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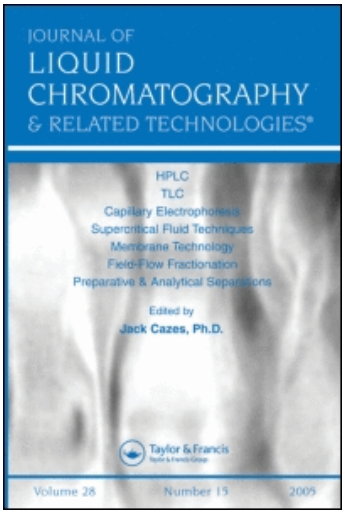

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ENANTIOMER SEPARATION OF 2-ARYLPROPIONIC ACIDS ON AN ERGOT ALKALOID-BASED STATIONARY PHASE MICROBORE COLUMN APPLICATION

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ABSTRACT

The separation of enantiomers of underivatized ketoprofen, as well as a number of other non-steroidal anti-inflammatory drugs, was studied by high performance liquid chromatography using a recently developed chiral stationary phase packed in a microbore column. Electrostatic and π-π interactions are assumed to control the retention of the analytes. The enantioselectivity remains constant in a wide range of organic modifier concentration, and is restricted to a pH window ranging from 2.5 to 5.0.

INTRODUCTION

2-Arylpropionic acids (profens) represent an important class of non-steroidal anti-inflammatory drugs (2-APA-NSAIDs), all commercialised as

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racemic mixtures, with the exception of naproxen, which is used as the pure (S)-isomer. It is well known that only the (+)(S)-form of 2-APA-NSAIDs exhibits biological activity (1). Recent pharmacodynamic studies have shown the (R)-isomer undergoes metabolic unidirectional chiral inversion to its pharmacologically active (S)-antipode (2-5). Use of optically pure profens should permit the reduction of the dosage as well as avoid any toxic effect arising from non-stereospecific mechanisms. Therefore separation methods of profen enantiomers are of growing interest, and chiral separations of these compound by HPLC have been in detail reviewed (6-8). Among such methods the direct ones appear to be of great applicability for the optical purity control in drug production, routine pharmacokinetic analyses, in the field of enzyme stereo-catalysed reactions, as well as in preparative scale separations. 2-APA-NSAIDs are well resolved by LC without derivatization on chiral columns based on α_1- acid glycoprotein (9), bovine and human serum albumin (10,11), ovomucoid (12), cyclodextrins (13), tris(3,5-dimethylphenylcarbamate) derivatives of cellulose and amylose (14). A CSP specially designed for the resolution of underivatized profens has been recently reported by Pirkle et al. (15).

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We have recently described the synthesis of an ergot alkaloid based chiral stationary phase for the resolution of optical isomers by HPLC (16,17) and interpreted by ^{1}H NMR spectroscopy the enantioseparation in terms of electrostatic and π-π interactions (18). This CSP showed to provide good resolution for a series of carboxylic group containing compounds, including amino acid derivatives (19) and underivatized dicarboxylic and arylpropionic acids. We now report the use of this CSP for the resolution of underivatized NSAIDs on a microbore column with the aim of studying the effect of mobile phase parameters on the resolution.

EXPERIMENTAL

Materials and methods

The CSP was prepared using silica gel Exsil 100 (Scientific Glass Engineering, Milton Feynes, UK) (particle size 5 μm, average pore diameter 100 Å), 3-glycidoxypropyltrimethoxysilane (Serva, Heidelberg, Germany) and (+) 1-(3-aminopropyl)-(5R,8S,10R)-terguride (AMP-TER) according to the previously described procedure (16).

Conventional slurry procedure was used to pack a microbore column (500 x 1.1 mm i.d.).

Racemic suprofen was purchased from Sigma (St. Louis, MO, USA). Ibuprofen and flurbiprofen were extracted from commercially available drugs (Nurofen® and Froben®, respectively) using $CHCl_3$ of analytical grade. (R,S)-Fenoprofen, (R,S)-ketoprofen, and (R,S)- and (S)-naproxen were a kind gift of Prof. E. Cernia, Department of Chemistry, "La Sapienza" University of Rome. All solvents were of HPLC grade.

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Instrumentation

The chromatographic apparatus consisted of a Perkin Elmer (Norwalk, CT, USA) Model Series 410 LC solvent delivery pump equipped with a Rheodyne Model 7520 internal loop injection valve (0.5μl), and connected to a Kontron (Milan, Italy) Model 433 UV capillary detector.

RESULTS AND DISCUSSION

The previously obtained results (17) led us to study in more detail the parameters influencing the enantioseparation of underivatized 2-APA-NSAIDs on AMP-TER based chiral stationary phase.

