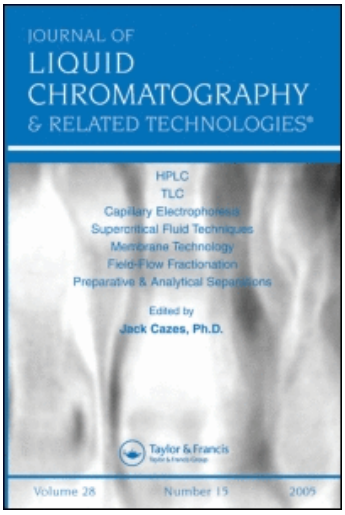

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Simultaneous Assay of Naproxen and Salicylic Acid in Serum Using High Pressure Liquid Chromatography

R. F. Burgoyne[a]; P. R. Brown[a]; S. R. Kaplan[b]
[a] Department of Chemistry, University of Rhode Island, Kingston, Rhode Island [b] Brown University Program in Medicine The Roger Williams General Hospital, Providence, Rhode Island

To cite this Article Burgoyne, R. F. , Brown, P. R. and Kaplan, S. R.(1980) 'The Simultaneous Assay of Naproxen and Salicylic Acid in Serum Using High Pressure Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 3: 1, 101 — 111
To link to this Article: DOI: 10.1080/01483918008060157
URL: http://dx.doi.org/10.1080/01483918008060157

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(1), 101-111 (1980)

THE SIMULTANEOUS ASSAY OF NAPROXEN AND SALICYLIC ACID IN SERUM USING HIGH PRESSURE LIQUID CHROMATOGRAPHY

R. F. Burgoyne and P. R. Brown
Department of Chemistry
University of Rhode Island
Kingston, Rhode Island 02881

and

S. R. Kaplan
Head, Subsection Rheumatology
Brown University Program in Medicine
The Roger Williams General Hospital
Providence, Rhode Island

ABSTRACT

A rapid, sensitive, reverse-phase high pressure liquid chromatographic assay was developed for the simultaneous determination of naproxen and salicylic acid. These compounds are extracted from serum and then separated on a reverse-phase column using an acidified methanol eluent. Utilization of a flow-through fluorescence detector in series with a variable wavelength micro-UV detector enhances the sensitivity of the assay. Application of the assay to therapeutic levels of the drugs in human serum is demonstrated.

INTRODUCTION

Naproxen (I), (+)-6-methoxy-α-methyl-2-naphthalene-acetic acid, was developed by Harrison, et al. (1) and its chemistry and pharmacology characterized by Dorfman (2) at Syntax Research. Initial clinical studies performed on laboratory animals (3-5) and humans (5-8) indicate that naproxen possesses potent analgesic and antipyretic activity. The drug is rapidly and completely absorbed and peak plasma levels attained within two hours of oral

administration of the drug (9). Katona (10) and Lussier (11) found that naproxen yielded effective relief of the symptoms of rheumatoid arthritis. Additional studies have demonstrated the anti-inflammatory activity of naproxen in the treatment of osteoarthrosis (12), ankylosing spondilitis (13), and acute gout (14). Furthermore, naproxen was found to produce fewer gastrointestinal side effects than are commonly found during long-term use of other anti-arthritic drugs (15,16).

With the increasing use of naproxen for arthritic conditions, there is a need for a rapid, sensitive assay of levels of the drug in serum to aid in the selection of the optimum treatment regimen and to monitor compliance. In addition, the method should be capable of assaying salicylic acid, the primary metabolite of aspirin, which is frequently prescribed concurrently with naproxen. Although naproxen is present in body fluids primarily as the parent drug (17), aspirin is rapidly converted to salicylic acid and is present in serum mainly as the salicylic acid metabolite (18). Consequently, the determination of serum levels of naproxen and salicylic acid are important if the most beneficial drug therapy is to be selected.

