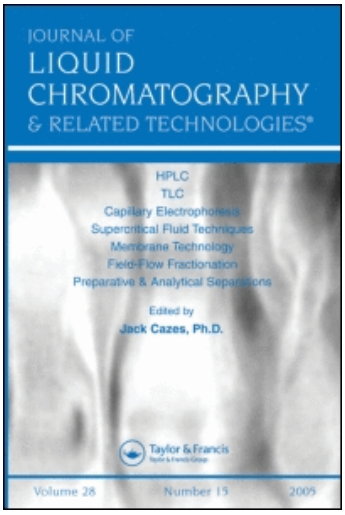

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COMPARATIVE STUDY ON THE ENANTIOMERIC SEPARATION OF SEVERAL NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON TWO CELLULOSE-BASED CHIRAL STATIONARY PHASES*

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ABSTRACT

Two cellulose-based chiral stationary phases were compared on their feasibility to resolve a representative number of 2-arylpropionic acids : a new experimental phase (Tolylcellulose, EXP B101) manufactured by Bio-Rad RSL and the Chiralcel OJ phase by Daicel. Both columns were tested under normal phase conditions, applying a n-hexane:isopropanol mobile phase. The Bio-Rad column was also assayed under reversed phase conditions using a methanol and perchlorate buffer system. Enantiomeric separation without prior derivatization of most 2-arylpropionic acids was far better on the Chiralcel OJ column than on the Bio-Rad column, the latter performing somewhat better under reversed phase conditions. Prior derivatization of the carboxylic acid group with an amine (naphthylmethylamine, (2-methyl)benzylamine) or an alcohol (benzylalcohol) permitted to separate the tested drugs to different extents on both columns.

* Part of this work was presented as a poster communication at the Vth International Symposium on Chiral Discrimination, Sept. '94, Stockholm

INTRODUCTION

2-Arylpropionic acids (2-APAs) are a group of non steroidal anti inflammatory (NSAI) drugs that are characterised by a chiral carbon atom adjacent to the carboxylic acid moiety. With the exceptions of naproxen and flunoxaprofen, they are marketed as racemic compounds and widely prescribed in relief of acute and chronic rheumatoid arthritis and osteoarthritis.

Numerous chiral stationary phases (CSPs) for the liquid chromatographic discrimination of these frequently used pharmaceuticals are described in literature [1-2]. Various protein columns with excellent selectivity towards underivatised 2-APAs have been developed and commercialised [3-4]. Efficient separations were afforded by immobilised phases of Human and Bovine Serum Albumin [5-6], α_1- Acid Glycoprotein [7-11], Ovomucoïd [12-14] and Avidine [15-16].

The first commercially available "brush type" phases for HPLC, designed by the groups of Pirkle (CSP1, DNB-phenylglycine derivatives) [17-24,53] and Ôi [19-20], permitted a separation of profens only after derivatization of the carboxylic acid moiety, necessary to meet the requirements for stereoselective interaction. Several other versions have been created, still asking for a pre-column derivatization of the 2-APAs with an amine or an alcohol [27-32]. An extensive overview of the numerous phases designed by Pirkle and his group has recently been published [33] and includes the successfully improved Pirkle concept that separates the profen family into their enantiomers as such [34-37].

Interesting results have also been obtained using diverse modified and immobilised cyclodextrins [38-41].

The possibilities offered by different derivatised cellulose columns to discriminate the enantiomers of 2-arylpropionic acids and many other classes of drugs have extensively been examined by many researchers. A variety of derivatised cellulose phases have been synthesised since the successful introduction of the cellulose triacetate phases [38-46]. Pioneering studies in the development of cellulose columns were performed by Okamoto and his group [47-51]. Many of these chiral stationary phases have been commercialised by Daicel (Tokyo, Japan) [52].

