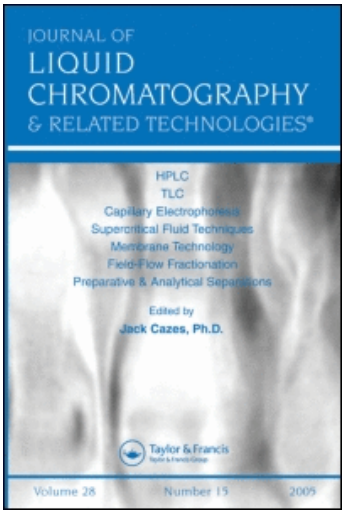

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Chromatographic Separation of Underivatized Naproxen Enantiomers

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CHROMATOGRAPHIC SEPARATION OF UNDERIVATIZED NAPROXEN ENANTIOMERS

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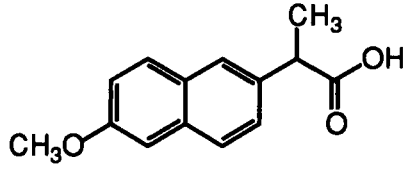
ABSTRACT

The enantiomers of underivatized Naproxen are resolved on a previously described commercially available HPLC chiral stationary phase (CSP). This same CSP also separates the enantiomers of a variety of ester and amide derivatives of Naproxen.

INTRODUCTION

Naproxen, shown below, is the fifth largest selling prescription drug in the world[1], being widely used in the treatment of arthritis. Commercial



preparations of the drug consist of the nearly pure, pharmacologically active (*S*) enantiomer[2]. As the only member of the important α-aryl propionic acid family to be sold as a single enantiomer, Naproxen has received considerable attention from the practitioners of asymmetric synthesis and separation science. Indeed, with the increasing regulatory interest in enantiomerically pure drug formulations,[3] Naproxen has become something of a standard in the field of enantiomer separations. A variety of strategies for the asymmetric synthesis or classical resolution of Naproxen enantiomers have been reported. Concurrently, a number of analytical techniques for the chromatographic separation of Naproxen enantiomers have been developed. A recent review describes many of the methods reported to date for the chromatographic separation of the enantiomers of Naproxen and other non-steroidal anti-inflammatory drugs (NSAIDs).[4] Many of these techniques require derivatization. Herein is described a convenient method for the determination of enantiomeric purity of either underivatized or derivatized Naproxen by HPLC using a previously reported chiral stationary phase (CSP) which is now available commercially in either enantiomeric form.

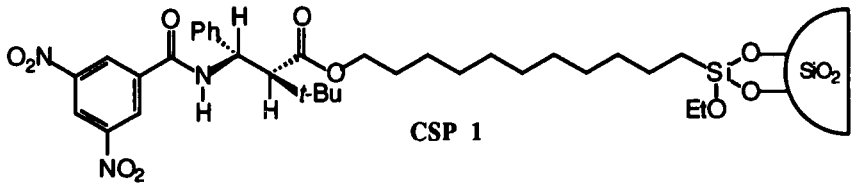
BACKGROUND

Derivatization in Chromatographic Analysis

Ideally, analytical procedures for the determination of enantiomeric purity would require no prior derivatization of the sample since this would entail an increase in time and cost, and introduce a possible source of error. In practice, derivatization is used to enhance detection and to improve chromatographic behavior. Most of the methods reported to date for the chromatographic determination of the enantiomeric purity of Naproxen require derivatization, some even employing a chiral derivatizing reagent so as to form diastereomers. Enantiomeric purity determinations which utilize

chiral derivatizing reagents suffer, in principle, from several drawbacks including: *i*) the rate of reaction of the chiral reagent with individual enantiomers may be different, leading to a ratio of diastereomers which does not reflect the initial ratio of analyte enantiomers; *ii*) the enantiomeric purity of the chiral reagent must be scrupulously maintained; furthermore, partial racemization of the analyte or reagent during the course of the derivatization procedure must be avoided; and *iii)* the diastereomeric products obtained may give non-identical detector responses, thus requiring additional validation procedures.

