High-Performance Liquid Chromatographic Assay for Suprofen, a Potent New Analgesic, in Plasma

K. B. ALTON [x] **and J. E. PATRICK**

Received August 19, 1977, from *Ortho Pharmaceutical Corporation, Raritan, NJ 08869.* Accepted for publication November 3, 1977.

Abstract □ A simple, rapid, high-performance liquid chromatographic (HPLC) determination of therapeutic levels of suprofen, a potent new analgesic, in human plasma is described. After a simple extraction, the compound is analyzed by HPLC using a reversed-phase column and a UV detector. Quantitation is accomplished using an external standard; peak areas are determined by computer. An average recovery of 80.0 ± 8.5% *SD* of the drug was obtained over the 0.2–20.0-μg/ml range. Maximum sensitivity is approximately 0.1 μg/ml. No interference is encountered from any known metabolite of suprofen.

Keyphrases □ Suprofen—high-performance liquid chromatographic analysis in plasma □ High-performance liquid chromatography—analysis, suprofen in plasma □ Analgesics—suprofen, high-performance liquid chromatographic analysis in plasma

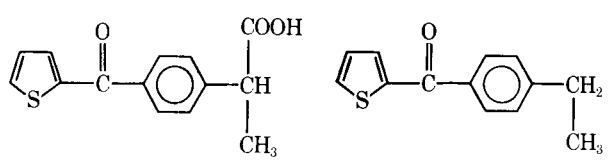
Suprofen, 2-[4-(2-thienoyl)phenyl]propionic acid (I), is a potent inhibitor of prostaglandin synthetase (1) and is currently being studied clinically as a new analgesic agent.

A rapid, precise method for the quantitation of suprofen in plasma was required to determine the pharmacokinetic parameters relating to its absorption, distribution, and elimination in animals and humans.

This paper describes a simple high-performance liquid chromatographic (HPLC) method for the rapid estimation of suprofen in human plasma. The maximum sensitivity is approximately 0.1 μg/ml.

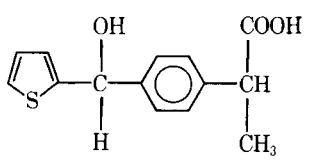
EXPERIMENTAL

Apparatus—The liquid chromatograph[1], equipped with a differential UV detector[2] (254 nm), was operated at ambient temperature. Chromatograms were traced on a strip-chart recorder[3], and peak area integration was performed by computer[4]. Separations were accomplished on a 30.5 × 0.64-cm microparticulate reversed-phase column[5]. A 25-μl syringe[6] was used for the introduction of samples onto the column through a septumless injector[7].

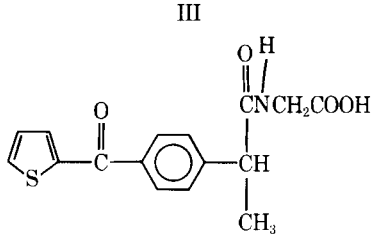


I

II



III



IV

The radioactive content of various samples was determined by liquid scintillation spectrometry. Aliquots of the samples were dissolved in scintillation fluid[8] and assayed for radioactivity in a liquid scintillation spectrometer[9]. Internal standardization was used to determine counting efficiency.

Reagents and Solvents—^{3}H-Suprofen (specific activity of ~65 μCi/mg), unlabeled suprofen (I), and three possible suprofen metabolites, 1-ethyl-4-(2-thienoyl)benzene (II), 2-[4-(α-hydroxy-2-thenyl)phenyl]-propionic acid (III), and 2-[4-(2-thienoyl)phenyl]propionic acid glycine conjugate (IV), were used[10]. All chemicals and reagents, except acetonitrile[11], were reagent grade. Acetonitrile, tetrahydrofuran, and methanol were redistilled before use.

Chromatographic Conditions—The mobile phase was 0.01% (v/v) aqueous acetic acid–acetonitrile (70:30 v/v); the flow was adjusted to 2.5 ml/min, generating a pressure of ~2500 psi. The solvent mixture was prepared daily and degassed under slight vacuum before use.

