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Enantiomeric separation of amide derivatives of some 2-arylpropionic acids by HPLC on a cellulose-based chiral stationary phase

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Abstract: A reversed phase high-performance liquid chromatographic method has been developed for the determination of the *R*- and *S*-enantiomers of ibuprofen, flurbiprofen, ketoprofen and tiaprofenic acid. Separation has been achieved using a tris(4-methylbenzoate)cellulose phase after derivatization into their amides. Flurbiprofen could also be partially resolved into its enantiomers without prior derivatization.

Keywords: *Chiral HPLC; tris(4-methylbenzoate)cellulose column; non steroidal anti-inflammatory drugs; 2-arylpropionic acids.*

Introduction

2-Arylpropionic acids, a group of non-steroidal anti-inflammatory (NSAI) drugs, are extensively prescribed in a wide range of inflammatory conditions. All are chiral and, with the exception of naproxen, marketed as racemates.

The direct separation by HPLC of the profen-enantiomers has been successfully achieved in literature using various types of chiral stationary phases (CSP). Several protein columns such as α_1-acid glycoprotein [1–5], ovomucoid [6–8], HSA (Human Serum Albumin) [9], BSA (Bovin Serum Albumin) [10] and avidine [11] have been applied; cyclodextrin-based columns have enabled the stereoselective resolution of ketoprofen [12], ibuprofen [13] and suprofen [14].

The use of Pirkle type CSP requires prior derivatization of the carboxylic group into the corresponding amide [4, 15–21]. Naproxen however was separated without prior formation of an amide using adjusted mobile phase conditions [22].

The possibilities offered by different derivatized cellulose columns have been examined extensively by various researchers [23–31]. Columns like cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD(-R), Daicel/Baker), cellulose tricinnamate (Chiralcel OK), cellulose triphenylcarbamate (Chiralcel OC) and cellulose tris(4-methylbenzoate) (Chiralcel OJ) separate the profen enantiomers, some only after amidation or esterification of the carboxylic group.

In this study an experimental tris(4-methylbenzoate)cellulose column (Bio-Rad RSL) was investigated for the chiral LC-analysis of four NSAI drugs (Fig. 1), testing various mobile phase compositions. The attempt to separate the acids enantiomerically without prior derivatization was succeeded partially for flurbi-

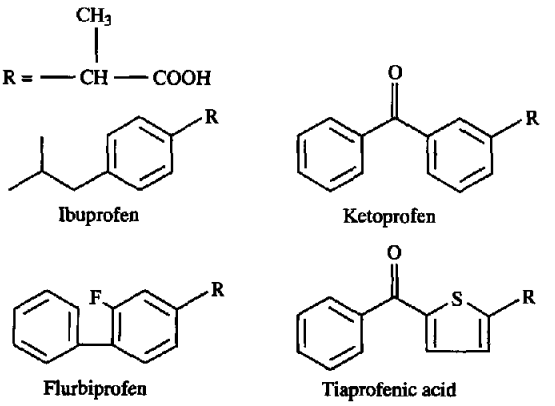

Figure 1
Chemical structures of the NSAI-drugs under investigation.

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profen only. The profens have been derivatized into amides. The optically inactive amines chosen were 1-naphthylmethylamine and benzylamine, frequently used for derivatization prior to enantiomeric resolution of acids [15, 20, 24–26].

Experimental

Apparatus

Chromatography was performed with a Varian 9010 SDS pump using a Rheodyne injector with a 10 μl loop. The diode array detector (series 1050) and integrating system were from Hewlett–Packard. The following parameters were measured: $k'1$ — capacity factor of the first eluted enantiomer, $(t_1 - t_0)/t_0$; $k'2$ — capacity factor of the second eluted enantiomer, $(t_2 - t_0)/t_0$, where t_0 is the time at which the first baseline disturbance by the solvent peak occurred; Rs — resolution factor, $Rs = 1.18\ (t_2 - t_1)/(w_1 + w_2)$, where w is the width at half-height of the peak based on peak area and height; α — selectivity factor, $k'2/k'1$.