We recently reported[5,6] the separation of the enantiomers of Naproxen and a number of other NSAIDs as anilide derivatives on a recently developed π-acidic CSP (CSP 1).[7] We have subsequently discovered that



CSP 1

this CSP is also capable of separating the enantiomers of simple ester and amide derivatives of Naproxen. Under certain mobile phase conditions, the baseline resolution of underivatized Naproxen enantiomers is also possible.

Direct Chromatographic Separation of Naproxen Enantiomers

Several other methods for the chromatographic separation of underivatized Naproxen enantiomers have been reported. Oi and coworkers reported modest enantioselectivities for several underivatized NSAID enantiomers using the previously described DNB phenylglycine CSP,[8] while Okamoto and coworkers reported separation of several underivatized NSAIDS using polysaccharide-derived CSPs.[9] Impressively, Hermansson and Eriksson[10] report an α of greater than four using an α-1-acid glycoprotein CSP and an achiral ion pairing reagent. In a personal

communication, Hermansson has informed us that using optimized conditions with an improved version of the α-1-AGP CSP an α of 15 can be obtained for this separation. While this is impressive enantioselectivity, separation of enantiomers using stationary phases derived from proteins or other biopolymers suffers from several drawbacks. Since proteins are available in only one enantiomeric form, one cannot easily reverse elution orders, something which is often desirable in analytical determinations of enantiomeric purity. Protein-derived CSPs are generally usable with only a relatively narrow range of mobile phases and are oftentimes relatively short-lived. In addition, owing to the extremely low concentration of binding sites on the protein, preparative scale resolutions are not feasible.

The fact that a protein-based CSP has generated a separation factor of 15 for the enantiomers of Naproxen is encouraging. A synthetic receptor containing the same disposition of the functional groups responsible for chiral recognition in the protein would be expected to afford still better separations owing to the fact that it, unlike the protein, need contain no superfluous functionality which may give rise to achiral or reverse-sense retention of the analyte.

Diederich's Naproxen Receptors

In addition to the chromatographic methods discussed thus far, some mention should be made of the work of Diederich and co-workers, which has focused on the enantioselective inclusion of Naproxen guest molecules by specially designed chiral hosts.[11-15] To date, moderate association constants ($K_a = 2.5 \times 10^3$) and modest enantioselectivities ($\alpha = 1.32$) have been seen for underivatized Naproxen, whereas the results are somewhat better for esters and amides of Naproxen ($\alpha = 1.76$ in the best case).

We herein report the separation of underivatized Naproxen enantiomers as well as simple ester or amide derivatives of Naproxen using the now commercially available CSP 1. On this CSP, the observed enantioselectivity exceeds any yet reported for amide derivatives of Naproxen, and is suitable for quantitation of the enantiomeric purity of underivatized Naproxen.

MATERIALS AND METHODS

Apparatus

Chromatographic analysis was performed using a Beckman-Altex 100-A pump, a Rheodyne model 7125 injector with a 20μl sample loop, a Beckman 153 UV absorbance monitor (254 nm), and a Hewlett-Packard HP 3394-A integrating recorder.

Materials

The preparation of CSP 1 was described previously.[7] In addition, this CSP is now commercially available from Regis Chemical Company, Morton Grove, IL in either enantiomeric form. Optically pure (*S*) and racemic Naproxen were kindly provided by Sepracor, Inc., Marlborough, MA. Naproxen ester derivatives were prepared by Fisher esterification with the appropriate alcohol, followed by chromatographic purification. Naproxen amide derivatives were prepared by reaction of the appropriate amine with either Naproxen acid chloride or the N-hydroxysuccinimide active ester of Naproxen, followed by chromatographic purification. All Naproxen derivatives were suitably characterized by ^{1}H NMR. Solvents used were of HPLC grade or were distilled prior to use.

Methods

All chromatographic experiments were carried out at a nominal flow rate of 2.00 ml/min. Column void time was determined by injection of tri-*t*-butylbenzene.[16]

RESULTS AND DISCUSSION

The separation of the enantiomers of some simple derivatives of Naproxen on CSP 1 are shown below in Table 1. All of the derivatives were baseline resolved, with amide derivatives generally showing greater

TABLE 1
Enantiomer Separations for Naproxen Derivatives on CSP 1

Compound	k'1	α
methyl ester	1.31	1.11
ethyl ester	1.15	1.20
n-butyl ester	1.03	1.22
n-butyl amide	9.69	2.45
t-butylamide	9.31	2.59
dimethylamide	4.77	2.66
diethylamide	3.31	3.60
di *n*-hexylamide	1.31	4.11
piperidine amide	7.46	3.17

Conditions: Flow rate = 2.0 ml/min; mobile phase = 20% 2-propanol in hexane; room temperature.

enantioselectivity than the esters. Among the amide derivatives, those formed from secondary amines were seen to give higher enantioselectivities than those formed from primary amines.