Extraction Procedure—Aliquots (1.0 ml) of plasma were transferred to 15-ml screw-capped vials (16 × 125 mm), acidified with 3.0 ml of 0.1 *N* HCl, and extracted with 8.0 ml of ether. The phases were separated by centrifugation for 2–3 min at ~1000 rpm, and the organic layer was removed with a Pasteur pipet and then transferred to another 15-ml screw-capped vial.

A second extraction was performed similarly.

The ether layers were pooled for each sample and then taken to dryness under a nitrogen stream in a water bath (40°). An aliquot (2.0 ml) of 1 *N* NaOH was added to the ether residue and mixed with a vortex mixer. The sodium hydroxide solution was then extracted twice with 2.0 ml of ether, and the organic layers were discarded after aspiration. The remaining aqueous fraction was acidified with 3.0 ml of 2 *N* HCl (saturated with sodium chloride), and this mixture again was extracted with 8.0 ml of ether.

The pooled ether extracts were dried over anhydrous sodium sulfate and taken to dryness under nitrogen in a water bath (35°). The residues were transferred with ether to 2.5-ml conical centrifuge tubes and concentrated as already described. The final residue was dissolved in tetrahydrofuran (0.1–0.5 ml) for injection onto the HPLC column.

Extraction Efficiency Studies—The recoveries of both ^{3}H-suprofen and unlabeled suprofen from control plasma samples, prepared to contain known drug concentrations, were investigated in two studies.

In the first study, 0.2 μg of ^{3}H-suprofen (~32,200 dpm) in 10 μl of methanol was added to each of 16 1.0-ml aliquots of control plasma from untreated volunteers. These samples were divided into four equal groups, and total suprofen concentrations of 0.2, 0.5, 1.0, and 5.0 μg/ml were achieved by the subsequent addition of 0, 0.3, 0.8, and 4.8 μg of unlabeled drug from methanolic stock solutions, respectively. The added drug was mixed thoroughly, allowed to equilibrate for 30 min at room temperature, and then extracted as described. Total radioactivity in the final residues (100 μl) was evaluated by assaying aliquots (20 μl) of the tetrahydrofuran solutions for radioactive content.

In a second study, aliquots of methanolic suprofen solutions (described later) were added to four groups, each consisting of at least 10 control

[1] Model ALC 202, Waters Associates, Milford, Mass.
[2] Model 440, Waters Associates, Milford, Mass.
[3] Sevo/riter II, Texas Instruments, Houston, Tex.
[4] Model 3352B, Hewlett-Packard, Avondale, Pa.
[5] μBondapak C_{18}, Waters Associates, Milford, Mass.
[6] Pressure-Lok B-110, Precision Sampling Corp., Baton Rouge, La.
[7] Model U6K, Waters Associates, Milford, Mass.
[8] Prepared from Liquifluor, New England Nuclear, Boston, Mass.
[9] Model SL-30, Teledyne Intertechnique, Westwood, N.J.
[10] Suprofen and its possible metabolite standards were synthesized and supplied by Janssen Pharmaceutica, Beerse, Belgium.
[11] Ninety-nine mole percent pure, Fisher Scientific Co., Fair Lawn, N.J.

0022-3549/78/0700/0985$01.00/0

Table I—HPLC Retention Times and Relative Separation (Resolution, *R*) of Suprofen and Three Possible Metabolites

Compound	t_r, min	*R*
I	7.7	—
II	14.8	22.4
III	4.5	16.5
IV	2.9	25

plasma samples (1.0 ml), to achieve final concentrations of 0.2, 0.5, 5.0, and 20.0 μg/ml of plasma. Samples were allowed to equilibrate and were extracted as described.

Since the prepared plasma drug concentrations ranged over two orders of magnitude, varying volumes of the final tetrahydrofuran dilutions were injected for HPLC analysis. When the drug concentration was 0.2 μg/ml, one-fifth (20 μl/100 μl) of the total extract volume was analyzed; at a concentration of 20 μg/ml, only 5 μl from a 500-μl dilution was injected. In general, samples were analyzed in duplicate. Quantitation of recovered suprofen, after separation by HPLC, was accomplished by the use of an external standard method contained in the software of the computer.