Chemicals

1-Naphthylmethylamine, benzylamine, 1-ethyl-3-dimethylaminopropyl-carbodiimide. hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBT) were purchased from Sigma–Aldrich.

Ibuprofen was obtained from Alpha Pharma (Belgium), ketoprofen from Sigma–Aldrich, tiaprofenic acid from Erfa (Belgium); flurbiprofen was a kind gift of Upjohn (Kalamazoo, MI, USA). The various alcohols used, hexane, dichloromethane, sodium acetate, acetic acid, sodium perchlorate and perchloric acid (70%) were all of analytical grade. De-ionized water was used throughout.

Derivatization procedure

The derivatization procedure applied in this study was based on a method used elsewhere [32–34]. To 1 ml of a solution of 10 mg acid in 100 ml dichloromethane were added HOBT (300 μl of a 1 mg ml^{-1} dichloromethane solution containing 1% w/v pyridine), EDC (300 μl of a 1 mg ml^{-1} dichloromethane solution) and the appropriate amine (300 μl of a 1–1.5 mg ml^{-1} dichloromethane solution). The mixture was vortexed, left at room temperature for 1.5 h, then washed with 1 ml 0.25 M HCl solution and 1 ml of water. The dichloromethane layer was evaporated to dryness under a stream of nitrogen and the residue was taken into 0.5 ml methanol or other alcohol, composing the mobile phase.

Chromatographic conditions

An experimental 4-methylbenzoate cellulose stationary phase (EXP B101, Bio-Rad RSL, Nazareth, Belgium) was investigated (Fig. 2). The cellulose layer is covalently bound onto a 10 μm silica gel with mean pore size of 300 Å. The coverage is about 10%. A range of mobile phases, reversed as well as normal phase systems have been tried out at a flow rate of 1.0 ml min^{-1}. For preparing the buffer solutions, the amount of salt needed to obtain the stated molarity was weighed and after adjusting the pH-value of the solution with the appropriate acid, water was added up to the desired volume. Acetate and perchlorate buffers of various molarities and pH-values were used. Chromatography was carried out at ambient temperature.

Results and Discussion

As the polar carboxylic group of the 2-arylpropionic acids was likely to hamper an enantiomeric resolution, a derivatization with optically inactive amines was performed, creating structures with possibilities of dipole, H-bonding and π–π interactions. The amines chosen were 1-naphthylmethylamine and benzylamine. The analysed samples contained a small amount of free acid besides their amide derivatives. Thanks to the presence in the mobile phase of an acid, the underivatized profen and their amide could be chromatographed in the same run. As the area under the curve (AUC) of the derivatives was practically the same for both enantiomers, the derivatization procedure was thought to have no stereospecific effect. The washing step with diluted hydrocholoric acid enabled the removal of excesses of derivatization reagents, which interfered particularly using mobile phases with higher pH values.

Working with a mobile phase consisting of methanol–acetate buffer (95:5, v/v) with a pH-value ranging from 3 to 6 and a molarity increasing from 0.05 to 0.1, showed that the retention of the acids could easily be reduced by lowering the pH-value or by increasing the molarity of the buffer fraction in the eluent. Ibuprofen and tiaprofenic acid could be separated into their enantiomers after derivatiz-

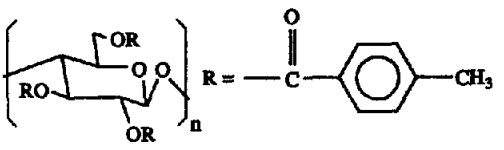

Figure 2
Chemical structure of the tris(4-methylbenzoate)cellulose layer.

ation with 1-naphthylmethylamine. Ketoprofen and flurbiprofen could only be resolved as the corresponding benzylamides. The problem of coelution of a free acid with one of their amides could be resolved by changing the methanol to buffer ratio of the mobile phase. This resulted in an increase of capacity and selectivity factors of the amides as the buffer fraction was enhanced. These general tendencies are illustrated for the 1-naphthylmethylamine derivative of ibuprofen (Table 1).