The most common methods of analysis used presently for naproxen are gas chromatography (19,20) and liquid scintillation spectrometry (21). Recently, spectrofluorometric and high pressure liquid chromatographic (HPLC) assays were reported. Antilla developed a naproxen fluorometric assay which is highly sensitive but is incapable of simultaneous determinations of naproxen and salicylic acid (22). Using HPLC, Thompson and Collins (23) developed a gradient elution ion-exchange chromatographic procedure for measuring urinary metabolic profiles of naproxen. Since reverse-phase HPLC is easier to use and has more versatility for monitoring drugs and their metabolites (24), Westerlund and Theodorsen (25) used this mode to study the retention behavior of three naphthaleneacetic acid derivatives on two hydrophobic supports. However, the method did not include the separation of

naproxen from salicylic acid and was not optimized for clinical studies.

By using a reverse-phase system and optimizing the separation conditions, a quantitative analysis for naproxen and salicylic acid in serum was developed. The naproxen was separated from the salicylic acid with high resolution in 7 minutes. Sensitive and selective detection of the solutes in the column effluent was achieved through the use in series of flow-through UV absorption and fluorescence detectors.

The identification of chromatographic peaks is of particular importance in HPLC analyses of biological samples. Since identifications based on retention time data alone can be misleading, additional methods of peak identification which are simple, reliable, and easily amenable to routine analysis must be employed. Methods utilizing instrumentation on line with the chromatographic separation are preferred in order to eliminate further handling of trace quantities in biological samples. By using micro-UV and fluorescence detectors to monitor the column effluent, positive identifications of chromatographic peaks can be obtained.

EXPERIMENTAL

Apparatus

A Waters Associates Model ALC 202 liquid chromatograph equipped with a 254 nm fixed wavelength micro-UV detector was operated at ambient temperature. Variable wavelength UV and flow-through fluorescence detectors (Schoeffel Instrument Corp.) were used in series for additional detection of the column effluent. Chromatograms were recorded on a dual pen recorder (Waters Associates) and quantitated electronically by an Autolab Minigrator (Spectra-Physics). Separations were obtained on a μBondapak C_{18} (4 mm x 30 cm) reverse-phase column.

Reagents and Solvents

The analytical method was developed using reference standards of naproxen (Syntex) and aspirin (Glenbrook) in tablet form,

acetylsalicylic acid (Heyden), and salicylic acid (Eastern Scientific). Pure naproxen was obtained directly from Syntex. All solvents were reagent grade except methanol (Burdick and Jackson) which was spectral grade.

HPLC Conditions

The optimal wavelengths for the detection of naproxen and salicylic acid were determined on the basis of UV spectra (Cary Instruments) of solutions of reference standards in the column eluent. Wavelengths of 254, 240, and 235 nm were selected to maximize absorption of the standards and minimize interferences by methanol. The fluorometer was operated with excitation at 240 nm and with a 340 nm cutoff filter. The mobile phase was methanol-0.1 M acetic acid (70:30, v/v); a flow rate of 1.0 ml/min was maintained in the isocratic mode at ambient temperature. The solvent mixture was prepared daily, filtered through glass microfibre paper (Whatman) and degassed under slight vacuum.

Peak Identification

The peaks of naproxen and salicylic acid were identified by a combination of methods. Initially retention times of sample components were compared with those of reference standards. Secondly, the characteristic absorption at different wavelengths was utilized by comparing the peak height ratios of reference standards with the ratios of sample components. In addition, the effluent was monitored by fluorescence.

Extraction Procedure

Conditions for the extraction of naproxen from serum were reported by Antilla (21). The procedure was tested by extractions of multiple 100 µl aliquots from serum pools containing 10, 25, 50, 75, and 100 µg/ml of naproxen and salicylic acid standards. Successful application of the procedure to therapeutic drug levels in serum was demonstrated by analyses of clinical samples collected from five female volunteers who were administered naproxen orally

on a daily basis for treatment of rheumatoid arthritis. The regimen of patient 5 included concurrent daily administration of 10 aspirin tablets. Blood samples were collected at random times in the course of individual regimens, processed to separate the serum from the formed elements of the blood, and the serum immediately frozen. In preparation for HPLC, the serum was thawed and three 100 µl aliquots were removed from each sample. The drugs were then extracted from each aliquot according to Antilla's procedure (22) and analyzed by HPLC.

