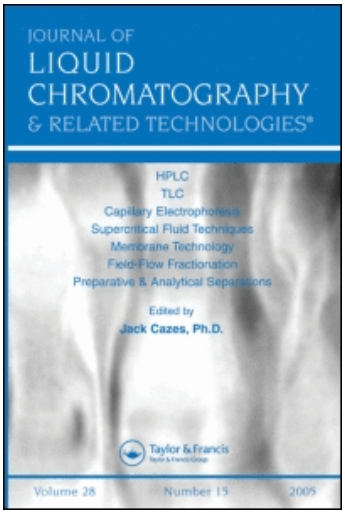

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Fluorometric High Performance Liquid Chromatography for Quantitation of Naproxen in Serum

Hearan Suh[a]; H. W. Jun[a]; G. W. Lu[a]
[a] Department of Pharmaceutics, College of Pharmacy The University of Georgia Athens, Georgia

To cite this Article Suh, Hearan , Jun, H. W. and Lu, G. W.(1995) 'Fluorometric High Performance Liquid Chromatography for Quantitation of Naproxen in Serum', Journal of Liquid Chromatography & Related Technologies, 18: 15, 3105 — 3115
To link to this Article: DOI: 10.1080/10826079508010436
URL: http://dx.doi.org/10.1080/10826079508010436

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(15), 3105-3115 (1995)

FLUOROMETRIC HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR QUANTITATION OF NAPROXEN IN SERUM

HEARAN SUH, H. W. JUN*, AND G. W. LU
Department of Pharmaceutics
College of Pharmacy
The University of Georgia
Athens, Georgia 30602-2352

ABSTRACT

A simple, sensitive and reliable HPLC method for the determination of naproxen in serum is presented. Samples were prepared by a protein precipitation method using acetonitrile. A reversed-phase C-18 column was used with a mobile phase consisting of 47% acetonitrile and 53% pH 2.5 buffer solution. A fluorescence detection with excitation wavelength of 230 nm and emission wavelength of 370 nm provided high sensitivity and specificity with no interference from normal serum constituents. The limit of quantitation was 2.0 ng/ml with a CV of 10.6 %. The standard plots were highly linear ($r > 0.999$) over the range of 10.0-200.0 ng/ml. The average recovery was 97% and the CVs of inter- and intra-day variabilities were 2.0% and 1.2%, respectively, for the entire calibration range. The method was used for the measurement of the time course of naproxen in dog serum after administration of a topical gel.

* Corresponding author

INTRODUCTION

Naproxen, (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid, is a potent antiinflammatory agent often used for the symptomatic treatment of rheumatoid arthritis, ankylosing spondylitis, acute gout and related diseases [1,2]. The drug also possesses analgesic properties which can effectively relieve moderate pain following orthopedic, dental, or surgical procedures. It is well absorbed orally and has an elimination half-life of about 13 hrs in man. The bioavailability of naproxen after topical application was reported to be low [3], despite its high lipophilicity.

In contrast to an oral route, topical administration of nonsteroidal antiinflammatory drugs (NSAIDs) could offer therapeutic benefits while avoiding gastrointestinal side effects. Recently, it was shown that high drug levels in local tissues such as muscle, ligament and synovial fluid could be achieved after topical application of several NSAIDs [4,5] in animals. Since the amount of drug entering the systemic circulation through the skin is usually low due to the barrier function of the stratum corneum, pharmacokinetic studies of drug administered by a topical route require a highly sensitive analytical method.

Many HPLC methods for the quantitation of naproxen in biological samples have been reported, employing a different detection capability such as spectrophotometric [6,7,8], fluorescence [9,10] or electrochemical detection [11]. However, most of these methods either required a time consuming extraction procedure for the sample preparation or lacked the sensitivity and reproducibility needed for a complete pharmacokinetic study of this drug.

Generally, fluorometric methods of detection provide an excellent sensitivity, often exceeding the sensitivity of most spectrophotometric methods. It has been shown that fluorescence detection gave higher sensitivity than UV [6,10], diode-array [10], or GC-MS [10] method for the determination of naproxen. In addition, fluorometry offers an advantage of having less interferences in the chromatogram due to fewer fluorescent compounds present

in the biological fluids than UV absorbing molecules. Approximately 10 % of all absorbing compounds are known to emit radiation via fluorescence.