Carbamate derivatives and tris(3,5-dimethylphenylcarbamate) cellulose in particular (Chiralcel OD), offer many possibilities for enantioselective interactions and have proved to be efficient in separating most profen enantiomers [53-55]. For some 2-APAs, chiral resolution could only be obtained after derivatization of the carboxylic acid moiety. Among

the ester cellulose derivatives coated on varied silica gel supports, the methylbenzoate CSPs have also found many applications [56-60]. These polymers have likewise proved their enantioselective capabilities configured as pure beads offering a much higher loadability [61-64].

A new experimental cellulose-based CSP, called Tolylcellulose column, manufactured by Bio-Rad RSL (Nazareth, Belgium) contains a similar tris(4-methylbenzoate) cellulose polymer as the Chiralcel OJ phase (Daicel, Tokyo, Japan) and has already been examined on its enantioselectivity towards several NSAI drugs [65-66]. In order to obtain an acceptable chiral separation for most analytes, a derivatization of the carboxylic group was necessary. Due to the fact that the cellulose layer is bound onto the modified silica support rather than being adsorbed as is the case for the Chiralcel OJ column, this CSP was used under reversed phase conditions.

The mobile phase recommended for analyses with the Chiralcel OJ column consists mainly of a mixture of n-hexane and an alcoholic modifier, preferably isopropanol. Pure ethanol however can also be applied and even aqueous acetonitrile has been tested [67]. Its analogue for reversed phase conditions, similar as the Chiralcel OD-R column, has not been commercialised yet.

In this paper the chiral discriminative properties towards ten 2-arylpropionic acids (Fig. 1) on a Chiralcel OJ column were compared with the experimental Tolylcellulose phase using a mobile phase of n-hexane:isopropanol:acetic acid. The same Tolylcellulose column was also used with a methanol:perchlorate buffer system. As only poor or no resolution of the free acids was obtained on this CSP [65-66], several derivatives (amides and esters) were prepared and analysed on both columns.

EXPERIMENTAL

Chemicals

Benzylamine, 1-naphthylmethylamine, 2-methylbenzylamine, 1-ethyl-3-dimethylamino-propyl-carbodiimide, 1-hydroxybenzotriazole, *rac*-2-phenylpropionic acid and *rac*-ketoprofen were purchased from Sigma-Aldrich (Bornem, Belgium); benzylalcohol from UCB (Leuven, Belgium). *Rac*-carprofen was a kind gift of Produits Roche (Brussels,