Table 1
Changes in capacity and selectivity factors of the 1-naphthylmethylamine derivatives of ibuprofen with increasing fraction of acetate buffer (0.05 M, pH 5) in methanol as mobile phase

% Buffer in methanol	$k'1$	$k'2$	Rs	α
10	0.83	1.11	1.01	1.34
15	1.40	2.01	1.50	1.44
20	2.64	4.04	2.21	1.53
25	5.29	8.48	2.34	1.60
30	11.63	19.37	2.65	1.67

The separation of the 1-naphthylmethylamine derivatives of ibuprofen and tiaprofenic acid and the benzylamine derivatives of ketoprofen and flurbiprofen were further investigated using buffer solutions with lower pH-values such as a perchlorate buffer pH 2. This enabled the acids to elute faster and to avoid coelution with one of their amide derivatives. Ibuprofen and tiaprofenic acid could be baseline separated as naphthylmethylamides, ketoprofen and flurbiprofen as benzylamine derivatives showed poorer separation results (Fig. 3).

Similarly as seen with acetate buffer composing the mobile phase, the enantiomeric separation of the amides was hardly affected by changes in perchlorate buffer composition. Lowering the molarity of the buffer fraction caused a minor increase of retention and selectivity factors; a decrease of the pH-value tended to decrease them slightly. A better resolution could only be obtained by increasing the buffer portion of the mobile phase at the expense of longer retention times (Table 2). The elution order of the enantiomers was not determined.

Using a perchlorate buffer, a partial resolution of the underivatized flurbiprofen was observed. Changes of mobile phase composition towards higher buffer portions led to longer retention times of flurbiprofen, but only

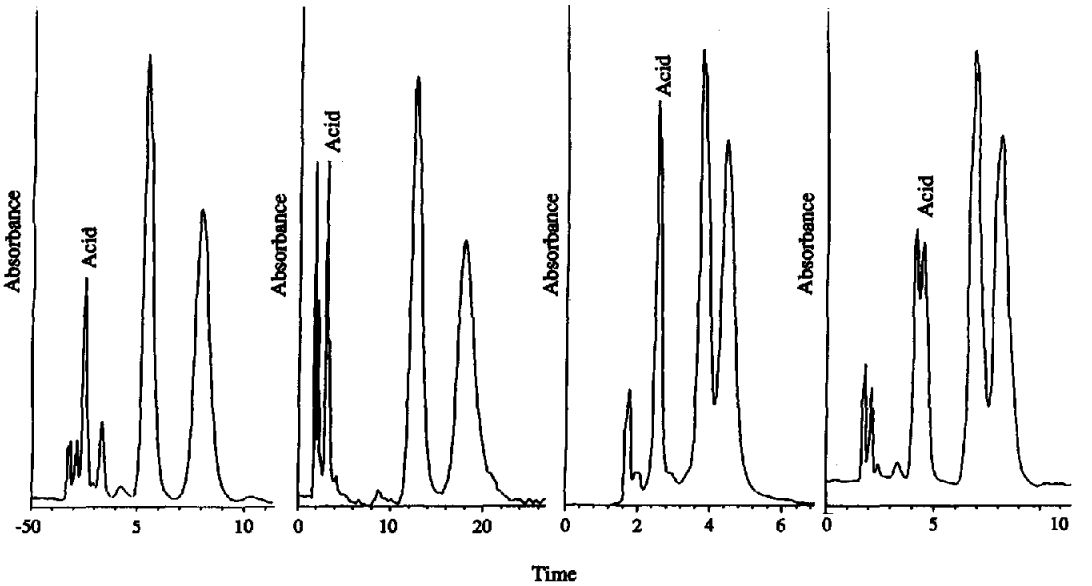

Figure 3
(a) Ibuprofen, 1-naphthylmethylamine derivative. (b) Tiaprofenic acid, 1-naphthylmethylamine derivative. (c) Ketoprofen, benzylamine derivative. (d) Flurbiprofen, benzylamine derivative. Mobile phase: methanol–perchlorate buffer (0.05 M, pH 1.5) (80:20, v/v). Detection: 230 nm.

