of I.

RESULTS AND DISCUSSION

A UV spectrum of a solution of I (1 mg/ml) in the mobile phase showed the absorption maximum to be at 272 nm with shoulders at 265 and 279 nm.

Table I gives the extraction efficiency of I from plasma. The UV response (as noted by peak height) of absolute standards of 5, 15, 30, and 60 μg of I/ml were compared to the same concentrations of extracted standards from plasma. These data showed ~95% recovery of I from plasma.

The day-to-day variability of the calibration curves was determined by preparing and assaying spiked standards of 5, 15, 30, and 60 μg of I/ml of plasma on 3 successive days (Table II). The calibration curves were linear and reproducible over the concentration range of 5–60 μg/ml; the mean (*SD*) linear regression equation was $y = 0.07132\ (0.00224)x + 0.02779\ (0.04308)$, and the mean (*SD*) correlation coefficient was 0.99970 (0.00028).

The within-day and between-day variabilities of the assay were determined by utilizing plasma samples obtained during a bioavailability study. These samples were mixed into four pools on the basis of their drug concentrations. Each *in vitro* pool then was assayed in quintuplicate on 3 different days (Table III). The within-day variability was <4% *RSD* for the four pools, which ranged from 4 to 23 μg of I/ml; however, the between-day variability of the 4.28- and 5.66-μg/ml pools was larger, 13.1 and 14.2 *RSD*, respectively.

Quantitation of I in plasma samples that were assayed immediately and again after several months of storage at 4° showed that there was no significant drug loss.

Chromatograms of extracts from a plasma sample containing III at 0 hr, a plasma sample spiked with I and III, and a plasma sample taken 0.5 hr after administration of a single 300-mg dose of I plus added III are shown in Fig. 1. Compounds I and III (R_t of 8 and 12 min, respectively) were well separated from endogenous interferences.

The elution time of the major metabolite, II (R_t ~6 min), was sufficiently different from I so as not to interfere in the assay. Plasma samples from subjects receiving 300 mg of I contained only traces of unconjugated II.

Injection of 50 μl of 0.1 *N* NaOH containing I had no effect on the elution time or the peak height of I when compared with a 50-μl injection of I in the mobile phase. The amount of acetic acid (0.38 *M*) in the mobile phase was more than sufficient to neutralize rapidly the sodium hydroxide (0.1 *N*) in the injection solvent. The sodium hydroxide solution was used in the assay of hundreds of samples with no deleterious effect on column resolution.

The plasma concentration–time profile of I from three subjects in the bioavailability study is shown in Fig. 2. The among-subject variability

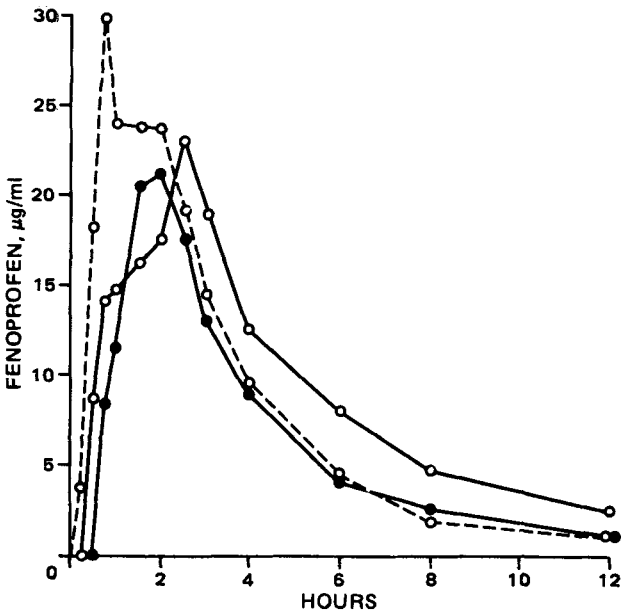

Figure 2—*Plasma time–concentration profiles of fenoprofen after administration of a single oral dose of 300 mg to three subjects. Key:* o—, *Subject RB;* ●, *Subject ML; and* o---, *Subject DP.*

was evident by examination of the differences in the concentration maxima and time to reach the peak blood level concentration.

Ibuprofen and benoxaprofen had identical elution times as I under the assay conditions described. Therefore, this assay cannot be used to distinguish between these drugs in clinical samples. Naproxen, with an elution time of 7.8 min, can be distinguished from I.

The described assay was used routinely during the past year to study the bioavailability of fenoprofen from dosage forms containing 50–300 mg of I. The method is simple, sensitive, and reproducible.

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ACKNOWLEDGMENTS

The authors thank Dr. L. D. Bechtol of the Lilly Clinic for the plasma samples of fenoprofen from the bioavailability study and Mr. P. R. Page for analysis of these samples.