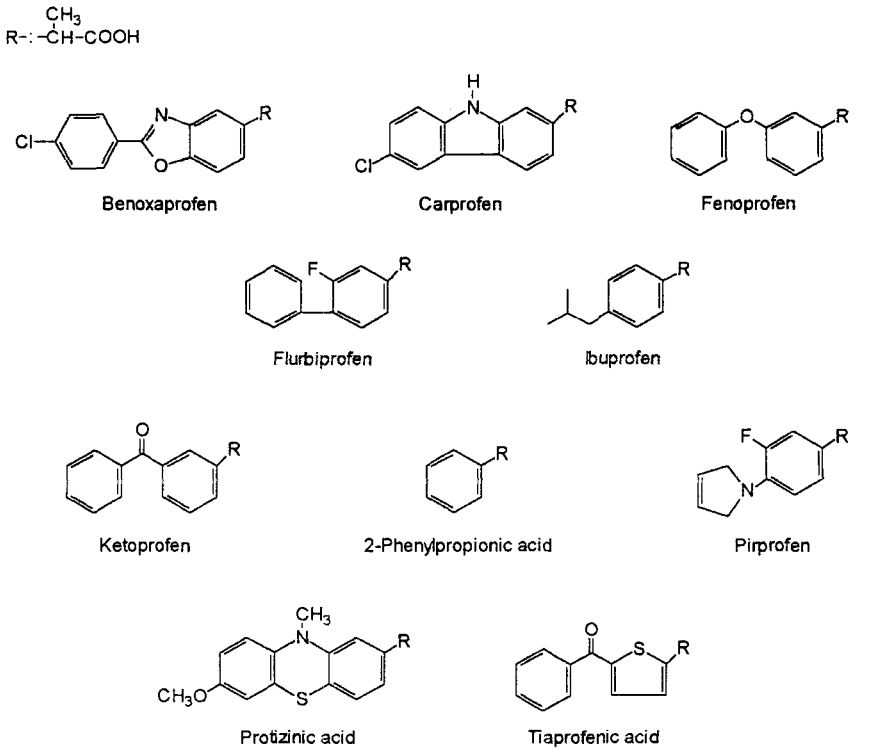

FIGURE 1. Chemical structures of the molecules under investigation

Belgium), *rac*-flurbiprofen of Upjohn Co. (Kalamazoo, MI, USA), *rac*-pirprofen of Ciba-Geigy (Groot-Bijgaarden, Belgium), *rac*-calcium fenoprofen of Eli Lilly Co. (Indianapolis, IN, USA), *rac*-protizinic acid of Rhône-Poulenc Rorer (Brussels, Belgium) and *rac*-tiaprofenic acid of Erfa (Brussels, Belgium). *Rac*-benoxaprofen was obtained from Eli Lilly Co. (Windelsham, UK) before withdrawal from the market and *rac*-ibuprofen from Profarma (Oud-Turnhout, Belgium).

Sodium-perchlorate (Merck, Darmstadt, Germany) perchloric acid 70 % aqueous solution (UCB, Leuven, Belgium), methanol (Labscan, Dublin, Ireland) and dichloro-

methane (UCB, Brussels, Belgium) were all of analytical grade. n-Hexane and isopropanol (HPLC-quality) were from J.T. Baker (The Netherlands). Deionised water was used throughout.

Apparatus

Chromatography was performed with a Varian 9010 SDS pump (Varian Associates Inc., Walnut Creek, CA, USA) using a Rheodyne injector with a 20 µl loop. Detection was achieved at two wavelengths simultaneously (230 and 254 nm) with a Hewlett Packard 1050 DAD (Waldbronn, Germany). Integration of the more intense chromatogram was made with the Hewlett Packard software package (1990). 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 : $(t_2-t_0) / t_0$.

Tri-*t*-butylbenzene was used to determine the t_0-value under normal phase conditions on both columns [68]. As it was retained on the Tolylcellulose column under reversed phase conditions, the elution time of methanol was used as t_0-value.

α : selectivity factor : k'2 / k'1.

Rs : resolution factor : $Rs = 1.18 (t_2-t_1) / (w_1+w_2)$; w is the width at half-height of the peak based on peak area and height.

Rp : Kaiser's peak separation index : the ratio of mean valley height between two peaks and the mean peak height, which rises to 1 for perfectly separated peaks. This factor is therefore measured of enantiomeric peaks that were not baseline separated.

Chromatographic conditions

The columns under investigation (250 mm x 4.6 mm ID) are both characterised by a tris (4-methylbenzoate) cellulose layer (Fig. 2). In case of the Tolylcellulose column, EXP B101 (Bio-Rad RSL, Nazareth, Belgium) the polymer is covalently bound onto a 10 µm aminopropylsilinazed silica gel with mean pore size of 300 Å; coverage is about 10 %.

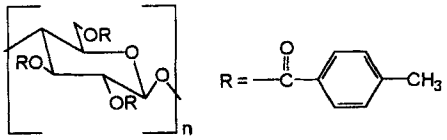

FIGURE 2. Chemical structure of the tris (4-methylbenzoate) cellulose layer

This phase has been compared with a commercially available Chiralcel OJ column (Daicel Chemical Industries, Tokyo, Japan, purchased from J.T. Baker, The Netherlands), adsorbed onto macroporous (1000 Å) 10 μm aminopropylsilinazed silica gel. The mobile phase for normal phase (NP) conditions consisted of n-hexane and isopropanol with 0.5 % (V/V) acetic acid (HAc), applied on both columns. The Tolylcellulose column was also used with methanol and perchlorate buffer 0.1 M pH 2.0 (Reversed phase, RP). The eluents were mixed in varying ratios and ultrasonicated and degassed before they were pumped at a flow rate of 1 $ml.min^{-1}$. For preparation of the buffer solution, 14.05 g sodium perchlorate was dissolved in water and after pH adjustment with a concentrated perchloric acid solution, water was added up to 1 litre. Chromatography was carried out at ambient temperature.