Calibration and Standard Preparation—Approximately 0.1 mg of ^{3}H-suprofen was dissolved in 5.0 ml of methanol. Analysis of this solution by HPLC and liquid scintillation spectrometry indicated that the solution contained ~32,200 dpm/0.2 μg/10 μl of solution. Unlabeled suprofen (25 mg) was dissolved in about 10 ml of methanol and diluted to volume in a 25.0-ml volumetric flask to achieve a concentration of 1.0 μg/μl. An aliquot (2.5 ml) of this solution was transferred to another 25.0-ml volumetric flask and then diluted to volume with methanol to obtain a concentration of 0.1 μg/μl. A third stock solution (0.01 μg/μl) was prepared by diluting the last one in the same manner. Three additional standard solutions (0.01, 0.1, and 1.0 μg/μl) were prepared similarly with tetrahydrofuran.

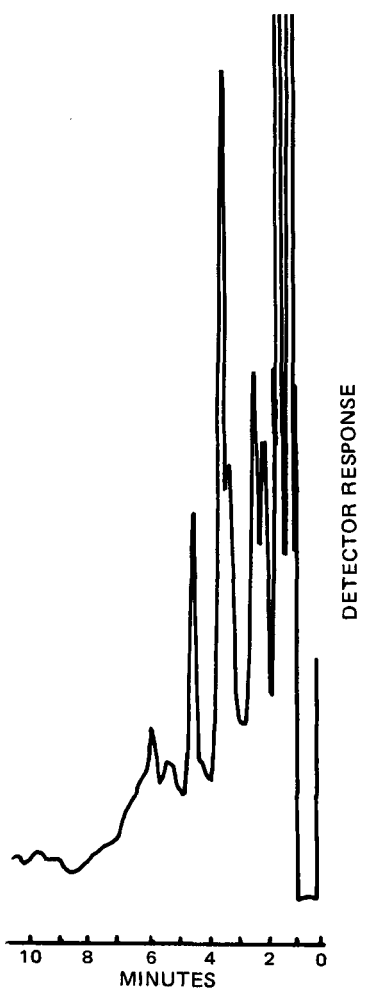


Figure 1—*Chromatogram of a residue from a control plasma sample extracted with ether.*

Table II—Precision and Recovery Data for Both ^{3}H-Suprofen and Unlabeled Suprofen Added to 1.0 ml of Drug-Free Plasma

Initial Concentration Added, μg/ml	*n*	Amount Found, μg/ml ± *SD*	Recovery, % ± *SD*
0.2[a]	4	0.175 ± 0.006	87.3 ± 3.23
0.5[a]	4	0.416 ± 0.007	83.1 ± 1.68
1.0[a]	4	0.727 ± 0.077	72.7 ± 10.6
5.0[a]	4	4.08 ± 0.187	81.6 ± 4.58
			$\bar{\bar{x}}$ = 81.2 ± 4.82
0.2[b]	18	0.158 ± 0.022	79.2 ± 11.0
0.5[b]	18	0.395 ± 0.04	79.0 ± 8.1
5.0[b]	18	4.03 ± 0.32	80.5 ± 6.4
20.0[b]	16	15.9 ± 1.7	80.0 ± 8.49
			$\bar{\bar{x}}$ = 80.0 ± 8.5

[a] Recovery of added ^{3}H-suprofen as determined by liquid scintillation spectrophotometry. [b] Recovery of added unlabeled suprofen as determined by HPLC.

Since the external standard method prepared from the software section of the computer assumes linearity between micrograms injected and peak area (microvolt seconds as reported by the integrating computer), this relationship was initially evaluated by repeated HPLC analyses of 0.2, 0.15, 0.05, 0.025, and 0.01 μg of suprofen in tetrahydrofuran (0.01 μg/μl). Thereafter, an average calibration response factor was established by the computer following triplicate injections of 0.15 μg of suprofen. As a final check, the accuracy of the computer-generated response factor was verified by analyzing known quantities of suprofen (0.05 and 0.2 μg).

Specificity—Assay specificity was determined by injecting a solution containing a mixture of suprofen and its potential metabolites, II–IV (1 μg/μl in methanol), onto the HPLC column.