Injection of underivatized Naproxen free acid under these mobile phase conditions gives some separation of enantiomers. However, unlike the ester and amide derivatives, the resolution is quite poor owing to band broadening. Addition of a small amount of acetic acid has a dramatic effect upon bandshape and near baseline resolution of underivatized Naproxen becomes possible as is shown in Table 2.

Actual chromatograms of racemic and (*S*) Naproxen are shown in Figure 1. It can readily be seen that resolution is sufficient for precise enantiomeric purity determination without derivatization. Furthermore, we report that these chromatograms were obtained with a column which has been in daily use in our laboratories for several years. Thus, in contrast to some biopolymer derived CSPs, CSP 1 is quite durable.

Using a mobile phase consisting of 20% 2-propanol in hexane containing 0.1% acetic acid and 0.1% triethylamine, improved separation of underivatized Naproxen enantiomers is obtained (k'1 = 6.68; α =1.27).

TABLE 2
Effect of Added Acetic Acid on Resolution of Naproxen Enantiomers

% HOAc	k'1	α	Rs
0	5.23	1.19	0.3
0.01	4.80	1.21	1.2
0.10	4.56	1.22	1.3

Conditions: Column = CSP 1; Mobile phase = 2.5 % 2-propanol in hexane with varying amounts of added acetic acid; Flow rate = 2.0 ml/ min; room temperature.

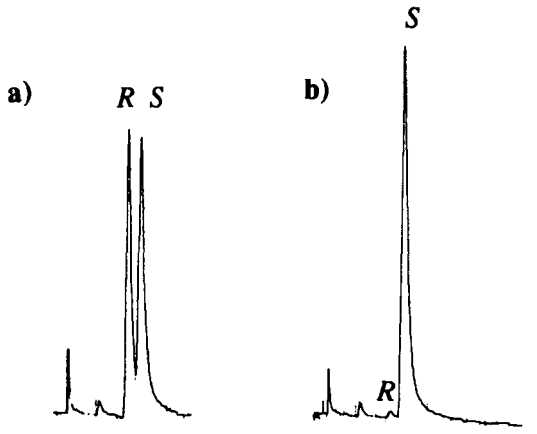


Figure 1: Separation of racemic (a) and (*S*) enriched (b) underivatized Naproxen enantiomers. Column = (*R*,*R*) CSP 1; Mobile phase = 2.5% 2-propanol in hexane with 0.10% added acetic acid; Flow rate = 2.0 ml/min.; temperature = room temperature; detection = UV at 254 nm; injection size = 5 μg Naproxen with 5 μg tri-*t*-butylbenzene added as a void volume marker.[16]

Reversed phase separations of underivatized Naproxen enantiomers on CSP 1 have also been briefly investigated. To date, with mobile phases such as 70% MeOH/H_2O containing 0.1 % HOAc, marginal separations ($\alpha = 1.05$) and poor resolutions ($R_S < 0.1$) have been obtained. Improved reversed phase resolution of the enantiomers of amino acid derivatives have been achieved by the use of achiral ion pair reagents as mobile phase additives.[17] Similar studies involving Naproxen are currently underway.

The Challenge of Developing Improved Naproxen Receptors

Even though CSP 1 is capable of separating the enantiomers of underivatized Naproxen, it is important to develop selectors of even greater enantioselectivity for use in preparative applications. Whereas extremely high enantioselectivity can actually be a hindrance in an analytical determination of enantiomeric purity, preparative chromatographic separations, as well as enantioselective membrane-based separations,[18] greatly benefit from large separation factors. By studying the mechanism of chiral recognition of Naproxen on CSP 1 and similar selectors, one might hope to gain sufficient mechanistic insight as to allow the design of selectors of greater enantioselectivity. Such studies are currently underway.