Derivatization procedure

Ester and amide derivatives were formed using 1-ethyl-3-dimethylaminopropyl-carbodiimide (EDC) as coupling agent in combination with 1-hydroxybenzotriazole (HOBT). The amines used were 1-naphthylmethylamine and benzylamine as applied in former studies [28-29] and 2-methylbenzylamine. Esters were formed with benzylalcohol.

Taking the derivatization of ibuprofen with benzylamine as an example, the given recipe was followed

To 1 ml of a solution of ibuprofen (1.0 $mg.ml^{-1}$ dichloromethane) were added HOBT (300 μl of a 1.0 $mg.ml^{-1}$ dichloromethane solution containing 1 % pyridine), EDC (300 μl of a 11 $mg.ml^{-1}$ dichloromethane solution) and benzylamine (300 μl of a 2.6 $mg.ml^{-1}$ dichloromethane solution). The mixture was vortexed and left for about 1.5 h. The

dichloromethane layer was evaporated to dryness under a stream of nitrogen and the residue was taken into 5.0 ml isopropanol (NP) or methanol (RP).

Solutions of other acids and amines or alcohol were prepared relative to their molecular weight. About 1.5 molar amounts of amine or alcohol are used relative to acid. Calcium fenoprofen was converted to its acid form prior to derivatization by an acid extraction.

RESULTS AND DISCUSSION

Although a tris(4-methylbenzoate) cellulose derivative forms the chiral layer of both columns in the present study, the stereoselective properties towards the tested analytes differ tremendously. A major difference between the two applied CSPs is the way the polymeric layer is coated onto its silica support, covalently in case of the Tolylcellulose and via adsorption for the Chiralcel phase. Differences in the solvents used in the synthesising process, washing steps, packing procedures, etc. may certainly be of influence as well on the stereoselective characteristics of the resulting CSP.

Enantiomeric separation of free 2-arylpropionic acids

The necessity to derivatise the carboxylic moiety in order to obtain a chiral discrimination of the 2-APAs on the Tolylcellulose column as observed in former experiments [65-66], was demonstrated not to be required for some analytes on the Chiralcel OJ column. An enantiomeric resolution of several (underivatised) 2-APAs using the Chiralcel OJ column has been described in literature, of ketoprofen and fenoprofen [52], of 2-(10,11-dihydro-10-oxodibenzo-[*b,f*]thiepin-2-yl)propionic acid [69], and of a methylester derivative of flurbiprofen [52]. Of all analytes considered in this paper, only flurbiprofen and 2-phenylpropionic acid could not be resolved on the Chiralcel OJ column. Table 1 summarises the results using n-hexane:isopropanol:HAc (80:20:0.5) as mobile phase (Fig. 3). Upon decrease of the isopropanol portion, resolution increases at the expense of higher capacity factors.

