Macro- and Micromethods for High-Performance Liquid Chromatographic Analysis of Oxaprozin in Plasma

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Abstract □ A high-performance liquid chromatographic method for the analysis of oxaprozin in the presence of ketoprofen (the internal standard) was developed. Sample preparation involved macro- and microextraction procedures. An octadecylsilane reversed-phase system with an acetonitrile–phosphate buffer (pH 3.9) mobile phase was used to separate the compounds from the extracted plasma components and the oxaprozin metabolites. Linear UV detector response over a wide concentration range resulted in rapid and reproducible quantitation.

Keyphrases □ Oxaprozin—high-performance liquid chromatographic analysis with macro- and microextraction methods, comparison with GLC, human and rat plasma □ High-performance liquid chromatography—analysis, oxaprozin, macro- and microextraction methods, comparison with GLC, human and rat plasma □ Anti-inflammatory agents—oxaprozin, high-performance liquid chromatographic analysis, comparison with GLC

Oxaprozin (4,5-diphenyl-2-oxazolepropionic acid), a nonsteroidal, anti-inflammatory compound (1), was analyzed previously in plasma using GLC (2). This paper describes both macro- and micromethods for the high-performance liquid chromatographic (HPLC) determination of plasma oxaprozin concentrations. Time–concentration profiles obtained by each method also are presented.

EXPERIMENTAL

Materials—Anhydrous sodium acetate[1], monobasic and dibasic sodium phosphate[1], absolute ether[1], acetic acid[2], and hydrochloric acid[2] were analytical reagent grade. ACS grade 85% phosphoric acid[3] was used. Glass-distilled acetonitrile[4] was used in the mobile phase. The internal standard was ketoprofen[5].

Macromethod—Aliquots (0.5 ml) of unknown plasma or plasma spiked with standard oxaprozin were placed in 20 × 125-mm screw-capped test tubes to which were added 2.5 ml of distilled water and 1.0 ml of ketoprofen solution in 0.1 *M* acetate buffer (pH 4.8). The concentration of the internal standard ranged from 20 μg/ml for the low range samples and standards (1–10 μg of oxaprozin/ml) to 60 μg/ml for oxaprozin concentrations up to 200 μg/ml, but it was held constant in all cases for each set of samples analyzed. The control plasmas were treated with an identical volume of pH 4.8 buffer without the internal standard.

The samples were adjusted to pH 2 with 1.0 *N* HCl (~ 0.2 ml), and 8 ml of ether was added to each tube. The tubes then were capped, shaken mechanically for 10 min, and centrifuged to 2000 rpm for 5 min. Aliquots (7 ml) of the ether phase were transferred to clean test tubes and evaporated to dryness under reduced pressure at room temperature. The dried extracts were redissolved in 0.5 ml of the mobile phase, and 10-μl aliquots were injected onto the chromatographic column.

Analyses were performed using a high-performance liquid chromatograph[6] equipped with an octadecylsilane column[7]. Separation was achieved by reversed phase using a mobile phase of 62% acetonitrile in 0.05 *M* NaH_2PO_4 (adjusted to pH 3.9 with phosphoric acid) at a flow rate of 1.5 ml/min. The samples were introduced onto the column by means of a syringe-loaded sample injector[8]. At 290 nm, the detection range was 0.02 absorbance unit full scale (aufs) for the lower standards and 0.2–0.5 aufs for those of higher concentration.

A standard curve was generated by plotting the known concentrations of oxaprozin in the extracted standards against the ratios calculated by dividing the oxaprozin peak height (millivolts) by the internal standard peak height (millivolts). Quantitation of the unknowns was achieved by comparing the peak height ratios to this standard curve.

Micromethod—Aliquots (50 μl) of unknown or control plasma were placed in 6 × 50-mm disposable glass tubes. The control plasmas were spiked with 50-μl aliquots of oxaprozin standards (1.0–60 μg/ml) in 0.1 *M* phosphate buffer (pH 7.0). The unknowns were treated with an equal volume of buffer without the standard.