RESULTS

The HPLC method was evaluated to determine reproducibility and linearity with respect to component concentration. Table 1 presents the reproducibility and peak height ratios obtained for a series of ten analyses of standard solutions of naproxen and salicylic acid over a two day period. Excellent reproducibility and linearity were obtained for reference standards in aqueous solution with relative standard deviations of less than 4% for all samples and a coefficient of linearity greater than 0.95 over a concentration range of 1-100 µg/ml. The minimum detectable limits of naproxen and salicylic acid by UV absorption detection were 1 ng and 10 ng, respectively. Figure 1 presents the chromatogram obtained of reference standards monitored at absorbance wavelengths of 235 nm (A) and 254 nm (B) and by fluorescence (C). The detection of the effluent by the fluorescence detector greatly enhanced the specificity and sensitivity of the assay. Under the fluorescence conditions selected, both naproxen and salicylic acid gave stronger detector responses and lower detection limits than those obtained by UV detection. Note in Figure 1C that the fluorometer attenuation is 1.0 µA full scale compared to the UV absorption at 0.02 AUFS. Since the fluorescence detector is capable of measurements of 0.01 µA full scale, it is therefore possible to increase the sensitivity of detection 100 fold and to determine, if necessary, much lower concentrations of the naproxen in the serum.

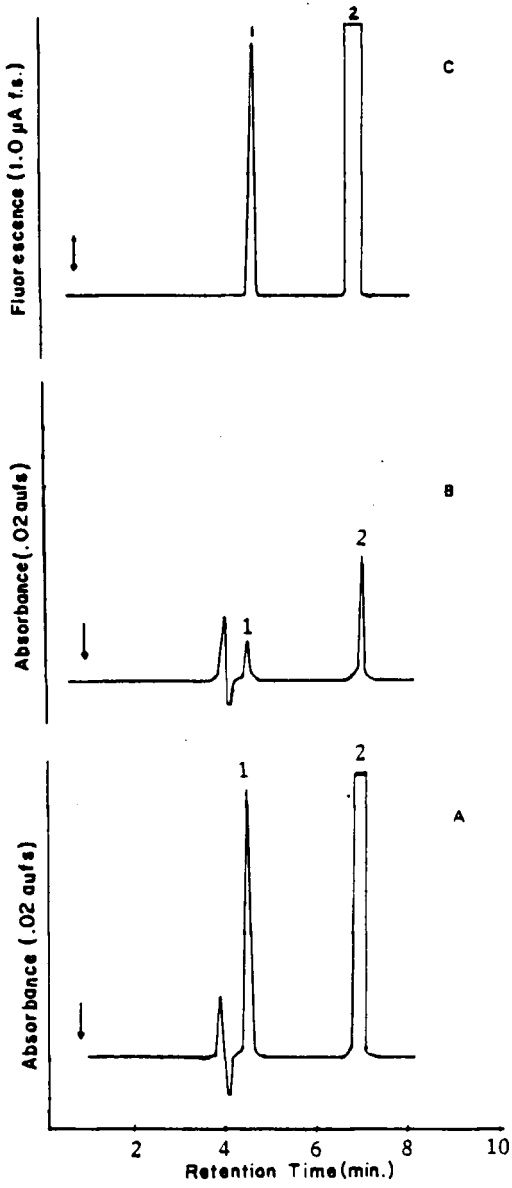

FIGURE 1

(A) Chromatogram of a standard mixture of salicylic acid (1) and naproxen (2) in 0.1N NaOH, detected by absorbance at 235 nm.

(B) Chromatogram of the mixture detected by absorbance at 254 nm.

(C) Chromatogram of the mixture detected by fluorescence excitation at 240 nm and emission greater than 340 nm.

Chromatographic conditions: column; μBondapak C_{18}, eluent; methanol-0.1M acetic acid (70:30, v/v), mode; isocratic, flow rate; 1.0ml/min, temperature; ambient.