As mentioned before, the chiral discriminative capability for underivatised acids on the Tolylcellulose column turned out to be far worse. The mobile phase, as an essential and dynamic part of the chiral system, modifies the enantioselective properties substantially. Under normal phase conditions, no enantiomeric separation was obtained. The capacity

TABLE 1. Resolution of Underivatised Acids on Chiralcel OJ Column

analyte	k'1	α	Rs	Rp
ibuprofen	0.27	1.14	0.67	0.27
ketoprofen	1.65	1.35	3.04	>1
flurbiprofen	1.16	1.00	0.00	0.00
tiaprofenic acid	2.69	1.06	0.65	0.23
fenoprofen	1.23	1.23	2.02	0.99
2-phenylpropionic acid	1.42	1.00	0.00	0.00
pirprofen	1.04	1.33	2.62	>1
protizinic acid	4.09	1.25	2.31	>1
carprofen	5.85	1.38	3.55	>1
benoxaprofen	2.86	1.13	1.17	0.83

Mobile phase : n-hexane:isopropanol:HAc 80:20:0.05

factors were a multiple fold of those obtained on Chiralcel OJ. Under reversed phase conditions, using methanol:perchlorate buffer 0.1 M, pH 2 mixtures, some of the free acids were only slightly resolved into their enantiomers on Tolylcellulose.

By increasing the buffer fraction of the mobile phase, the acids were retained longer. If a mobile phase with e. g. 40 % buffer was applied, ibuprofen (Rp=0.36) and tiaprofenic acid (Rp=0.38) were enantiomerically resolved as well to a small extent. Piketoprofen, an amide of ketoprofen with 2-amino, 4-methylpyridine (Almirall, Barcelona, Spain) was partially resolved on the Tolylcellulose column under normal phase conditions only (n-hexane:isopropanol 95:5; k'1=6.30, Rp=0.38).

Enantiomeric separation of derivatives of 2-arylpropionic acids

As no acceptable resolution was obtained for the free acids on the Tolylcellulose column, the carboxylic acid group was derivatised to improve possible stereoselective interactions with the CSP. Amides were formed with 1-naphthylmethylamine, 2-methylbenzylamine and benzylamine; esters were formed with benzylalcohol. These four series of derivatives were chromatographed on the Chiralcel OJ column and on the Tolylcellulose column, the latter in NP and RP.

Comments that hold for all ten of the studied acids, cannot easily be provided. It was obvious that if the free acids eluted at higher retention times, their derivatives showed the same relative behaviour. This may indicate that the cyclic moiety on the chiral carbon atom