The purpose of this study was to develop a simple and sensitive HPLC method for the quantitation of naproxen in blood samples. The applicability of the assay was demonstrated by the complete elucidation of the time course of serum concentrations of naproxen in a dog after receiving a topical gel.

MATERIALS AND METHODS

Materials

Naproxen (Sigma Chemical Co., St.Louis, MO), phenylsalicylate (Eastman Organic Chemicals, Rochester, NY), potassium chloride (Merck & Co., Rahway, NJ), and hydrochloric acid (EM Science, Gibbstown, NJ) were analytical or reagent grade and used as received. HPLC-grade acetonitrile was purchased from J.T.Baker Inc. (Phillipsburg, NJ). Distilled water was filtered by means of a Millipore system (Continental Water Systems Corp., El Paso, TX).

Instrumentation

The chromatographic analysis was performed on a modular HPLC system consisting of a reciprocating pump (Beckman model 110A), equipped with a fluorescence detector (Kratos FS 970) and an integrator (Shimadzu C-R3A). The fluorometer was set at an excitation wavelength of 230 nm and an emission wavelength of 370 nm. The detector was operated at the sensitivity scale of 0.1 μA. Injections were made with a 50-μl sample loop using a syringe-loading injection system (Rheodyne model 7125). The analytes were eluted on the reverse phase C-18 column (250 x 4.6 mm), packed with Partisil 5 μm ODS-3 (Whatman^R) with a guard column (C-18, Perisorb 30 μm).

Mobile Phase

Acetonitrile and pH 2.5 hydrochloric acid buffer (0.04 M) were thoroughly mixed at a ratio of 47:53 (v/v) and degassed by sonication prior to

use. The mobile phase was pumped through an inlet filtering system at a flow rate of 1.2 ml/min at ambient temperature. The column was washed with the mobile phase by maintaining the flow rate of 0.2 ml/min overnight.

Stock Solutions

The stock solutions of naproxen and phenylsalicylate (internal standard) were prepared at the concentrations of 100 μg/ml and 1000 μg/ml, respectively, in the mobile phase. The working stock solutions of naproxen for the calibration and recovery studies were prepared by diluting the stock solution with the mobile phase to obtain the concentration range of 0.1 to 1.5 μg/ml.

Sample Preparation

Samples for the quantitation of naproxen in dog serum were prepared by precipitating proteins with acetonitrile. To 0.2 ml of dog serum (dosed or spiked) in a 5.0-ml glass test tube was added 20 μl of the stock solution of the internal standard and vortexed briefly. After adding 0.56 ml of acetonitrile, the mixture was vortexed for 30 sec and then centrifuged at 3500 rpm for 10 min. Fifty μl of the clear supernatant was loaded into the sample loop of the Rheodyne injector for quantitation.

Validation

Blank dog serum (0.2ml) in a 5-ml glass test tube was spiked with a varying amount of the working stock solution of naproxen and a constant volume of the phenylsalicylate stock solution to prepare seven concentrations within the calibration range of 10.0-200.0 ng/ml for naproxen and 100 μg/ml for the internal standard. The samples prepared according to the procedure described earlier were chromatographed, and the standard calibration graphs were constructed by plotting the peak height ratios of naproxen to internal standard against the respective concentrations of naproxen. For each of the intra-day (n=3) and inter-day (n=9) standard plots obtained, the correlation coefficient, slope and intercept

were determined. The reproducibility of the assay was evaluated by comparing the least-square linear regression data obtained from the replicate intra- and inter-day calibration plots. The accuracy of assay was periodically confirmed by analyzing several quality control samples during analyses of unknown samples.

Quantitation

The calibration plot for naproxen was constructed daily by analyzing spiked serum samples with known amounts of naproxen and internal standard. The concentrations of naproxen in unknown samples were calculated by interpolation of the calibration plot using the peak height ratios (naproxen/phenylsalicylate) obtained from the prepared samples.