A 50-μl aliquot of a 5-μg/ml solution of ketoprofen in 0.1 *M* phosphate buffer (pH 7) was added to each sample and standard of the low range (0.1–2 μg/ml). Internal standardization of the high range (3–60 μg/ml) samples and standards was achieved with the addition of 50 μl of a 20-μg/ml ketoprofen solution in the same buffer. Two hundred microliters of fresh absolute ether was added to each sample, which then was mixed for 5 sec using a vortex mixer and centrifuged to break up the emulsion. The ether phases were transferred nonquantitatively to fresh tubes, and the extraction was repeated twice. The combined ether extracts were

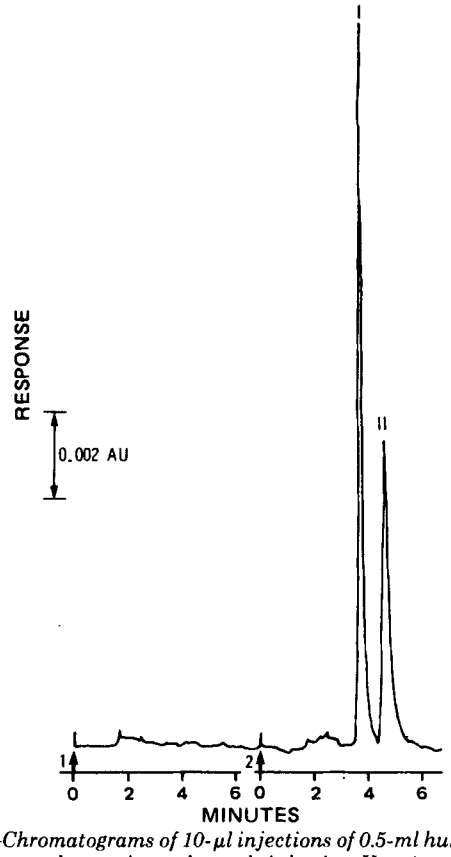

Figure 1—*Chromatograms of 10-μl injections of 0.5-ml human plasma extracts. Arrows denote times of sample injection. Key: 1, control plasma extract; and 2, extract of plasma spiked with ketoprofen (I) (30 μg/ml) and oxaprozin (II) (5 μg/ml).*

[1] Mallinckrodt, St. Louis, Mo.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] Fisher Scientific Co., Fair Lawn, N.J.
[4] Burdick & Jackson Laboratories, Muskegon, Mich.
[5] Courtesy of Ives Laboratories, New York, N.Y.
[6] LDC analyst series 7800 with a Spectromonitor III UV monochrometer (or equivalent), a model 308 computing integrator, and a model 3401 strip-chart recorder, Laboratory Data Control, Riviera Beach, Fla.
[7] Chromegabond C_{18}, 10-μm particle size, 30 cm × 4.6 mm i.d., E.S. Industries, Marlton, N.J.
[8] Model 7120, Rheodyne, Berkeley, Calif.

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Table I—Concentrations of Oxaprozin in Human Plasma after a Single 600-mg Oral Dose of Oxaprozin

Hours	Oxaprozin, μg/ml[a]
0	0
1	49.4
2	57.9
4	73.0
6	71.2
9	65.9
12	60.5
24	49.3
48	32.1
96	17.4
144	9.1
168	6.7
240	3.0

[a] Concentrations represent the means for three normal human volunteers.

taken to dryness under a nitrogen stream. The dried extracts were redissolved in 50 μl of the mobile phase, and a 20-μl injection was made.

The HPLC instrument[9] was equipped with a precolumn in addition to the analytical column[7]. The precolumn (5 cm × 2.1 mm i.d.) was filled with dry octadecylsilane[10] by the tap and fill method. The mobile phase was similar to that used in the macromethod, except that the acetonitrile concentration was decreased to 60% to optimize separation. At 280 nm, the detection range was 0.002–0.016 aufs and the peak heights were measured manually. The method of quantitation was the same as for the macromethod.

RESULTS

Macromethod—The chromatograms in Fig. 1 illustrate the absence of interference from control plasma, the peak shape symmetry, and the sensitivity of the detector response. The method achieved good peak separation in <6 min with a lower limit of detectability of 0.5 μg of oxaprozin/ml of plasma. Peak height ratios of the standards over the oxaprozin concentration range of 1–200 μg/ml gave a linear regression line with a correlation coefficient of 0.9993.