The influence of the organic modifier content in the mobile phase on the separation of naproxen enantiomers was examined under constant values of both, ionic strength and pH. The corresponding capacity and enantioselectivity factors are reported in Table I. The plot of ln k' vs acetonitrile content (Fig. 1) indicates decreasing retention with increasing concentration of organic solvent, which is in good agreement with reversed phase mechanism.

The influence of the buffer pH on the retention for six 2-APA-NSAIDs was examined and chromatographic data are summarised in Table II. Data show that chiral recognition operates in a pH range between 2,5 - 5.0, and is influenced by the nature of the aryl moiety and dissociation degree (pK_a) of the carboxylic acids. Separation of all profens was observed in the whole examined pH range, with the exception of ibuprofen and flurbiprofen, which have not been resolved at pH < 3. Separation factors (α) remain substantially constant for all compounds. An example of baseline enantioseparation of fenoprofen is given in Figure 2.

The plots of ln k' vs pH of the buffer for ibuprofen, fenoprofen and suprofen enantiomers are shown in Figure 3. All pairs of enantiomers eluted with similar retention trend (maximum at pH 4) which is the most probably the result of two interactions involved in the adsorption process. First one could be ascribed to the increased dissociation of the analyte acids, in accordance with the pK_a values. (9), resulting in a stronger electrostatic interaction with the protonated nitrogen atoms in the aminopropyl-terguride molecule. At pH > 4 electrostatic interaction becomes less significant owing to the lower protonation of the selector, and second interaction (hydrophobic) prevails thus resulting in an overall

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TABLE 1
Effect of Acetonitrile Content in Mobile Phase on Capacity Factors (K'), Enantioselectivity (α) and Resolution (R) for Naproxen Enantiomers. Buffer, 0.05 potassium acetate, pH = 4.0.

% AcN	K_R'	K_S'	α#	R∅
30	12.6	13.1	1.04	0.85
40	7.7	8.0	1.04	0.81
50	5.3	5.6	1.04	0.77
60	3.3	3.5	1.06	1.04

$\alpha = K_S / K_R$, ∅ $R = \sqrt{(N/4)} \times [(\alpha - 1)/\alpha] \times [K'/(K'+1)]$

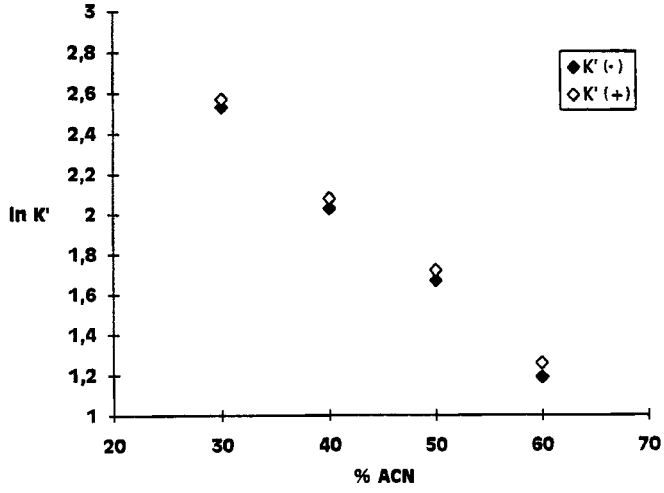

FIGURE 1 Plot of ln k' *vs* acetonitrile content of the mobile phase for naproxen enantiomers. Chromatographic conditions: column, 50 x 0.1 cm i.d.; buffer, 0.05 M potassium acetate at pH 4.0; flow rate, 80 μl / min; UV detector set at 254 mn.

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TABLE 2

Capacity (K') and Enantioselectivity Factors (α) of NSAIDs Enantiomers as a Function of pH. Chromatographic Conditions: column, 50 X 0,1 cm I.D.; buffer, 0.05 M potassium acetate - acetonitrile (50:50 v/v); flow-rate, 80 µl/min; detection UV, 254 nm.

