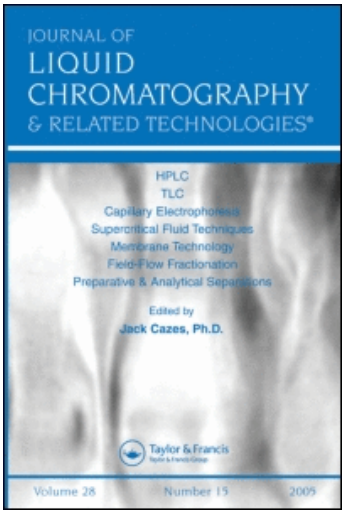

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THE SEPARATION OF THE ENANTIOMERS OF A VARIETY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) AS THEIR ANILIDE DERIVATIVES USING A CHIRAL STATIONARY PHASE

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ABSTRACT

Widespread demand for accurate determinations of the enantiomeric purity of pharmaceuticals used in synthetic and pharmacological research has increased the need for reliable and efficacious methods for separating the stereoisomers of a variety of biologically active compounds. Here we present a procedure for the separation of the enantiomers of a number of non-steroidal anti-inflammatory drugs (NSAIDs), as their anilide derivatives, by HPLC using chiral stationary phases. The chromatographic conditions, the chiral stationary phase, and the choice of the derivitizing amine can be altered to accomodate various requirements.

INTRODUCTION

It is widely recognized that the enantiomers of a given pharmaceutical will often show pronounced differences in their

biological activity. Consequently, the need arises frequently to determine the enantiomeric purity of such compounds, often when there are but traces of the enantiomeric impurity present. The non-steroidal anti-inflammatory drugs (NSAIDs) are one class of pharmaceutical compounds which exhibit this enantiospecific activity. As such, a number of methods have been developed which utilize gas or liquid chromatography to determine the enantiomeric purity of these compounds (1,2). In some methods, the racemic or optically enriched NSAID sample is derivitized with a single enantiomer of a chiral reagent to afford a diastereomeric mixture of derivatives which can be analyzed by HPLC or GC. Such procedures, however, can be prone to error (3).

The use of HPLC with chiral stationary phases (CSPs) to effect the separation of the enantiomers of many NSAIDs, especially the profens, has been reported (4,5). Using chiral stationary phases developed in these laboratories, some of which are now commercially available, one may easily separate the enantiomers of anilide derivatives of a number of NSAIDs. The derivatization can be performed in a straightfoward manner using inexpensive achiral arylamines. While the use of 3,5-dimethylaniline is emphasized herein, other aromatic amines can be employed to tailor selectivity, detectability, or retention for a particular purpose. The derivatization may be performed in either organic or aqueous media, and either normal or reverse phase mobile phases can be used for the chromatography.

EXPERIMENTAL

Apparatus

All chromatography was performed using an Anspec-Bischoff Model 2200 isocratic high performance liquid chromatography pump, a Rheodyne model 7125 injector with a 20 μl sample loop, and 250 x 4.6 mm stainless steel columns packed with chiral stationary phases bonded to 5 μ spherical silica particles. The column containing CSP III was obtained from the Regis Chemical Company. Two Milton-Roy UV Monitor D fixed wavelength detectors (254 and 280 nm) were connected in series, followed by a Kipp & Zonen Model BD 41 dual pen chart recorder.

Derivatization

The NSAIDs, most of which are α-aryl proprionic acids, were, in the early stages of this work, converted to the corresponding anilide through the agency of 1,3-dicyclohexylcarbodiimide (DCC) as described below. Midway through the project, however, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, (EDC), was adopted as the coupling reagent. This reagent, as well as its byproducts, are water soluble and can be easily removed from the reaction mixture by extraction with dilute acid.

General synthesis of the NSAID anilides

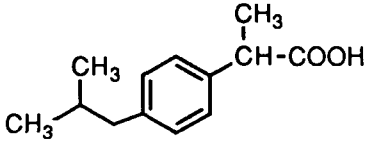
Equal quantities (*ca* 10 mg) of the NSAID and the coupling reagent, either DCC or EDC, were added to a 5-ml screw-capped test

tube followed by two drops of the aniline and 1.5 mL of dichloromethane. After 30 min., 1 ml of 1 M hydrochloric acid was added, the mixture shaken vigorously, and the aqueous upper layer removed with a Pasteur pipet. The lower layer was washed with water, and can then be dried over anhydrous magnesium sulfate for normal phase analysis or injected directly for reverse phase analysis.

