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Journal of Chromatography A, 772 (1997) 185–194

JOURNAL OF CHROMATOGRAPHY A

Enantiomeric resolution study by capillary electrophoresis Selection of the appropriate chiral selector

Salvatore Fanali*, Claudia Desiderio, Zeineb Aturki

Istituto di Cromatografia del Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo Roma, Italy

Abstract

The enantiomeric separation of several arylpropionic acids, namely carprofen, cicloprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, naproxen and suprofen has been studied by capillary zone electrophoresis using different chiral selectors added to the background electrolyte with the aim to find the optimum experimental conditions for both qualitative and quantitative purposes. The chiral selectors used included two β-cyclodextrin derivatives and a glycosidic antibiotic, namely 2,3,6-tri-O-methyl-β-CD, heptamethylamino-β-CD and vancomycin. When the CDs were used the chiral selector was present in both capillary and electrode compartments while for vancomycin the partial filling method was used (the chiral selector was not present at the detector window in order to improve the sensitivity; vancomycin is strongly absorbing at low wavelengths). Enantiomeric resolution was recorded for all the compounds studied except for ibuprofen when heptamethylamino-β-CD was used; resolution generally increased by increasing the chiral selector concentration. Good sensitivity and good precision for both migration times and corrected peak areas (A_{sample}/migration time) were achieved using the three chiral selectors. Among the three chiral additives employed vancomycin proved to be the most effective for the enantiomeric separation of the studied arylpropionic acids. The optimized method achieved analysis with the shortest time (<6 min), and the highest efficiency (270 000 plates×meters).

Keywords: Enantiomer separation; Chiral selectors; Buffer composition; Arylpropionic acids

1. Introduction

Enantiomeric separations represent an important topic of research in analytical chemistry, especially in the pharmaceutical field where a wide number of drugs, having one or more than one asymmetric centre, exist as a couple of enantiomers. Very often the pharmacological activity and metabolism of two enantiomers of a certain drug may be different and thus analytical methods are required for, e.g., chiral purity control, pharmacokinetic studies etc.

Several analytical methods have been so far used for the separation of chiral compounds, e.g., high-performance liquid chromatography (HPLC) [1,2], thin-layer chromatography (TLC) [3], gas chromatography (GC) [4] and recently capillary electrophoresis (CE) [5–14].

The separation of enantiomers by CE is achieved mainly using the direct separation method where the chiral selector is simply added to the background electrolyte (BGE). Among the different chiral selectors studied in CE for enantiomeric resolutions, cyclodextrins, cyclodextrin derivatives and antibiotics exhibited successful stereoselective effects towards a wide number of analytes [13,14].

Cyclodextrins (CDs) are cyclic oligosaccharides

*Corresponding author.

PII S0021-9673(97)00128-3

composed of several D-(+)-glucopyranose units with a shape similar to a truncated cone with a relatively hydrophobic cavity able to accommodate a wide number of compounds. The exterior of CDs is hydrophilic due to the presence of hydroxyl groups at the two entrances of the oligomers. The hydroxyl groups at positions 2, 3 and 6 of glucopyranose can easily be modified by chemical reactions producing CD derivatives with different properties to the parent cyclodextrin, e.g., increased solubility, different depth, introduction of charged/chargeable groups, possibility for different secondary bonds between CDs and analytes, etc. It has been reported that when CDs are used, the main enantiomeric resolution mechanism is of an inclusion type [15].

The presence of several stereogenic centres and substituent groups, as well as the possibility of having electrostatic, hydrogen bonding, hydrophobic interactions and possibly inclusion complexation can account for the stereoselective properties of vancomycin.

Recently we showed the enantiomeric resolution of several aryl propionic acids (APAs) using either an uncharged β-cyclodextrin derivative (2,3,6-tri-OMe-β-CD) or positively charged β-cyclodextrins (6A-methylamino- or heptamethylamino-β-CD) [16]. The resolution of racemic APAs into their enantiomers has also been achieved using vancomycin as a chiral selector added to the BGE [17,18].

For the optimization of enantiomeric separation methods several parameters have to be considered in order to obtain, e.g., the shortest analysis time, the best sensitivity, the optimum of repeatability for both qualitative and quantitative purposes, etc.

As a continuation of our researches in the field of chiral separations by CE, different chiral selectors were tested for the optimization of the enantiomeric resolution of eight arylpropionic acids. The aim of this paper was the comparison of different chiral selectors (CD derivatives and vancomycin) with diverse enantioseparation mechanism evaluating some of the parameters, e.g., chiral selector concentration, buffer pH, which affect their stereoselective capability. Furthermore, validation data of the optimized methods such as repeatability of migration times and corrected peak areas, linearity and detection limit, have also been investigated.