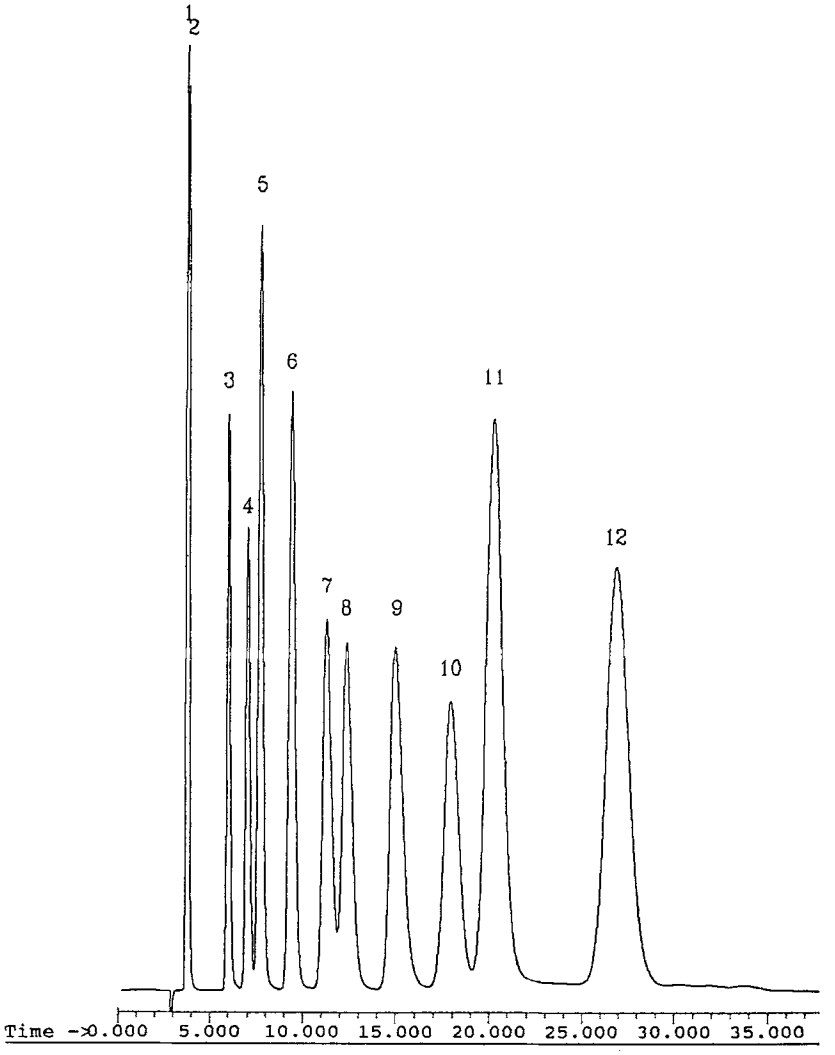

FIGURE 3. Mixture in isopropanol of 0.5 mg.ml^{-1} ibuprofen (1,2), 0.5 mg.ml^{-1} pirprofen (3,4), 0.5 mg.ml^{-1} ketoprofen (5,6), 0.5 mg.ml^{-1} benoxaprofen (7,8), 0.5 mg.ml^{-1} protizinic acid (9,10), 0.3 mg.ml^{-1} carprofen (11,12). Mobile phase: n-hexane:isopropanol:acetic acid 80:20:0.5. Detection wavelength: 230 nm.

TABLE 2. Resolution of Underivatised Acids on Tolylcellulose Column

analyte	k'1	α	Rs	Rp
flurbiprofen	1.24	1.10	0.61	0.25
protizinic acid	3.15	1.05	0.43	0.06
carprofen	1.85	1.07	0.52	0.11
benoxaprofen	2.24	1.10	0.68	0.28

Mobile phase : methanol:perchlorate buffer 0.1 M, pH 2 80:20

interacts, e.g. via inclusion phenomenon, with the stationary phase. On the other hand, a good resolution of the free acid does always not imply good chiral discrimination of the derivatives.

As for the *1-naphthylmethylamine derivatives,* the best results were obtained using the Tolylcellulose column under reversed phase conditions. All the samples under investigation with the exception of the ketoprofen derivative were separated yielding a resolution Rs of at least 0.8.

Under normal phase conditions, better results were obtained for Chiralcel OJ. However, only half of the investigated analytes could be enantiomerically discriminated, the obtained resolutions on the other hand were mostly better than in RP on Tolylcellulose. The bicyclic amides had higher capacity factors than the *(2-methyl)benzylamine derivatives.* Most NSAI drugs showed stereospecific interaction following derivatization with these smaller amines. The addition of a methyl group on benzylamine was thought to improve the stereoselective interactions with the cellulose layer. As a weak electron donating group, the methyl function may increase the electron density of the aromatic ring (promoting $\pi-\pi-$ interactions with the methylbenzoate group of the CSP) and favour hydrogen bonding interaction with the ester group of the CSP, thus contributing to the electron negativity of the carbonyl group or the proton character on the amine moiety. A consistent improvement however of the separations induced by the o-substitution on the aromatic amine moiety was obvious for the Tolylcellulose column only; for the Chiralcel OJ no clear preference could be assigned to either of the series. More recent studies in our lab on other derivatives using the Tolylcellulose phase have shown that a methyl group in *meta-* or *para*-position leads to a decrease in resolution versus *ortho*-substitution.