		K_R' (ln K_R')	K_S' (ln K_S')	α
pH = 2.9	IBUPROFEN	1.9 (0.64)	1.9 (0.64)	1.00
	FENOPROFEN	3.5 (1.25)	3.7 (1.31)	1.06
	NAPROXEN	3.9 (1.36)	4.0 (1.39)	1.03
	KETOPROFEN	5.8 (1.76)	6.1 (1.81)	1.05
	FLURBIPROFEN	5.7 (1.74)	5.7 (1.74)	1.00
	SUPROFEN	6.1 (1.81)	6.3 (1.84)	1.03
pH = 3.2	IBUPROFEN	2.4 (0.87)	2.5 (0.92)	1.04
	FENOPROFEN	4.2 (1.43)	4.5 (1.50)	1.07
	NAPROXEN	4.8 (1.57)	5.0 (1.61)	1.04
	KETOPROFEN	---	---	---
	FLURBIPROFEN	7.0 (1.94)	7.2 (1.97)	1.03
	SUPROFEN	8.8 (2.17)	9.1 (2.21)	1.03
pH = 3.6	IBUPROFEN	2.7 (0.99)	2.9 (1.06)	1.05
	FENOPROFEN	---	---	---
	NAPROXEN	5.4 (1.69)	5.7 (1.74)	1.05
	KETOPROFEN	---	---	---
	FLURBIPROFEN	6.7 (1.90)	6.9 (1.93)	1.03
	SUPROFEN	9.3 (2.23)	9.8 (2.28)	1.04
pH = 4.0	IBUPROFEN	4.0 (1.37)	4.2 (1.42)	1.04
	FENOPROFEN	5.5 (1.70)	5.8 (1.76)	1.05
	NAPROXEN	5.3 (1.67)	5.6 (1.72)	1.04
	KETOPROFEN	7.5 (2.01)	7.9 (2.07)	1.05
	FLURBIPROFEN	7.5 (2.01)	7.7 (2.04)	1.03
	SUPROFEN	9.9 (2.29)	10.3(2.33)	1.04
pH = 4.5	IBUPROFEN	2.5 (0.92)	2.6 (0.95)	1.04
	FENOPROFEN	4.3 (1.46)	4.6 (1.53)	1.07
	NAPROXEN	5.5 (1.70)	5.7 (1.74)	1.04
	KETOPROFEN	6.0 (1.79)	6.3 (1.84)	1.05
	FLURBIPROFEN	5.4 (1.69)	5.6 (1.72)	1.04
	SUPROFEN	5.8 (1.76)	6.0 (1.79)	1.03

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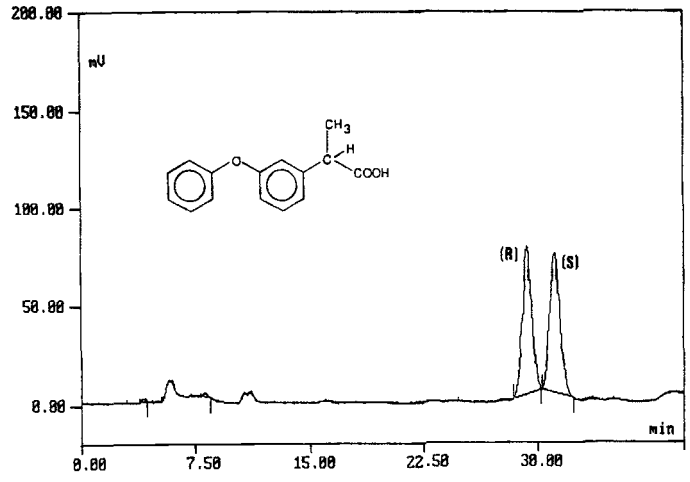

FIGURE 2 Chiral separation of (R)- and (S)- fenoprofen enantiomers. Chromatographic conditions: 0.05 M potassium acetate buffer (pH 4.6) / acetonitrile (60:40 v/v); flow-rate 80 µl/min; λ, 254 nm.

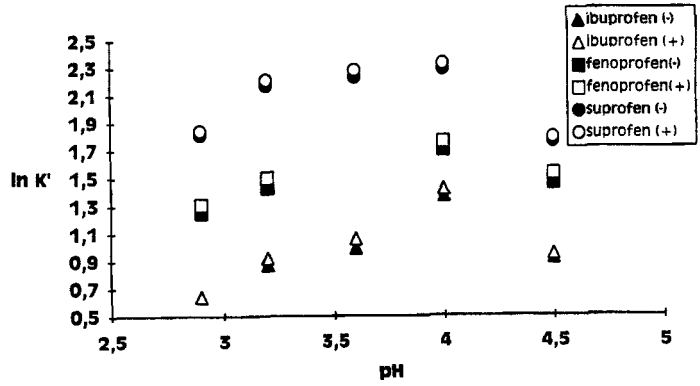

FIGURE 3 Plots of ln k' vs pH values of the acetate buffer for a serie of 2-APAs enantiomers. Chromatographic conditions as on figure 1.

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decrease of retention. These observations are consistent with ^1H NMR spectroscopic data (18), obtained for aqueous equimolar mixtures of AMPTER and (R,S)- and (R)-naproxen, individualising the positively charged nitrogen N(6)-CH_3 and H(12), H(13),and H(14) of the ergine skeleton as the most significant sites of interactions. Accordingly, experimental results may be explained assuming the formation of diastereoisomer complexes. N(6) represents an anchoring point for the carboxylic group of the analyte, allowing to its aryl moiety a π-π stacking with the ergoline structure. Study in progress, separation of different derivatives of amino acids (19), should further confirm this proposed mechanism of chiral recognition process.

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