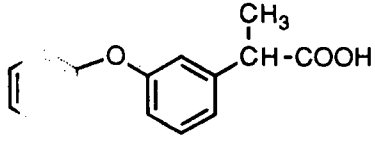
The synthesis of anilides via carbodiimide coupling reagents is of relatively wide scope, and has been used extensively in earlier studies. Many chiral acids, including several of the profens, were previously derivatized on a larger scale by the method described (4). All NMR and elemental analysis data are in accord with the expected reaction products.

RESULTS AND DISCUSSION

For convenience, the structures of the NSAIDS surveyed are reproduced below:



IBUPROFEN



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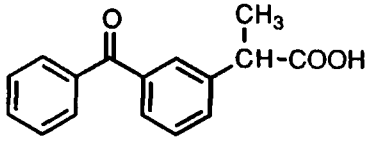
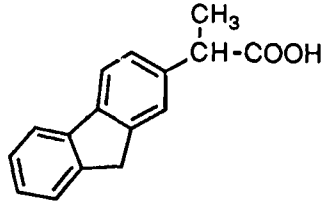
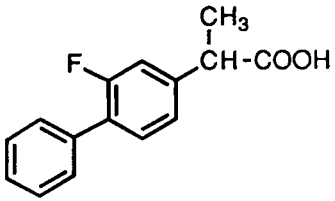


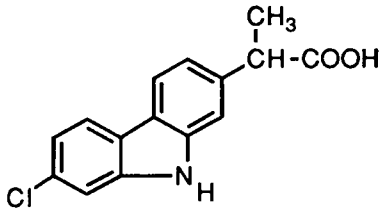
KETOPROFEN



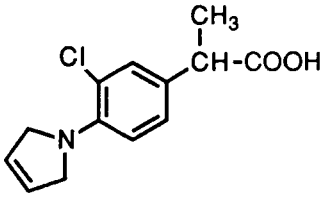
CICLOPROFEN



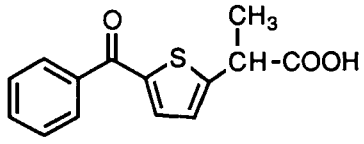
FLURBIPROFEN



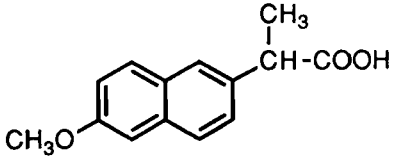
CARPROFEN



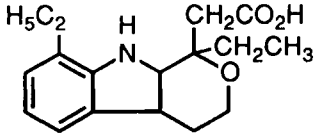
PIRPROFEN



TIAPROFENIC ACID

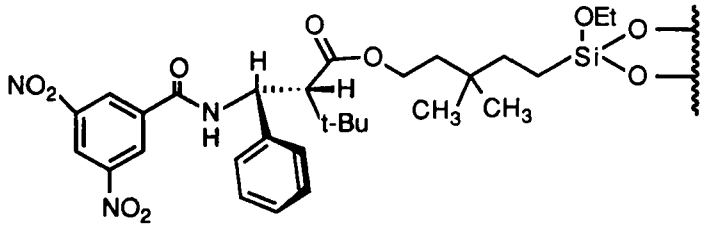


NAPROXEN

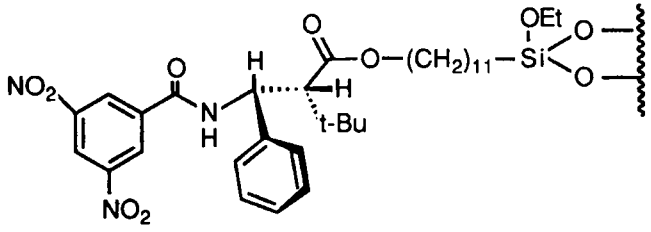


ETODOLAC

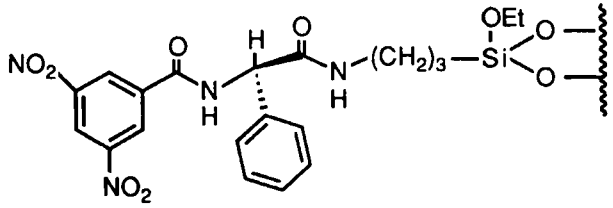
Three chiral stationary phases were used in the study, the structures of which are shown below:



CSP I



CSP II



CSP III

The synthesis and evaluation of CSPs II and III have been reported (6,7). CSP I was made in a manner analogous to that reported for CSP II. The details concerning the preparation and evaluation of this CSP will be discussed elsewhere. Note that CSP I differs from CSP II only in the structure of the connecting arm.

