Fluorometric Determination of Naproxen in Serum

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Abstract □ The fluorometric measurement of naproxen following its extraction from serum by a two-step procedure is described. Different parameters in the analysis were examined. The method was useful in biopharmaceutic and pharmacokinetic studies of naproxen.

Keyphrases □ Naproxen—fluorometric analysis, human serum □ Fluorometry—analysis, naproxen in human serum □ Anti-inflammatory agents—naproxen, fluorometric analysis, human serum

Naproxen, (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, is a potent nonsteroidal anti-inflammatory, analgesic, and antipyretic drug (1). The pharmacokinetics of naproxen in various animal species and in humans have been reported (2). These studies were based on an assay method, described briefly previously (2), which involves GLC with flame-ionization detection. In the present study, a fluorometric method was developed; it is more sensitive and, because of its simplicity, may be more widely applicable than the GLC method.

EXPERIMENTAL

Materials and Equipment—Naproxen was synthesized[1], and all other chemicals were analytical grade and used as supplied[2]. Standard solutions were prepared by dissolving naproxen in 0.1 *M* NaOH. Fluorescence spectra (uncorrected) and measurements were made with a fluorescence spectrophotometer[3].

Procedure—Serum or plasma, 0.10 ml, was added to 1 ml of 1 *M* hydrochloric acid and 5.00 ml of benzene containing 1.5% isoamyl alcohol in 10-ml screw-capped[4] tubes. These tubes were then shaken for 10 min on a reciprocating shaker. Following a brief centrifugation (5 min at 2000×*g*), 4.00 ml of the organic phase was transferred to a second tube containing 3.00 ml of 0.1 *M* sodium hydroxide.

After shaking and centrifuging again, the organic phase was aspirated and discarded. The fluorescence of the water phase was measured at excitation and emission maxima of 330 and 355 nm, respectively. The concentrations of naproxen in the samples were estimated by reference to standard solutions of naproxen in serum prepared simultaneously.

RESULTS AND DISCUSSION

Naproxen showed strong native fluorescence in aqueous solution with three distinct excitation maxima at wavelengths of 240, 275, and 330 nm and an emission maximum at 355 nm (Fig. 1). The fluorescence was insensitive to changes in pH (the fluorescence changed by less than 3% over the pH 0.1–14.0 range). For routine assay, the excitation maximum at 330 nm was used because fluorescence remained linearly related to naproxen concentration up to 10 μg/ml. At excitation maxima of 240 and 270 nm, linearity was preserved only up to 2.5 and 1 μg/ml, respectively.

The extraction of naproxen from serum into several organic solvents was tested. Quantitative recovery was obtained with heptane and benzene (both containing 1.5% isoamyl alcohol); 99 ± 2% (mean ± *SD*, *n* = 10) of the drug added at a concentration of 50 μg/ml to serum samples was found in the aqueous phase after two extractions. Benzene was selected for the assay because it produced a lower background fluorescence.

The optimum conditions for extraction of naproxen from aqueous solution into organic solvents were found by studying the distribution of the compound between various buffer solutions and benzene. For the transfer of naproxen from an aqueous into an organic phase, a pH lower than 7 was required; reextraction into water required a pH above 9.