The ester analogues of the benzylamide derivatives were formed with *benzylalcohol,* having no capabilities for hydrogen bonding interactions. In most cases, better separations were obtained with the amide analogues. The retention times of the ester derivatives were

TABLE 3.a. Under the applied normal phase conditions, *ibuprofen* turned out to be a too small a molecule for its derivatives to be sufficiently retained on either column. Consequently, best chiral separations were noted following derivatization with a naphthylgroup. (ND because of overlapping with blank peaks)

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	0.84	1.36	0.95*	ND	-	-	ND	-	-	ND	-	-
TNP	2.98	1.22	0.91*	ND	-	-	ND	-	-	ND	-	-
TRP	1.31	1.53	2.36	4.07	1.33	1.95	1.07	1.47	1.97	4.41	1.14	0.86*

TABLE 3.b. *Ketoprofen* enantiomers were better resolved as amide than as ester derivatives. The Tolylcellulose column could not separate the 1-naphthylmethylamide nor the ester derivatives.

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	8.94	2.15	3.95	2.31	1.86	4.46	1.92	1.43	0.97*	3.52	1.07	0.34*
TNP	2.75	1	0	4.97	1.20	0.98*	4.49	1.34	2.03	2.18	1	0
TRP	3.48	1	0	5.85	1.41	2.30	1.80	1.80	3.01	3.21	1	0

TABLE 3.c. *Flurbiprofen* enantiomers could not be separated as such on the Chiralcel OJ column, but were easily resolved after derivatization. In NP, a better resolution was obtained for ester derivatives; in RP, derivatization into amides was preferred.

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	3.64	1.46	1.67	2.30	1.33	2.21	2.08	1.37	0.99*	4.34	1.37	3.17
TNP	2.51	1.15	0.39*	4.41	1.17	0.96*	4.21	1.23	0.98*	1.42	1.21	0.98*
TRP	6.89	1.10	0.32*	3.22	1.23	0.98*	4.15	1.45	2.52	9.94	1.13	0.91*

TABLE 3.d. *Tiaprofenic acid* enantiomers were easily separated after derivatization, amides performing generally better than esters.

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	5.98	1.70	2.72	4.06	1.40	2.71	3.69	1.51	2.60	6.34	1.46	3.78
TNP	4.32	1.41	1.85	3.60	1.20	0.95*	9.81	1.33	2.32	4.31	1.25	2.21
TRP	3.73	1.43	2.27	1.82	1.19	0.87*	2.30	1.43	2.21	4.73	1.17	0.96*

TABLE 3.e. *Fenoprofen* enantiomers were easily separated after derivatization. Under NP conditions ester derivatives were not resolved.

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	3.74	2.60	4.62*	1.83	1.58	3.78	1.61	1.50	2.51	2.96	1	0
TNP	2.09	1	0	1.39	1.16	0.77*	2.92	1.33	2.28	0.95	1	0
TRP	4.46	1.20	0.91*	1.88	1.33	1.88	2.42	1.88	3.73	4.50	1.12	0.74*

(continued)

TABLE 3.f. *2-Phenylpropionic acid* probably is too small an acid to bring about an efficient interaction with the cellulose polymer. On the Tolylcellulose column, only derivatives of 1-naphthylmethylamine were partially resolved. On the Chiralcel OJ phase, all other derivatives were poorly chirally discriminated.

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	1.14	1	0	1.25	1.09	0.29*	1.05	1.14	0.47*	2.32	1.08	0.61*
TNP	8.68	1.11	0.74*	2.16	1	0	1.88	1	0	ND	-	-
TRP	1.06	1.78	0.72*	ND	-	-	0.74	1	0	1.73	1	0

TABLE 3.g. *Pirprofen* enantiomers could be separated after derivatization with an amine or an alcohol. Some analyses however suffered from interferences of impurities or degradation products present in the acid samples.

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	2.44	1.20	0.64*	1.81	1.38	0.98*	1.70	1.35	0.98*	2.75	1.35	3.15
TNP	2.10	1.07	0.07*	1.79	1.11	0.34*	3.75	1.17	0.93*	1.26	1.12	0.66*
TRP	3.73	1.22	0.96*	2.10	1.27	1.43	2.58	1.32	0.95*	5.97	1.16	0.95*

TABLE 3.h. *Protizinic acid* enantiomers were most efficiently separated as free acid on the Chiralcel OJ column. Generally, no chiral discrimination was obtained for amide derivatives, ester derivatives were only partially resolved.