The enantiomers of each of the ten NSAIDs studied are separable as their 3,5-dimethylanilides or their α-naphthylamides on one or more of the CSPs. Table I provides chromatographic data for the normal phase separation of these analytes on CSP I, while Table II shows the reverse phase behavior of the same analytes on CSP I utilizing two different mobile phases. Resolution values listed in the tables were calculated as $2t / w_1 + w_2$, where t = the distance between the peaks and w = the width of each peak at its base.

TABLE I

Normal Phase Separation on (2*R*, 3*R*)-CSP I of Some NSAIDs As Their 3,5-Dimethylanilide Derivatives

NSAID	α^a	$k'_1{}^b$	$R_s{}^c$
IBUPROFEN	1.68	1.53	5.33
NAPROXEN	2.21	4.60	12.30
FLURBIPROFEN	1.80	2.10	6.40
CARPROFEN	1.78	2.93	7.16
FENOPROFEN	1.54	2.27	8.00
PIRPROFEN	1.99	2.87	8.00
CICLOPROFEN	2.09	3.17	11.55
ETODOLAC	1.23	0.87	*
TIAPROFENIC ACID	1.66	3.90	7.80
KETOPROFEN	1.60	2.63	5.87

[a] Chromatographic separation factor

[b] Capacity factor for the first eluting enantiomer using 20% (v/v) 2-propanol in hexane as the mobile phase; flow rate 2 ml/min

[c] resolution ; * indicates resolution less than 1.5

Baseline resolution ($R_S > 1.5$) was achieved under normal phase conditions for all NSAIDs but etodolac, which differs significantly in its structure from the other NSAIDs.

Under reverse phase conditions using a mobile phase of 9:1 methanol-water on CSP I, ketoprofen was barely resolvable, but the enantiomers were readily separated when the more polar 8:2 methanol-water mobile phase was utilized. It is interesting to note that, as usual, the separation factors, α, for the enantiomers tend to be larger under normal phase conditions than under reverse phase conditions, and that retention is lessened using reverse phase conditions, shortening analysis time. Comparing capacity factors, then, it becomes apparent that one can alter retention with slight

TABLE II

Reverse Phase Separation on (2*R*, 3*R*)-CSP I of Some NSAIDs As Their 3,5-Dimethylanilide Derivatives

NSAID	α^a	$k'_1{}^b$	$R_s{}^c$	α^d	$k'_1{}^e$	$R_s{}^f$
IBUPROFEN	1.24	1.40	2.50	1.25	2.50	3.27
NAPROXEN	1.30	2.83	4.00	1.52	2.50	4.10
FLURBIPROFEN	1.13	2.50	2.86	1.22	2.97	3.00
CARPROFEN	1.24	2.83	1.74	1.33	4.00	5.57
FENOPROFEN	1.11	2.00	*	1.15	2.67	2.20
PIRPROFEN	1.25	2.40	2.31	1.77	3.53	3.73
CICLOPROFEN	1.26	1.73	3.25	1.42	4.10	5.55
ETODOLAC	*	2.40	*	1.04	3.07	*
TIAPROFENIC ACID	1.07	2.37	2.00	1.34	2.93	1.33
KETOPROFEN	*	2.26	*	1.42	4.10	2.33

[a] Chromatographic separation factor using 9:1 (v/v) methanol-water as the mobile phase

[b] Capacity factor for the first eluting enantiomer using 9:1 (v/v) methanol-water as the mobile phase; flow rate 1 ml/min

[c] Resolution using 9:1 (v/v) methanol-water as the mobile phase

[d] Chromatographic separation factor using 8:2 (v/v) methanol water as the mobile phase

[e] Capacity factor for the first eluting enantiomer using 8:2 (v/v) methanol-water as the mobile phase; flow rate 1 mL/min

[f] Resolution using 8:2 (v/v) methanol-water as the mobile phase

* Enantiomers barely resolvable; resolution less than1.5

modifications in the mobile phase. The advantage of this capability becomes clear when other interferring materials are present, as might be the case when analyzing urine or plasma samples.