RESULTS AND DISCUSSION

Ether extracts of control plasma were free of endogenous UV (254 nm) absorbing peaks that interfered with the assay (Fig. 1). Under the chro-

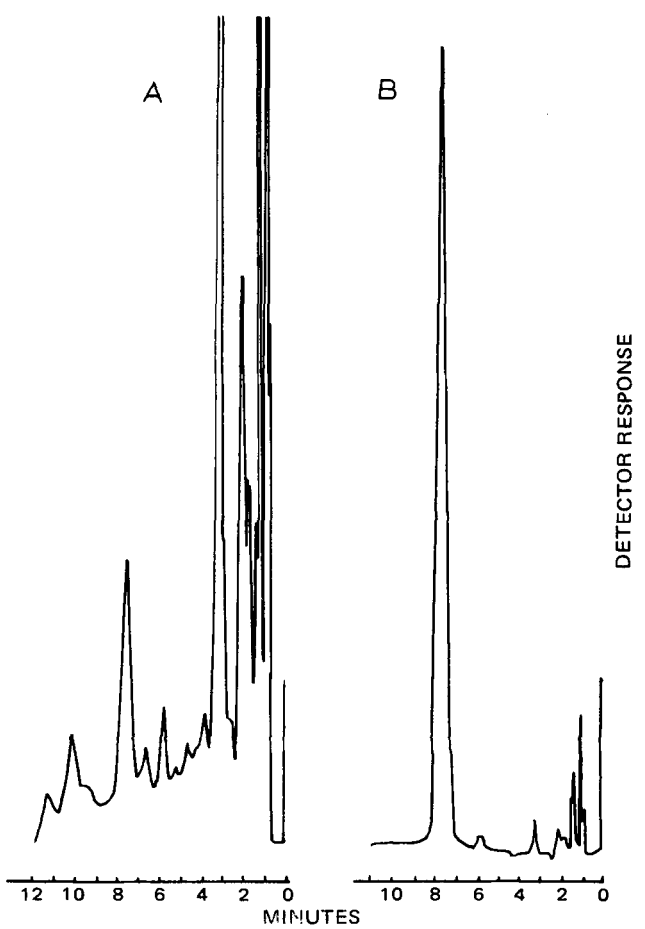


Figure 2—*Chromatograms of ether residues from plasma samples containing 0.2 μg of suprofen/ml (A) and 20 μg of suprofen/ml (B).*

matographic conditions described, baseline resolution between suprofen and the three potential metabolite reference standards was achieved. Retention times and the relative separation of these potential metabolites from suprofen are reported in Table I.

Linear regression analysis of the curve, described by plotting microvolt seconds *versus* micrograms injected, indicated a virtual linear fit of the data ($r^2 = 0.99992$).

The response factor, F_i, generated by the computer is described by $F_i = W_i/A_i$, where W_i is the amount injected and A_i is the area in microvolt seconds. It was shown to be linear over the concentration range evaluated (0.05–0.2 μg/injection). Over a 2-month period of analysis, this response factor demonstrated little change, with a coefficient of variation equal to 2.4% ($n = 23$).

The results from the extracted control plasma to which ^{3}H-suprofen had been added are shown in Table II. The average extraction efficiency based on total recovered radioactivity was 81.2 ± 4.8% *SD*. The average recovery of suprofen from plasma samples to which unlabeled suprofen had been added was 80.0 ± 8.5% *SD* (Table II). Representative chromatograms of extracts from plasma containing 0.2 μg/ml (20 μl from a 100-μl dilution with tetrahydrofuran) and 20.0 μg/ml (5 μl from a 500-μl dilution with tetrahydrofuran) are shown in Fig. 2. A multicomparison analysis of the percent recovery data shown in Table II was performed using the two-tailed Dunnett (2) and Scheffé (3) test. No statistically significant differences were found in extraction efficiencies among the four groups of plasma samples with added suprofen.

These data suggest that there is no concentration dependence in extraction efficiencies over the range of expected plasma drug[12] levels following oral administration of 200 mg of suprofen. The results of this study will be the subject of a separate report.

REFERENCES

(1) P. A. J. Janssen, *Arzneim.-Forsch.*, **25**, 1495 (1975).