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	7.45	1	0	8.11	1	0	7.81	1	0	10.9	1.21	0.97*
TNP	6.40	1	0	15.6	1.10	0.53*	5.55	1	0	5.39	1.13	0.77*
TRP	13.0	1.14	0.56*	9.50	1	0	12.5	1	0	22.1	1.07	0.19*

TABLE 3.i. The best separation for *carprofen* was obtained on the Chiralcel OJ column as free acid. Chiral discrimination was generally better for ester than for amide derivatives on the Tolylcellulose phase. (ND: not detected within an analysis time of 2 hours)

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	ND	-	-	3.31	1.31	0.91*	3.46	1	0	5.63	1	0
TNP	3.67	1	0	10.5	1.05	0.09*	9.44	1.08	0.29*	7.75	1.13	0.90*
TRP	8.17	1.22	0.89*	6.57	1.18	0.87*	9.00	1	0	10.1	1.21	0.99*

TABLE 3.j. *Benoxaprofen* enantiomers could be largely baseline separated after derivatization with an amine. Esters were significantly worse resolved. (ND: not detected within an analysis time of 2 hours)

	naphthylMeamide			benzylamide			2-Mebenzylamide			benzylester		
MP	k'1	α	R	k'1	α	R	k'1	α	R	k'1	α	R
COJ	ND	-	-	5.72	2.66	7.11	4.95	2.64	4.24	8.82	1.12	0.77*
TNP	4.68	2.09	3.08	2.17	1.63	3.03	8.97	2.21	5.06	3.21	1.11	0.63*
TRP	16.1	2.81	7.01	9.03	3.01	7.68	10.5	4.04	8.80	18.3	1	0

1.5 to 2 times higher on the Chiralcel OJ and the RP-Tolylcellulose columns. In the case of Tolylcellulose column in NP, amide and ester derivatives interacted to similar extents, consistently worse than under RP conditions. This may indicate that for the Tolylcellulose phase, hydrogen bonding interactions are favoured using an aqueous mobile phase. This assumption may also be confirmed by former findings that the use of acetonitrile instead of methanol causes a faster elution of the amide derivatives with loss of resolution that could partly be compensated by increasing the buffer portion of the mobile phase [66]. The mobile phase modifies the CSP at both achiral and chiral sites. It functions as a dynamic part of the total chiral system and competes for interaction locations with the analytes.

Table 3 (subdivided for each individual acid) summarises the obtained resolutions for the four series of derivatives of the ten acids under various conditions on both columns. For the resolution R, either Rs or Rp* is given. (COJ stands for the Chiralcel OJ column, TNP for the Tolylcellulose column, both used with n-hexane:isopropanol:acetic acid, TRP for the Tolylcellulose column used with methanol:perchlorate buffer 0.1 M, pH 2). Plate numbers of the enantiomeric peaks eluting at comparable retention times, were of the same order for the Chiralcel OJ and the Tolylcellulose column in RP. Plate numbers were often slightly worse for Tolylcellulose in NP.

This selection of chromatographic results shows that there is a derivative to be formed of each acid that interacts stereoselectivily with tris(4-methylbenzoate)cellulose stationary phases.

CONCLUSIONS

In general, the feasibility of chiral separation of the investigated group of NSAI drugs turned out in favour of the Chiralcel OJ column as more 2-arylpropionic acids could be resolved without prior derivatization. The tested Tolylcellulose phase however has the advantage that it can be used under reversed phase conditions without significant deterioration of the phase over several months of experiments. Derivatization of the carboxylic acid moiety accounts for an improved resolution or an increased absorbance. Further experiments on derivatives with mono-, bi- and tricyclic amines and alcohols are currently under investigation on the Tolylcellulose column with promising results.

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