Another method of altering retention as well as increasing selectivity and detection sensitivity involves selection of the appropriate derivatizing amine. Table III presents data which allows comparison of derivatizations using α-naphthylamine

TABLE III

Reverse Phase Separation on (2*R*, 3*R*)-CSP II of Some NSAIDs As Their α–Naphthylamide Derivatives

NSAID	α^a	$k'_1{}^b$	$R_s{}^c$
IBUPROFEN	1.79	1.27	7.43
NAPROXIN	1.22	5.73	2.00
FLURBIPROFEN	2.00	1.80	6.00
CARPROFEN	2.27	3.53	9.67
FENOPROFEN	1.43	1.17	3.43
PIRPROFEN	2.17	2.93	8.40
CICLOPROFEN	2.40	4.33	8.18
ETODOLAC	*	2.46	*
TIAPROFENIC ACID	1.64	1.93	6.72
KETOPROFEN	1.55	1.63	4.16

[a] Chromatographic separation factor

[b] Capacity factor for the first eluting enantiomer using 9:1 (v/v) methanol-water as the mobile phase; flow rate 1.5 ml/min

[c] Resolution

* Enantiomers barely resolvable or resolution less than 1.5

instead of 3,5-dimethylaniline as the derivitizing amine. All data in Table III were collected reverse phase using CSP II. Resolution is good in every case except etodolac.

One advantage of derivatizing NSAIDs with α-napthylamine or similar polycyclic arylamines is that they contain strongly absorbing chromophores, which enhance sensitivity by ultraviolet detection and also permit the use of fluorescence detectors, allowing further reduction in detection limits. Detecting NSAID enantiomers or stereoisomeric metabolites of the NSAIDs at relatively low physiological concentrations should then be relatively straightfoward.

TABLE IV

Normal Phase Separation on D-CSP III of Some NSAIDs As Their 3,5-Dimethylanilide Derivatives

NSAID	α[a]	k'_1[b]	R_s[c]
IBUPROFEN	1.30	1.23	1.66
NAPROXEN	1.17	9.10	1.46
FLURBIPROFEN	1.26	2.27	3.00
CARPROFEN	1.09	6.90	0.50
FENOPROFEN	1.25	2.50	2.57
PIRPROFEN	1.27	4.30	2.06
CICLOPROFEN	1.22	6.00	1.86
ETODOLAC	1.35	1.33	1.87
TIAPROFENIC ACID	1.13	7.50	1.30
KETOPROFEN	1.18	4.97	1.53

[a] Chromatographic separation factor

[b] Capacity factor for the first eluting enantiomer using 20% 2-propanol in hexane as the mobile phase; flow rate 2.0 ml/min

[c] Resolution

A synthetic mixture of the four stereoisomeric ibuprofen diacid metabolites (formed by oxidation of one of the isobutyl substituent's methyl groups) was converted to the bis-3,5-dimethylanilides. These compounds are also readily separated from one another and from the earlier eluting enantiomers of the corresponding ibuprofen derivatives under the aforementioned conditions.

For comparative purposes, the separation of the enantiomers of the NSAID derivatives was undertaken using CSP III, a commercially available phase derived from phenylglycine. This CSP also permits facile separation of the anilide derivatives of the NSAIDs under consideration, as shown in Table IV. However, this CSP

affords poor separations for many of the NSAID enantiomers when used under reverse phase conditions.

CONCLUSION

The enantiomers of a wide variety of anilide derivatives of non-steroidal anti-inflammatory drugs are easily and rapidly separated by HPLC using chiral stationary phases. By altering the polarity of the mobile phase, the amine used in the derivatization, and the chiral stationary phase utilized, one can effectively tailor the separation to accomodate any number of circumstances. Since the derivitization can be performed in either organic or aqueous media, the method may prove valuable as a means for monitioring the pharmacokinetics of NSAID metabolism.

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