Table 2
Influence of composition and fraction of perchlorate buffer in methanol as the mobile phase on the separation of naphthylmethylamides of ibuprofen and tiaprofenic acid and of benzylamides of ketoprofen and flurbiprofen

Mobile phase			$k'1$	$k'2$	Rs	α
Ibuprofen						
pH 2,	0.1 M,	20%	2.92	4.94	2.41	1.69
	0.3 M,	20%	2.39	4.00	2.41	1.67
		15%	1.18	1.88	1.92	1.59
pH 1.5,	0.1 M,	15%	1.13	1.79	1.88	1.58
		20%	2.40	4.00	2.38	1.67
	0.05 M,	20%	2.37	3.95	2.42	1.67
		25%	5.15	8.91	2.86	1.73
Tiaprofenic acid						
pH 2,	0.1 M,	20%	8.04	11.95	1.91	1.49
	0.3 M,	20%	7.11	10.54	1.98	1.48
		15%	3.64	5.25	1.70	1.44
pH 1.5,	0.1 M,	15%	3.60	5.15	1.61	1.43
		20%	7.00	10.39	1.92	1.48
	0.05 M,	20%	7.16	10.63	1.89	1.49
		25%	13.87	21.54	2.69	1.55
Ketoprofen						
pH 2,	0.1 M,	20%	1.64	2.12	1.08	1.29
	0.3 M,	20%	1.36	1.76	1.01	1.29
		15%	0.84	1.04	0.80	1.23
pH 1.5,	0.1 M,	15%	0.81	0.98	0.67	1.22
		20%	1.43	1.83	1.01	1.28
	0.05 M,	20%	1.41	1.81	1.08	1.29
		25%	2.45	3.26	1.22	1.33
Flurbiprofen						
pH 2,	0.1 M,	20%	3.93	4.85	1.07	1.23
	0.3 M,	20%	3.29	4.04	1.06	1.23
		15%	1.66	2.01	0.86	1.21
pH 1.5,	0.1 M,	15%	1.67	2.00	0.88	1.20
		20%	3.32	4.08	1.05	1.23
	0.05 M,	20%	3.41	4.19	1.08	1.23
		25%	6.81	8.39	1.14	1.23

Table 3
Influence of composition and fraction of perchlorate buffer in methanol on the stereoselective separation of flurbiprofen

Mobile phase		$k'1$	$k'2$	Rs	α
pH 2, 0.1 M,	10%	0.66			1.0
	20%	1.81	2.08	0.694	1.148
0.3 M,	10%	0.63			1.0
	20%	1.62	1.85	0.666	1.144
	30%	5.44	6.29	0.774	1.157
0.5 M,	10%	0.62			1.0
	20%	1.66	1.90	0.667	1.143
	30%	5.49	6.34	0.801	1.156
0.7 M,	10%	0.61			1.0
	20%	1.57	1.80	0.651	1.142
	30%	5.19	6.00	0.776	1.155

to a small improvement of the resulting α-factor, varying the molarity of the buffer gave no significant difference. No separation was observed when a mobile phase with 10% perchlorate buffer was used (Table 3). None of the other three 2-arylpropionic acids showed any enantiomeric resolution under the applied conditions. Figure 4 shows the resolution obtained for flurbiprofen using methanol–perchlorate buffer (0.3 M, pH 2) (70:30, v/v).