The relative accuracy of the method was determined by comparing the HPLC results to the GLC results for a series of human plasma samples taken within 48 hr of an oral 600-mg dose of oxaprozin. The correlation between the two methods of analysis was 0.98. The accuracy of the GLC method was determined previously by comparison with radioactive monitoring of carbon 14 (2). The time–concentration profiles obtained by both HPLC and GLC are presented in Fig. 2. The recovery from plasma was 90.1 ± 3.7%. The coefficient of variation of the HPLC assay was 1.9%. Table I presents the mean drug concentrations in human plasma following a single oral 600-mg dose.

Micromethod—The chromatograms in Fig. 3 represent the analysis of rat plasma extracted by the micromethod. No interference from plasma components was observed, and oxaprozin was separated from its metabolites, including the hydroxylated compounds identified previously[11] (2). The lower limit of detectability was 0.1 μg of oxaprozin/ml of plasma, and the peak height of the standards between 0.1 and 60.0 μg/ml resulted

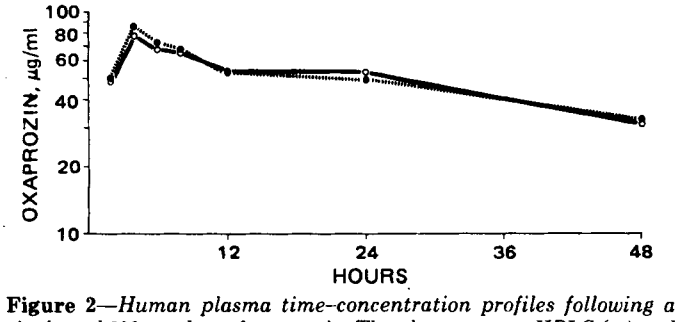

Figure 2—*Human plasma time–concentration profiles following a single oral 600-mg dose of oxaprozin. The plots compare HPLC (●) and GLC (○) results.*

[9] LDC model 2396 dual minipumps with a model 1203 UV-III monitor and a model 3401 strip-chart recorder.

[10] Co:Pell ODS, 37–45-μm particle size, Whatman, Clifton, N.J.

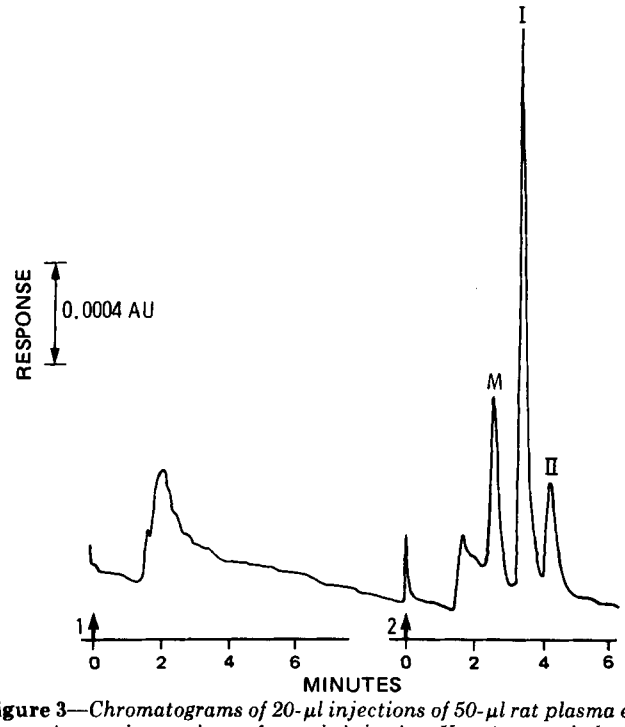

Figure 3—*Chromatograms of 20-μl injections of 50-μl rat plasma extracts. Arrows denote times of sample injection. Key: 1, control plasma extract; and 2, extract of rat plasma obtained 4 hr after a 20-mg/kg oral dose of oxaprozin. The peaks represent 20 μg of ketoprofen/ml (I), 1.9 μg of oxaprozin/ml (II), and a nonquantified amount of oxaprozin metabolites (M).*

in a linear regression correlation coefficient of 0.9994. The coefficient of variation of the micromethod was 3.3%.