REFERENCES

1. Haggin, J., Monsanto Advances Chiral Drug Synthesis, *Chem. Eng. News*, May 7, 58, 1990.

2. Ariens, E. J., Chiral Separations by HPLC, Applications to Pharmaceutical Compounds, Krstulovic´, A. (Ed.), John Wiley & Sons, New York. 1989.

3. DeCamp, W. H., The FDA Perspectives on the Development of Stereoisomers, *Chirality*, **1**, 2, 1989.

4. Bojarski, J., Chromatography of Enantiomers of 2-Arypropionic Acids, *J. Liq. Chrom.*, **12**, 2685, 1989.

5. Pirkle, W. H.; McCune, J., Improved Chiral Stationary Phase for the Separation of the Enantiomers of Chiral Acids as their Anilide Derivatives, *J. Chromatogr.*, **471**, 271, 1989.

6. Pirkle, W. H.; Murray, P. G., The Separation of the Enantiomers of a Variety of Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) as their Anilide Derivatives Using a Chiral Stationary Phase, *J. Liq. Chromatogr.*, **13**, 2123, 1990.

7. Pirkle, W. H.; McCune, J., An Improved Chiral Stationary Phase for the Facile Separation of Enantiomers, *J. Chromatogr.*, **441**, 311, 1988.

8. Oi, N.; Matsumoto, Y.; Kitahara, H.; Miyazaki, H., Direct Separation of Underivatized alpha-Methylarylacetic Acid Enantiomers by High Performance Liquid Chromatography with Chiral Stationary Phase, *Bunseki Kagaku*, **35**, 312, 1986 (abstract).

9. Okamoto, Y.; Aburatani, R.; Kaida, Y.; Hatada, K.; Inotsume, N.; Nakano, M., Direct Chromatographic Separation of 2-Arylpropionic Acid Enantiomers Using Tris(3,5-Dimethylphenylcarbamate)s of Cellulose and Amylose as Chiral Stationary Phases, *Chirality*, **1**, 239, 1989.

10. Hermansson, J.; Eriksson, M., Direct Liquid Chromatographic Resolution of Acidic Drugs Using a Chiral α 1-Acid Glycoprotein Column (Enantiopac®), *J. Liq. Chromatogr.*, **9**, 621, 1986.

11. Rubin, Y.; Dick, K.; Diederich, F.; Georgiadis, T., Chiral Recognition in Aqueous Solution *J. Org. Chem.*, **51**, 3270, 1986.

12. Dharanipragada, R.; Diederich, F., Diastereomeric Complex Formation Between a Novel Optically Active Host and Naproxen in Aqueous Solution,*Tet. Lett..*, **28**, 2443, 1987.

13. Dharanipragada, R.; Ferguson, S.; Diederich, F., A Novel Optically Active Host: Design, Computer Graphics, Synthesis, and Diastereomeric Complex Formation in Solution, *J. Amer. Chem. Soc.* **110**, 1679, 1988.

14. Castro, P.; Georgiadis, T.; Diederich, F., Chiral Recognition in Clefts and Cyclophane Cavities Shaped by the 1,1'-Binaphthyl Major Groove, *J. Org. Chem.*, **54**, 5835, 1989.

15. Georgiadis, T.M.; Georgiadis, M.M.; Diederich, F., Synthesis and Complexation Properties of a Water-Soluble Optically Active Cyclophane Incorporating a 4-Naphthyl-1,2,3,4-Tetrahydroisoquinoline Unit as a Chiral Spacer, *J. Org. Chem.*, **56**, 3362, 1991.

16. Pirkle, W. H.; Welch, C. J., A Convenient Void Volume Marker for Several Chiral HPLC Columns, *J. Liq. Chromatogr.*, 14, 1, 1991.

17. Pirkle, W. H.; Chang, J. P.; Burke, J. A., Use of Achiral Ion-Pairing Reagents with Chiral Stationary Phases, *J. Chromatogr.*, **479**, 377, 1989.

18. Pirkle, W. H.; Doherty, E. M., Enantioselective Transport Through a Silicone-Supported Liquid Membrane, *J. Amer. Chem. Soc.*, **111**, 4113, 1989.