Chromatographic peaks were identified using a combination of techniques. Initially the retention times of peaks of sample components were compared with those obtained for reference standards. After tentative identifications were made on this basis, the absorbance peak height ratios of standards and sample components were investigated so that more positive identifications could be made. Table 1 also presents the data obtained for the two major peaks present in the chromatograms of serum extraction products. Correlation of retention time and peak height ratio data indicate the presence of naproxen and salicylic acid in the serum analyzed. A third method of peak identification utilizes the fluorescence characteristics of the species of interest. By using a flow-through fluorescence detector in series with UV absorption detectors, measurements selective for naproxen and salicylic acid were obtained.

The recovery of naproxen and salicylic acid from serum was tested by a series (n=25) of extractions of known quantities of drug standards added at 10-100 μg/ml levels to serum pools. Recoveries of 78 ± 6% of naproxen were obtained. The same procedure yielded average recoveries of 69 ± 2% for salicylic acid from serum pools. There were no interferences from other serum components in the HPLC analysis of naproxen and salicylic acid.

TABLE 1
HPLC Retention Times and Peak Height Ratios of Naproxen and Salicylic Acid

	Naproxen	Salicylic Acid	Serum Peak 1	Serum Peak 2
t_r, min	7.00±0.12	4.44±0.14	4.46±0.16	6.82±0.08*
A_{235}/A_{254}	15.1±0.7	2.93±0.16	2.51±0.22	14.5±0.5
A_{235}/A_{240}	1.69±0.06	1.07±0.05	1.29±0.10	1.68±0.65

*mean ± standard deviation

Using the procedures described, the sera from five females who had been administered naproxen for rheumatoid arthritis conditions were assayed. Table 2 shows the daily treatment regimens and the naproxen serum concentrations obtained by HPLC analysis of triplicate aliquots of each sample. There was considerable range in the levels of naproxen in the sera of the 5 patients. The drug concentration in the serum of patient 3 was significantly lower than the serum drug concentrations of the other four patients even though patients 1 and 5 were on lower daily dosages. Patient 5 had taken aspirin concurrently with the naproxen and the salicylic acid peak was present in the chromatogram of the serum of that patient (Figure 2). The concentration of salicylate in the serum of patient 5 was 20 μg/ml.

CONCLUSIONS

The reverse-phase HPLC method described for routine determinations of serum levels of naproxen and salicylic acid can be useful in the clinical laboratory. Since it is fast, sensitive, and reproducible, the data obtained can be valuable to the clinician in monitoring compliance and in individualizing a program of optimal drug therapy for each patient. The assay will also be useful in pharmacokinetic studies and can be adapted for assays of other biopharmaceuticals used in the treatment of rheumatoid arthritis.

TABLE 2

	Naproxen Daily Dose Levels	Serum Concentrations
Patient	Daily Dose(mg)	Obtained by HPLC Analysis
1	500	30.0±1.8*
2	750	54.9±3.3
3	750	21.9±1.3
4	750	44.1±2.6
5	500	56.8±2.8