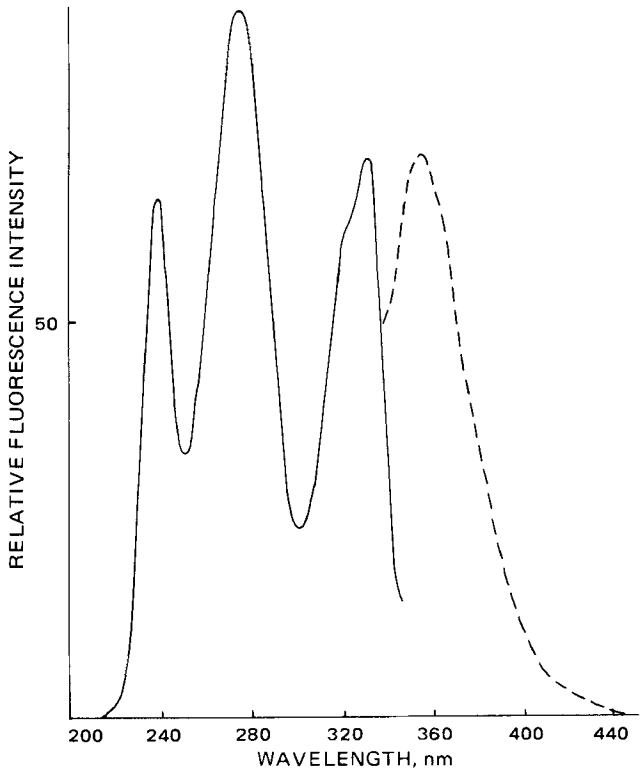

Figure 1—*Uncorrected excitation (—) and emission (- - -) spectra of the fluorescence of naproxen (1 μg/ml of 0.1 M NaOH).*

The detection limit of the present method is 0.5 μg/ml of serum. At this concentration, the fluorescence is twice that of the reagent blank. The sensitivity of the procedure is such that it will detect therapeutic plasma concentrations of naproxen. The sensitivity could be increased by using larger volumes (to 1 ml) of serum, since this change did not excessively increase background fluorescence and did not affect the recovery. Because

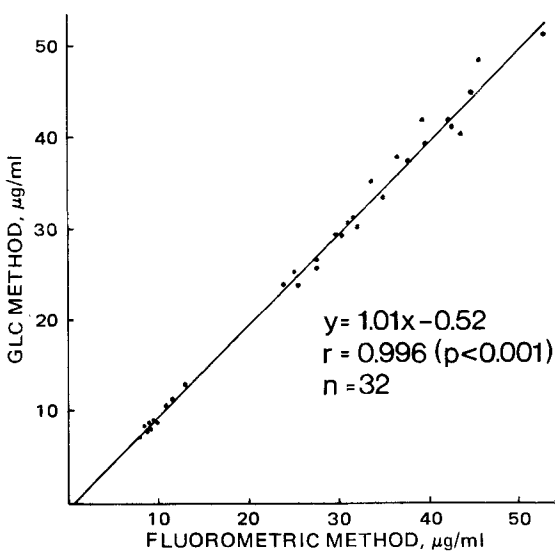

Figure 2—*Comparison of the serum levels of naproxen as determined by the fluorometric and GLC methods.*

[1] Synthetic laboratory of Farmos Group, Research Center, Turku, Finland.
[2] E. Merck, Darmstadt, Germany.
[3] Model MPF-2A, Perkin-Elmer Corp., Norwalk, Conn.
[4] Lined with Teflon (du Pont).

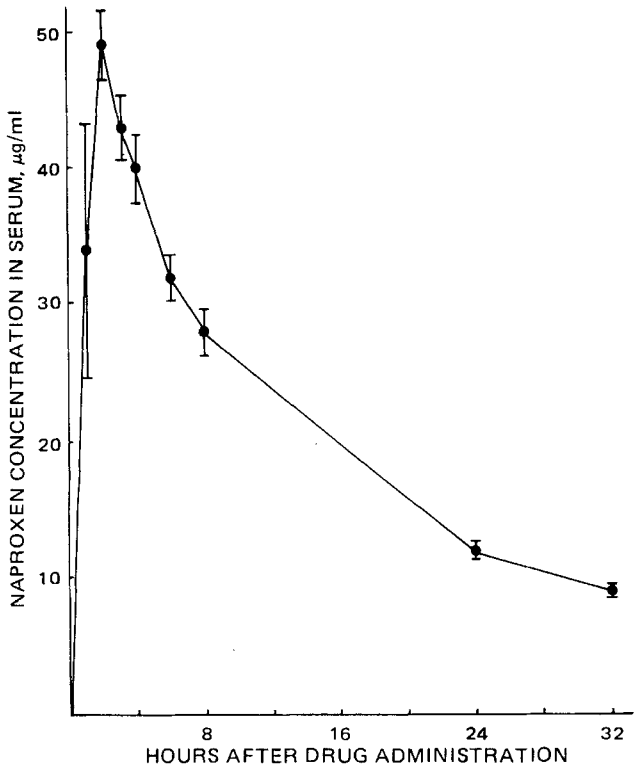

Figure 3—*Mean (±SEM) serum levels of naproxen in humans (n = 6) following single-dose oral administration of 250 mg of drug as a compressed tablet.*

of the linearity of the standard curve, serum concentrations up to 250 μg/ml can be measured using 0.1 ml of sample.