(2) C. W. Dunnet, *Biometrics*, **20**, 482 (1964).

(3) H. A. Scheffé, "The Analysis of Variance," Wiley, New York, N.Y., 1959.

ACKNOWLEDGMENTS

The authors thank Dr. E. McGonigle for useful discussions. They are grateful to Dr. J. Heykants and Dr. J. Wynants, Janssen Pharmaceutica, for providing preliminary information concerning plasma levels following oral administration of 100 mg of suprofen to humans and for supplying samples of suprofen and potential metabolites. The authors also thank Dr. H. Weintraub and Dr. L. Abrams, Ortho Pharmaceutical Corp., for providing preliminary information concerning plasma levels of total radioactivity following oral administration of 200 mg of ^{3}H-suprofen (specific activity of ~0.25 μCi/mg) to humans.

[12] Preliminary studies, conducted by Janssen Pharmaceutica and Ortho Pharmaceutical Corp., indicated that peak plasma levels of suprofen, equal to approximately 10–20 μg/ml, would be achieved following the oral administration of 200 mg of suprofen to normal human subjects.

Anticonvulsant and Antiproteolytic Properties of 2,5-Disubstituted Oxadiazoles and Their Inhibition of Respiration in Rat Brain Homogenates

SUNIL K. CHAUDHARY *§, SUSHIL KUMAR *, SURENDRA S. PARMAR ‡x, and MAHIMA CHAUDHARY *§

Received September 12, 1977, from the **Jawahar Lal Nehru Laboratory of Molecular Biology, Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow 226003, India,* and the ‡*Department of Physiology, School of Medicine, University of North Dakota, Grand Forks, ND 58202.* Accepted for publication November 7, 1977. §Present address: National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Abstract □ Eight 2-(3,4-methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles were synthesized, characterized by their sharp melting points, elemental analyses, and IR spectra, and evaluated for anticonvulsant activity. The protection afforded by oxadiazoles (100 mg/kg ip) against pentylenetetrazol (90 mg/kg sc)-induced convulsions ranged from 50 to 80%. All oxadiazoles inhibited the respiratory activity of rat brain homogenates during oxidation of pyruvate, α-ketoglutarate, and succinate. The presence of added nicotinamide adenine dinucleotide (NAD) to the reaction mixture during oxidation of pyruvate decreased the degree of inhibition. All oxadiazoles possessed antiproteolytic activity that was reflected by their ability to decrease trypsin-induced hydrolysis of bovine serum albumin. Such an inhibition was concentration dependent and ranged from 10.2 to 47.5 and from 15.7 to 71.8% by 0.5 and 1 m*M* oxadiazoles, respectively. All oxadiazoles competitively inhibited *in vitro* succinate dehydrogenase activity of rat brain homogenates.

Keyphrases □ Oxadiazoles, various—synthesized, evaluated for effect on enzyme activity in rat brain homogenates, anticonvulsant activity in mice, and antiproteolytic activity *in vitro* □ Enzyme activity—effect of various oxadiazoles in rat brain homogenates □ Anticonvulsant activity—various oxadiazoles evaluated in mice □ Antiproteolytic activity—various oxadiazoles evaluated *in vitro* □ Structure–activity relationships—various oxadiazoles evaluated for effect on enzyme activity in rat brain homogenates, anticonvulsant activity in mice, and antiproteolytic activity *in vitro*

Earlier studies indicated central nervous system depressant (1, 2), analgesic (3), muscle relaxant (4), and tranquilizing (5, 6) properties of substituted oxadiazoles. The inhibitory effects of 2,5-disubstituted 1,3,4-oxadiazoles on the respiratory activity of rat brain homogenates were reported (7–9). These observations led to the synthesis of 2,5-disubstituted oxadiazoles, which were evaluated for anticonvulsant activity.

In the present study, the ability of these oxadiazoles to inhibit respiratory activity of rat brain homogenates and trypsin activity during hydrolysis of bovine serum albumin was determined to investigate the biochemical mechanism of action for the anticonvulsant activity of these newer substituted oxadiazoles.

0022-3549/78/0700/0987$01.00/0