Recovery Studies

The absolute recoveries of the assay were assessed by comparing the peak height ratios of naproxen over phenylsalicylate which were obtained from spiked dog serum samples of different naproxen concentrations (10.0-200.0 ng/ml) to the peak height ratios of the samples containing the equivalent amounts of the drug and internal standard (100 μg/ml) which were directly dissolved in the mobile phase. For each naproxen concentration used, three replicate samples were prepared and analyzed.

In Vivo Application

The proposed procedure was employed to determine the serum concentration versus time profile of naproxen after topical administration of naproxen in a gel (10 mg/kg) on the medial thigh of a beagle dog. Blood samples (4ml) were withdrawn from the jugular vein using vacutainer tubes containing clot activator at the intervals of 0, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hrs and were centrifuged at 2500 rpm for 8 min. The serum was stored in a screw-capped plastic vial at -20°C until assay within a week.

RESULTS AND DISCUSSION

The goodness of an HPLC method for the determination of drugs in biological fluids depends on many criteria such as the extraction efficiency, chromatographic quality, simplicity and reliability of the method, among others. It was found in this study that naproxen was highly extracted from dog serum by precipitating serum proteins with acetonitrile. However, a slow drop-wise addition of acetonitrile to the serum sample with continuous vortexing during protein precipitation was crucial for achieving high and consistent recovery of naproxen from the sample. This procedure may be necessary for the extraction of other drugs in serum, especially for those which are highly bound to serum proteins.

Fig 1 shows typical chromatograms for blank dog serum (1-A) and dog serum containing 6.25 ng/ml of naproxen (1-B). Under the conditions described, the peaks corresponding to naproxen and phenylsalicylate were well resolved, sharp and symmetrical with no interference from endogeneous materials at their respective retention times of 5 and 10 min, thus facilitating accurate measurement of the peak height ratios. It was found that the composition and pH of the mobile phase significantly affected the chromatogram of naproxen. Naproxen is a weak acid (pKa= 4.2), and therefore the peak retention time was longer at the mobile phase pH of 2.5 as compared to those obtained at higher pH values, which could be attributed to the greater adsorptivity of the acidic form of naproxen to the nonpolar stationary phase of the column. As the pH was increased, naproxen was eluted quickly along with several interfering peaks. On the other hand, increased peak broadening and tailing were observed as the volume ratio of acetonitrile in the mobile phase decreased below 47 %. Consequently, pH 2.5 and 47 % (v/v) acetonitrile were chosen as the optimal conditions of the mobile phase used in this assay.

The standard calibration graphs obtained by plotting the peak height ratios of naproxen to internal standard against the respective naproxen concentrations were highly linear over the range of 10.0-200.0 ng/ml. Using linear regression

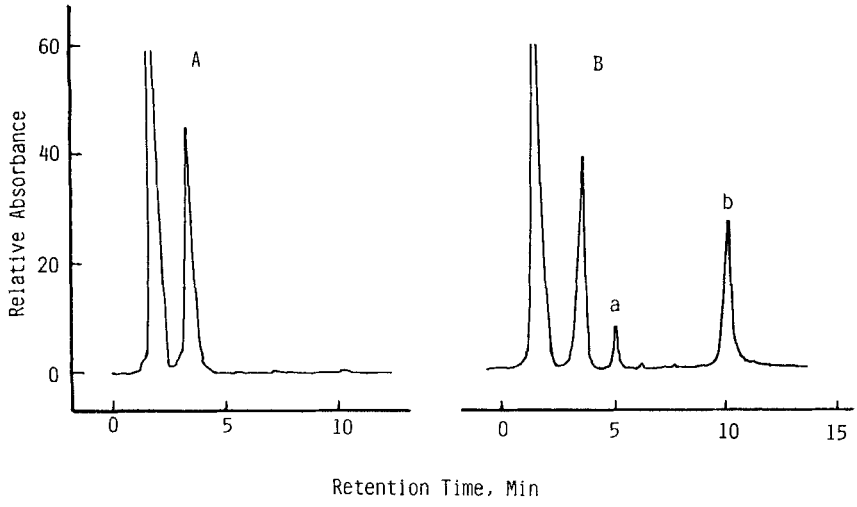

FIGURE 1. Chromatograms of naproxen in dog serum. A: blank dog serum; B: dog serum containing 6.25 ng/ml of naproxen (a) and internal standard (b).

analyses, the three standard plots obtained on the same day gave the average regression equation of $Y=-0.016+0.069X$ (CV of 1.59 % for the slope), where Y and X represent the peak height ratio and naproxen concentration, respectively. All of the intra- and inter-day standard graphs prepared for naproxen in dog serum had a correlation coefficient >0.999 (Tables 1 and 3).