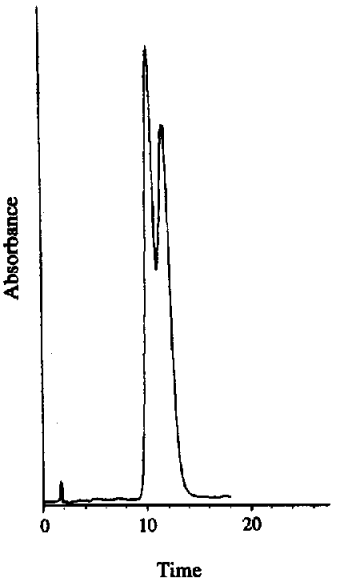

Figure 4
Separation of flurbiprofen enantiomers. Mobile phase: methanol–perchlorate buffer (0.3 M, pH 2) (70:30, v/v). Detection: 245 nm.

Substituting methanol by ethanol 95% reduced the retention and separation of the amide derivatives of the four profens. No separation of the flurbiprofen enantiomers was detected. Using acetonitrile instead of methanol caused faster elution of the free acids without being enantiomerically resolved. Ketoprofen and flurbiprofen were not resolved either as naphthylmethylamides or as benzylamides. The separation of naphthylmethylamides of ibuprofen and tiaprofenic acid was worse and an increase of the buffer fraction could not compensate for the decrease of α-factors.

Chiral recognition mechanisms of cellulose phases have not been fully explained so far in the literature. Wainer and Alembik [24] proposed that the chiral recognition mechanism of cellulose tribenzoate phases involves three important features. Besides the formation of diastereomeric solute complexes through attractive interaction between the amide moiety of the solute and the ester moiety of the CSP (involving hydrogen bonding π–π and dipole interactions), the positioning of the solute and the CSP within the complex and the steric fit of the asymmetric portion of the solute in the chiral cavity of the

CSP determine whether the amide enantiomers will be resolved. The profens under investigation could be separated as amide derivatives to some extent.

The nature of the mobile phase markedly influenced the separation. This was probably due to changes of the properties of the CSP, illustrated by the fact that acetonitrile did not separate the amide derivatives of either flurbiprofen or ketoprofen as could be achieved using methanol. Another chiral recognition mechanism appeared to be involved in the limited resolution of the underivatized flurbiprofen. An enantiomeric separation of this acid was only clearly seen at low pH-values of the applied buffer. Ionization of the carboxylic acid group seemed to hamper the enantioselective process.

Normal phase

A separation of derivatized ibuprofen enantiomers was investigated on the same cellulose column using hexane with different alcohols as organic modifiers. The free acid form could not be chromatographed under these conditions. The best separation (α = 1.170) of ibuprofen naphthylmethylamide was obtained using hexane-isopropanol (95:5, v/v). The *R*-enantiomer was found to elute first. Lowering the portion of organic modifier led to an increase of capacity and selectivity factors. Addition of primary alcohols, secondary or tertiary butanol to hexane gave poorer results. Benzylamine derivatives of ibuprofen could not be separated.

Conclusion

A wide range of solvents could be used on the investigated cellulose column, due to the fact that the tris (4-methylbenzoate) cellulose layer is bound onto a silica layer instead of being adsorbed. Of the various mobile phases tried out to separate enantiomerically four 2-arylpropionic acids after derivatization with achiral amines, a methanol–perchlorate buffer mixture was preferred. The attempt of resolving the enantiomers of the free acids was not successful except for one product. Apparently, the polar character of the carboxylic acid moiety adjacent to the chiral centre of the NSAI-drugs hampered an adequate interaction on the CSP in order to be resolved enantiomerically. Restraining the ionization of the acid led to a partial separation of the enantiomers of flurbiprofen only. Derivatization into amides showed to be successful for resolving the four acids within reasonable time limits. The described preliminary experiments permitted no more adequate interpretation of the interaction mechanisms on the applied column.

Further investigation into various derivatives of a larger group 2-arylpropionic acids and into the derivatization method is being carried out.

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