*mean ± standard deviation

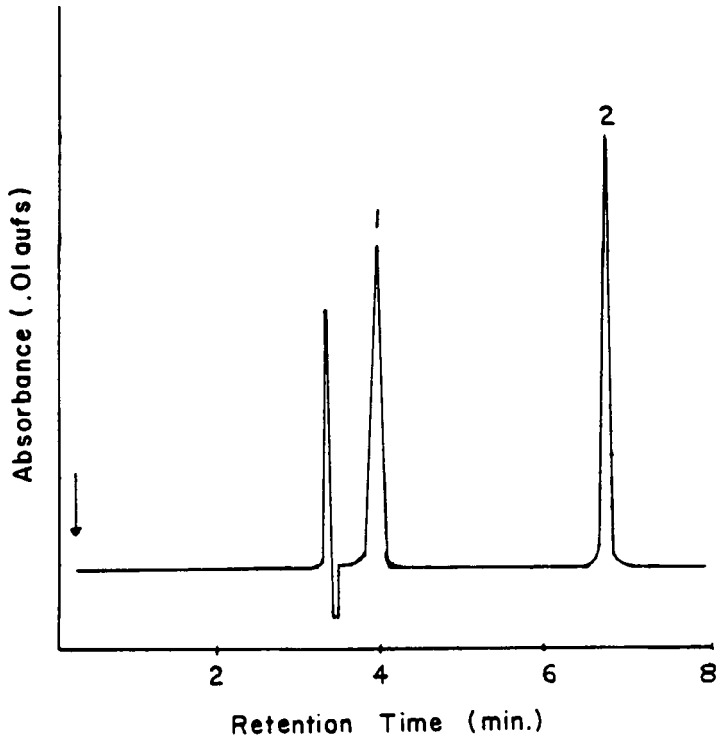

FIGURE 2

Chromatogram of extraction product from serum collected from a patient using naproxen and aspirin therapy for a rheumatoid arthritis condition. Chromatographic conditions same as in Figure 1A.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Jack Cohen of Syntex Research for providing a sample of pure naproxen and Mr. Hank Turner of the Roger Williams General Hospital for processing blood samples.

REFERENCES

1. Harrison, I. T., Lewis B., Nelson, P., Rooks, W., Roszkowski, A., Tomolonis, S., and Fried, J. H., J. Med. Chem., 13, 203 (1970).

2. Dorfman, R. I., Arzneimittel-Forsch, (Drug Res.), 25, 278 (1975).

3. Roszkowski, A. P., Rooks, W. H., Tomolonis, A. J., and Miller, L. M., J. Pharmacol. Exp. Ther., 179, 114 (1971).

4. Lombardino, J. G., Otterness, I. G., and Wiseman, E. H., Arzneimittel-Forsch, (Drug Res.), 25, Nr. 10, 1629 (1971).

5. Runkel, R., Chaplin, M., Boost, G., Segre, E., and Forchielli, E., J. Pharm. Sci., 61, 703 (1972).

6. Runkel, R., Forchielli, E., Sevelius, H., Chaplin, M. and Segre, E., Clin. Pharmacol. Ther., 15, 261 (1974).

7. Lussier, A., Mykal, A., Menard, D., Camerlain, M. and Arsenault, A., Arzneimittel-Forsch, (Drug Res.), 25, Nr. 2a, 294 (1975).

8. Messias, A. R., Brito, A. S. and DeOliviera, I. L., J. Clin. Pharmacol., 15, 324 (1975).

9. Segre, E. J., J. Clin. Pharmacol, 15, 316 (1975).

10. Katona, G., Pan American Congr. of Rheumatol., Punta del Este, Uruguay, Feb. 1971.

11. Lussier, A., Seventh Annual Mtg. of the Canad. Soc. of Chemotherapy, Mont Orford, Quebec, May 1971.

12. Barnes, C. G., Goodman, H. V., Eade, A. W. T., Misra, H. N., Cochrane, G. M., Clarke, A. K., and Stoppard, M., J. Clin. Pharmacol., 15, 347 (1975).

13. Hill, H. F. H. and Hill, A. G. S., J. Clin. Pharmacol., 15, 355 (1975).

14. Wilkens, R. F., Case, J. B., and Huix, F. J., J. Clin. Pharmacol., 15, 363 (1975).

15. Arsenault, A., Varady, J., Lebel, E. and Lussier, A., J. Clin. Pharmacol., 15, 340 (1975).

16. Roth, S. H., and Boost, G., J. Clin. Pharmacol., 15, 378 (1975).

17. Boost, V. G., Arzneimittel-Forsch, (Drug Res.), 25, Nr. 2a, 281 (1975).

18. Chignell, C. F., and Starkweather, D. K., Molecular Pharmacol., 7, 229 (1971).

19. Desager, J. P., Vanderbist, M., and Harvengt, C., J. Clin. Pharmacol., 16, 189 (1976).

20. Dromgoole, S. H., Furst, D. E. and Paulus, H. E., J. High Resolut. Chromatogr. Commun., 2, 82 (1979).

21. Chaplin, M. D., Biochem. Pharmacol., 22, 1589 (1973).

22. Antilla, M., J. Pharm. Sci., 66, 433 (1977).

23. Thompson, G. F., and Collins, J. M., J. Pharm. Sci., 62, 937 (1973).

24. Hartwick, R. A. and Brown, P. R., J. Chromatogr., 126, 679 (1976).

25. Westerlund, D., and Theodorsen, A., J. Chromatogr., 144, 27 (1977).