Several drugs at normal serum concentrations were examined for interference with this fluorometric assay of naproxen. Basic drugs were removed in the extraction procedure and caused no interference. The following acidic drugs also did not interfere: chlorpropamide, furosemide, ibuprofen, indomethacin, mefenamic acid, phenobarbital, phenylbutazone, phenytoin, sulfamethoxazole, and warfarin. Interference was observed with salicylic acid; 200 μg of salicylic acid/ml of serum gave a fluorescence value equivalent to about 10 μg of naproxen/ml. Therefore, this fluorometric assay method is not suitable for the determination of serum levels of naproxen in patients simultaneously receiving salicylates.

Interference by metabolites of naproxen was not studied, because the concentrations of metabolites in serum after naproxen administration are negligible (3).

The precision of the method was determined at three different concentrations. Naproxen solution was added to serum to give final concentrations of 5, 50, and 200 μg/ml. Ten 0.1-ml samples of each serum solution were analyzed by the proposed method at the same time. The relative standard deviations obtained were 2.6, 1.4, and 1.1%, respectively.

To test the accuracy of the method, 32 serum samples containing unknown concentrations of naproxen were assayed by both the present method and the GLC method (2). The results (Fig. 2) showed excellent correlation between the two methods.

To demonstrate the utility of this fluorometric method, serum concentrations of naproxen were measured in six normal human subjects after oral administration of a single dose of 250 mg of naproxen (one 250-mg tablet). The resulting serum levels (Fig. 3) agreed well with the serum levels of naproxen obtained by other workers using the GLC method (2).

The described fluorometric method is simple, rapid, and sensitive and can serve as a useful tool in the study of biopharmaceutics and pharmacokinetics of naproxen.

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Fluorescent Inhibitors of Thymidylate Synthetase

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Abstract □ Two fluorescent derivatives of 2′-deoxy-5-fluorouridine 5′-*p*-aminophenyl phosphate were prepared by treatment of this compound with fluorescein isothiocyanate in dimethyl sulfoxide or 5-(dimethylamino)naphthalenesulfonyl chloride in pyridine. The products of the reactions were isolated by diethylaminoethylcellulose chromatography and were shown to be homogeneous by polyacrylamide electrophoresis and TLC. Confirmation of the structure was provided by elemental analysis, absorption and fluorescence spectra, PMR measurements, and liberation of nucleotide upon hydrolysis with snake venom phosphodiesterase. The fluorescent derivatives are good competitive inhibitors ($K_i \simeq 10^{-6}$ *M*) of thymidylate synthetase from a methotrexate-resistant strain of *Lactobacillus casei*.

Keyphrases □ Thymidylate synthetase—inhibition by fluorescent derivatives of 2′-deoxy-5-fluorouridylate ester evaluated □ 2′-Deoxy-5-fluorouridylate ester—fluorescent derivatives synthesized, inhibition of thymidylate synthetase evaluated □ Enzyme inhibitors—fluorescent derivatives of 2′-deoxy-5-fluorouridylate ester synthesized, effect on thymidylate synthetase

The *de novo* enzymatic synthesis of thymidylate (2′-deoxy-5-methyluridylate) is achieved by transfer of the methylene substituent of 5,10-methylenetetrahydrofolate to 2′-deoxyuridylate with the associated conversion of the hypothetical methylene intermediate (1) to methyl using the inherent reducing power of the folate cofactor (Scheme I). A metabolic cycle is completed when the ensuing dihydrofolate is reduced to the tetrahydro level in the presence of dihydrofolate reductase, and an additional one-carbon unit is reintroduced enzymatically (2). Because

5,10-methylenetetrahydrofolate + 2′-deoxyuridylate $\xrightarrow{\text{thymidylate synthetase}}$ dihydrofolate + thymidylate

Scheme I