The recovery and precision of the assay were assessed by analyzing replicate samples of inter-day and intra-day assays at each concentration used, and results were summarized in Tables 2 and 4. The overall mean recovery of naproxen from serum in the entire calibration range was 97.2 % with a CV of 2.0 %, when serum was deproteinized using 2.8 volume of acetonitrile per volume of serum. The recovery fell substantially when the volume ratio of acetonitrile to blood was less than 2.5. Acetonitrile has been widely used as a protein precipitant in extracting drugs for HPLC techniques because of its popularity as a mobile

TABLE 1

Intra-day Regression Data for the Standard Plots of Naproxen in Dog Serum

Standard plot	Slope[a]	Intercept	Correlation coefficient
1	0.0172	-0.0297	0.9994
2	0.0169	-0.0197	0.9997
3	0.0176	+0.0006	0.9998

[a] Mean slope (% CV) = 0.0172 ml/ng (1.59)

TABLE 2

Intra-day Variation of Naproxen Assay in Dog Serum

Concentration (ng/ml)	Mean peak height ratio	SD	CV (%)[a]
10.0	0.1773	0.0009	0.49
20.0	0.3333	0.0010	0.30
40.0	0.6613	0.0174	2.63
80.0	1.3198	0.0206	1.56
120.0	2.0766	0.0156	0.75
160.0	2.7926	0.0479	1.72
200.0	3.4152	0.0389	1.14

[a]Average CV = 1.3 %

phase component and high deproteinizing efficiency. The CVs for the inter-day and intra-day recoveries of naproxen in plasma were 2.0 % and 1.2 %, respectively, in the range of 10.0 ng/ml-200.0 ng/ml. The low coefficients of variation and high recovery indicate the good precision and accuracy of the present method.

The limit of quantitation and the limit of detection for naproxen in serum using the present method were 2.0 ng/ml (CV of 10.6 %) and 1.0 ng/ml (the signal/noise ratio of 3), respectively, when 0.2 ml of serum was used. The

TABLE 3

Inter-day Regression Data for the Standard Plots of Naproxen in Dog serum

Standard plot[a]	Slope[b]	Intercept	Correlation coefficient
1	0.0172	-0.0145	0.9998
2	0.0174	-0.0116	0.9999
3	0.0173	-0.0162	0.9997

[a] Prepared over a period of one week
[b] Mean slope (% CV) = 0.0173 (0.50)

TABLE 4

Inter-day Recovery and Precision Data for Naproxen Assay in Dog Serum

Naproxen added (ng/ml)	Mean peak height ratio	SD	CV (%)[a]	Recovered (ng/ml)	Recovery (%)[b]
10.0	0.1692	0.0071	4.18	9.0	90.0
20.0	0.3344	0.0063	1.88	18.9	94.4
40.0	0.6599	0.0108	1.64	38.8	97.1
80.0	1.3515	0.0307	2.27	80.3	100.4
120.0	2.0810	0.0231	1.11	119.3	99.4
160.0	2.7874	0.0348	1.25	158.9	99.3
200.0	3.4200	0.0463	1.35	199.8	99.9

[a]Average CV = 2.0 %
[b]Overall Mean Recovery = 97.2 %

sensitivity could be increased, for example, by increasing the volume of serum used, combined with the use of a higher volume of sample to be injected for chromatography. Concentrating the supernatant of deproteinized serum extract by evaporation could further enhance the sensitivity of the assay.

The method was successfully applied to determine the time course of naproxen concentrations in serum samples of a dog after receiving a topical dose of naproxen in a gel. The serum concentration versus time profile obtained with this assay is shown in Fig 2.