A plasma time–concentration profile resulting from an oral dose of 20 mg of oxaprozin/kg to a rat is presented in Fig. 4. The plasma recoveries were similar to those obtained using the macromethod.

DISCUSSION

The HPLC methods outlined here represent several advantages over the GLC method employed previously. Since derivatization is no longer necessary, sample preparation time is shortened and the use of hazardous

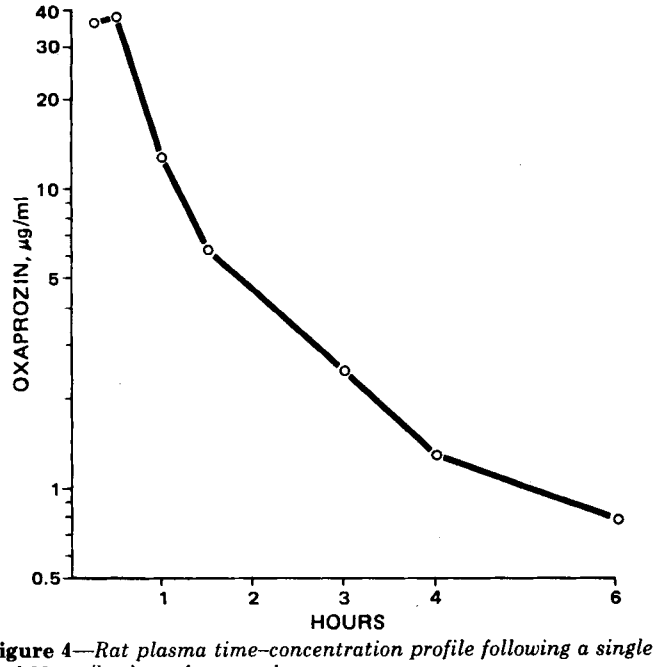

Figure 4—*Rat plasma time–concentration profile following a single oral 20-mg/kg dose of oxaprozin.*

diazomethane is eliminated. The precision of the macromethod is better than that reported for the GLC assay (2), while that of the micromethod is equal to that of the GLC method. The micromethod permits monitoring of drug concentrations in small volumes of plasma, making pharmacokinetic studies in rats and children feasible.

As reported for the GLC method (2), this procedure can be used to determine oxaprozin concentrations in urine by extracting the free drug at pH 7 rather than at pH 2. This modification is necessary in both urine and rat plasma[11] to avoid the partial extraction of oxaprozin glucuronide.

[11] F. W. Janssen and S. K. Kirkman, unpublished data.

Due to the chromatographic separation of oxaprozin from its major metabolites, no other modification in the extraction procedure is necessary.

REFERENCES

(1) K. Brown, J. F. Cavalla, D. Green, and A. B. Wilson, *Nature*, **219**, 164 (1968).

(2) F. W. Janssen, S. K. Kirkman, J. A. Knowles, and H. W. Ruelius, *Drug Metab. Dispos.*, **6**, 465 (1978).

Electronic Study of Receptor Binding of Analgesic Aryl Moiety II: Prodine Analogs

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Received April 12, 1979, from the *College of Pharmacy, University of Baghdad, Baghdad, Iraq.* Accepted for publication February 5, 1980.

Abstract □ Analogs of the prodine analgesics were prepared and tested for analgesic activity. A good correlation seems to exist between the energy level of the highest occupied molecular orbital and biological activity. The energy level of the highest occupied molecular orbital of the aryl moiety of these analogs may permit a charge transfer interaction between the aryl groups of the analgesic molecules and their receptors with the aryl groups acting as charge donors.

Keyphrases □ Prodine analogs—synthesis, testing for analgesic activity, correlation between activity and energy level of highest occupied molecular orbital of aryl moiety □ Analgesic activity—prodine analogs, correlation between potency and energy level of highest occupied molecular orbital of aryl moiety □ Charge transfer interactions—prodine analogs, correlation between analgesic activity and energy level of highest occupied molecular orbital of aryl moiety, complex formation between aryl moiety and receptor

The synthesis of 1-methyl-4-(3-thienyl)-4-propionoxypiperidine (I) and its 2-pyridyl analog (II) was reported previously (1). A similar type of replacement of the benzene ring by heterocyclic rings, such as pyridine, also was reported for propoxyphene, tetralins, and chromanes (2). Compounds I and II were designed to study the receptor binding of the analgesic aryl moiety. The ED_{50} data obtained for I and II at 3.9 and 16.0 mg/kg, respectively, using the mouse–hot plate method and subcutaneous administration pointed to the possible interaction of the aryl group by forces (1) other than van der Waals forces (3, 4) or hydrophobic bonding (5). No further attempt was made to identify these possible forces.

BACKGROUND

Organic compounds containing aromatic rings form charge transfer complexes with other compounds, and they may act as donors or acceptors of the charge (6). Some biochemicals were found to interact in the biological system through the formation of charge transfer complexes (7). Their role as charge donors or acceptors depends on their respective highest occupied molecular orbital or lowest empty molecular orbital energy levels. Correlations between electron densities and the energy levels of the highest occupied molecular orbital or lowest empty molecular orbital and biological activity were described for cholinesterase inhibitors (8, 9), several antimalarials (10), hallucinogens (11), benzothiadiazine antihypertensives (12), and other compounds (13).

Charge transfer complex formation between the aryl moiety of the analgesic molecules and the analgesic receptor is one possibility for explaining variations in analgesic potency. This possibility can be studied by the synthesis of analogs of I and II with aryl groups having different energy levels for their highest occupied molecular orbital or lowest empty molecular orbital. Study of the analgesic activity of these analogs, expressed as their ED_{50} values, together with that of I and II may give more understanding as to how these groups interact with the receptors.

This paper describes the synthesis and analgesic activity of some prodine analogs and correlates the activity with the energy state of the highest occupied molecular orbital or lowest empty molecular orbital of the aryl groups in these analogs.

EXPERIMENTAL[1]

Formation of Aryl Lithium—The aryl halide (0.1 mole) was dissolved in 100 ml of dry ether, placed in a three-necked 500-ml flask, and cooled with a dry ice–acetone bath. An equivalent amount of *n*-butyl lithium[2] (2 moles in 100 ml of hexane) was added dropwise, with stirring, to the aryl halide solution under nitrogen.

Formation of 1-Methyl-4-arylpiperidine-4-ols (IV–VIII)—To the prepared aryl lithium salts was added, with stirring and cooling at the temperature of a dry ice–acetone bath, a solution of 1-methyl-4-piperidone[2] (0.1 mole, 11.3 g) in 100 ml of dry ether over 20 min. The temperature of the reaction mixture then was allowed to rise to 0° and was maintained for 45 min. The reaction mixture then was poured over 100 g of ice–hydrochloric acid (1:1).

The ether layer was separated and washed twice (~50 ml) with dilute hydrochloric acid, and the washings were added to the aqueous acid layer. The mixture was made basic with 10% NaOH and extracted three times (~100 ml) with ether and once with 100 ml of chloroform. The organic washings were mixed and dried over anhydrous sodium sulfate. Removal of the organic solvents under vacuum gave the required alcohols.

Microanalyses were performed on the pure products after recrystallization from the appropriate solvent or on the quaternary salts. The quaternary salts were prepared by treatment of the alcoholic product with methyl iodide or benzyl bromide in methanol and recrystallization from an alcohol–ether mixture.

The IR spectra (chloroform solution) of IV–VIII showed absorption bands in the region of the alcoholic hydroxyl group at 3400–3150 cm^{-1} and at 3100–3000 and 1600–1500 cm^{-1} due to the aryl rings introduced in the piperidine ring.

Esterification of IV–VIII—A solution of IV–VIII (0.02 mole) in 50

[1] All melting points were recorded in an oil bath and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 257 spectrophotometer. All products had IR spectra in agreement with the assigned structures. The aryl halides (Fluka, AG, Bucks, Switzerland) and toluene were distilled before use. Ether was dried over anhydrous sodium sulfate. Microanalyses were performed at Midwest Microlab, Indianapolis, Ind., or by the Department of Medicinal Chemistry, University of Kansas, Lawrence, Kans.

[2] Fluka, AG, Bucks, Switzerland.

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