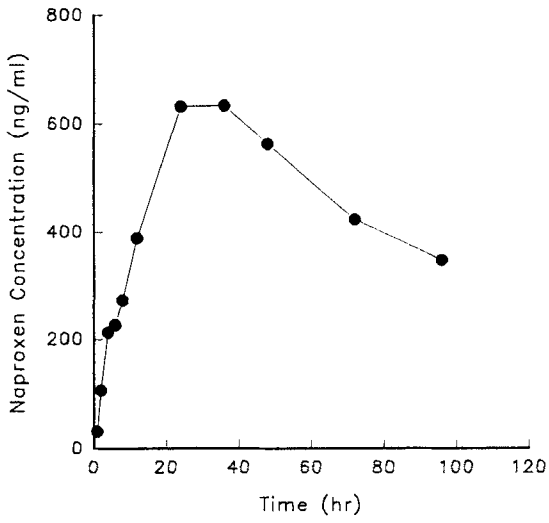

FIGURE 2. Serum concentration versus time profile of naproxen in a dog after administration of a topical gel (10mg/kg).

CONCLUSIONS

This report presented an improved HPLC method for the quantitation of naproxen in serum using a simple sample preparation procedure combined with the method of fluorometric detection. The method is accurate, sensitive and selective with no interference by endogeneous compounds. High and reproducible recoveries of the drug from serum were obtained due to the improved sample preparation procedure used. Since the method is simple and rapid, requiring no time consuming liquid or solid extraction procedures, it is recommended that this assay be used for routine therapeutic monitoring as well as for pharmacokinetic and bioavailability studies of naproxen in man and animals.

REFERENCES

1. Todd, P.A., and Clissold, S.P., Naproxen. A reappraisal of its phamacology and therapeutic use in rheumatic diseases and pain states., Drugs, 40: 91 (1990)

2. Syntex corporation, Naproxen. Proceedings of an international medical symposium., J. Clin. Pharmacol., 15: 305 (1975)

3. Van den Ouweland, F.A., Eenhoorn, P.C., Tan, Y., and Gribnau, F.W.J., Transcutaneous absorption of naproxen gel., Eur. J. Clin. Pharmacol., 36: 209 (1989)

4. Rabinowitz, J.L., Feldman, E.S., Weinberger, A., and Schumacher, H.R., Comparative tissue absorption of oral ^{14}C-aspirin and topical triethanolamine ^{14}C-salicylate in human and canine knee joints., J. Clin. Pharmocol., 22: 42 (1982)

5. Riess, V.W., Schmid, K., Botta, L., Kobayashi, K., Moppert, J., Schneider, W., Sioufi, A., Strusberg, A., and Tomasi, M., Die perkutane resorption von diclofenac., Arzneim-Forsch/Drug Res., 36: 1092 (1986)

6. Van Loenhout, J.W.A., Van Ginneken, C.A.M., Ketelaars, H.C.J., Kimenai, P.M., Tan, Y., and Gribnau, F.W.J., A high-performance liquid chromatographic method for the quantitative determination of naproxen and des-methyl-naproxen in biological samples., J. Liq. Chromatogr., 5: 549 (1982)

7. Nielsen-Kudsk, F., HPLC-determination of some antiinflammatory, weak analgesic and uricosuric drugs in human plasma and its applicaition to pharmacokinetics., Acta Pharmacol. et toxicol., 47: 267-273 (1980)

8. Satterwhite, J.H., and Boudinot, F.D., High-performance liquid chromatographic determination of ketoprofen and naproxen in rat plasma., J. Chromatogr., 431: 444 (1988)

9. Shimek, J.L., Rao, N.G.S., and Wahba Khalil, S.K., An isocratic high-pressure liquid chromatographic determination of naproxen and desmethyl naproxen in human plasma., J. Pharm. Sci., 71: 438 (1982)

10. Singh, A.K., Jang, Y., Mishra, U., and Granley, K., Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicylic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass chromatography. J. Chromatogr., 568: 351 (1991)

11. Kazemifard, A.G., and Moore, D.E., Liquid chromatography with amperometric detection for the determination of non-steroidal anti-inflammatory drugs in plasma., J. Chromatogr., 533: 125 (1990)

Received: March 13, 1995
Accepted: April 2, 1995