2. Experimental

2.1. Chemicals and reagents

Phosphoric acid (85%, w/w), acetic acid, boric acid, methanol and sodium hydroxide were purchased from Carlo Erba (Milan, Italy). Racemic ibuprofen, indoprofen, ketoprofen, flurbiprofen and vancomycin were from Sigma (St. Louis, MO, USA); racemic suprofen, carprofen, naproxen, cicloprofen, (−)- and (+)-suprofen, (−)- and (+)-flurbiprofen, (−)- and (+)-naproxen were kindly provided by Dr. Cecilia Bartolucci, Istituto di Strutturistica Chimica, C.N.R. (Montelibretti, Rome, Italy). Heptakis-2,3,6-tri-O-methyl-β-cyclodextrin (tri-OMe-β-CD) was purchased from Cyclolab (Budapest, Hungary). Heptamethylamino-β-cyclodextrin ([MeNH]7-β-CD) was kindly provided by Dr. Alexey Eliseev, Department of Chemistry, Moscow University (Moscow, Russian Federation).

Doubly distilled water (Menichelli, Rome, Italy) was used to prepare all the solutions. The background electrolytes were filtered through nylon filters with 0.45-μm pore size (Lida, Kenasha, WI, USA) after the addition of the chiral selectors.

Standard solutions of APAs (10^{-3}) *M* were prepared in methanol and diluted at the desired concentration with ten times diluted BGE without chiral selector.

The buffer used for all experiments contained 50 m*M* of phosphoric acid, 50 m*M* of acetic acid and 50 m*M* of boric acid in water after titration with concentrated sodium hydroxide (150 m*M* of Britton Robinson Buffer (B.R.B.) 75 m*M* B.R.B. was prepared by diluting 150 m*M* B.R.B. with water [19].

2.2. Apparatus

A Biofocus 3000 automated capillary electrophoresis apparatus (Bio-Rad, Hercules, CA, USA) equipped with a multi-wavelength UV–visible detector and a thermostating liquid system was used for the experiments.

Electrophoretic separations of enantiomers were performed using a fused-silica capillary 35 cm×50 μm I.D., with an effective length of 30.5 cm, coated with polyacrylamide using the modified method

described by Hjerten [20]. The measurements of electrophoretic mobility of vancomycin were done with an untreated fused-silica capillary tube 50 cm× 50 μm I.D., effective length 45.5 cm.

Fused-silica capillaries 50-μm I.D., 375-μm O.D. were from Composite Metal Services (Worcestershire, UK).

The capillaries were positioned into a capillary user assembled cartridge (Bio-Rad) after removing 0.5 cm of the polyimide layer with concentrated H_2SO_4 (at about 100°C). The temperature of both cartridge and carousel was kept constant at 25°C. Injection was done at 10 p.s.i.·s, (1 p.s.i.=6894.76 Pa) while the applied voltage was constant at −15, −18 or −20 kV.

2.3. Procedure

When cyclodextrin derivatives were used as chiral selectors the capillary was purged with water for 100 s and then with the running buffer containing the appropriate concentration of chiral selector for 120 s using high pressure.

When vancomycin was used as chiral selector a different procedure had to be performed; the capillary was purged between runs as follows: (1) water (100 s), (2) running buffer free of vancomycin (120 s), (3) running buffer containing the appropriate concentration of vancomycin at low pressure (175 p.s.i.·s) and (4) injection of samples at low pressure (10 p.s.i.·s) .

3. Results and discussion

The separation of enantiomers can be performed by CE using the direct separation method where the chiral selector can be either added to the BGE or bound to the capillary wall. So far the first approach has been widely used in CE because the method is simple to perform, low amounts of chiral selector are required and thus is inexpensive, a wide number of chiral selectors are commercially available etc. However, poor detection limits due to the strong absorbtion of the chiral selector is a drawback to be considered for the optimization of the CE method for qualitative and quantitative chiral analysis.

Three different chiral selectors, namely 2,3,6-tri-OMe-β-CD, heptamethylamino-β-CD and vancomycin, were separately added to the BGE in electrophoretic experiments for the enantiomeric separation of eight racemic aryl propionic acids (for their chemical structures see Fig. 1). A polyacrylamide coated capillary was selected for the experiments in order to eliminate/reduce the electroosmotic flow. Considering our previous studies using tri-OMe-β-CD, we selected a BGE of pH 5 that gave good enantiomeric resolution for APAs. At the selected pH all the studied compounds moved as anions in the direction of the anodic compartment. Preliminary experiments performed using the two modified β-CDs at a relatively low wavelength (206 nm) did not reveal any problem with detection of analytes while the use of vancomycin for the chiral separation of APAs under these conditions failed due to the strong absorbtion of the chiral selector.

When using the positively charged CD derivative (heptamethylamino-β-CD), the chiral selector moved in the opposite direction to the analytes and was

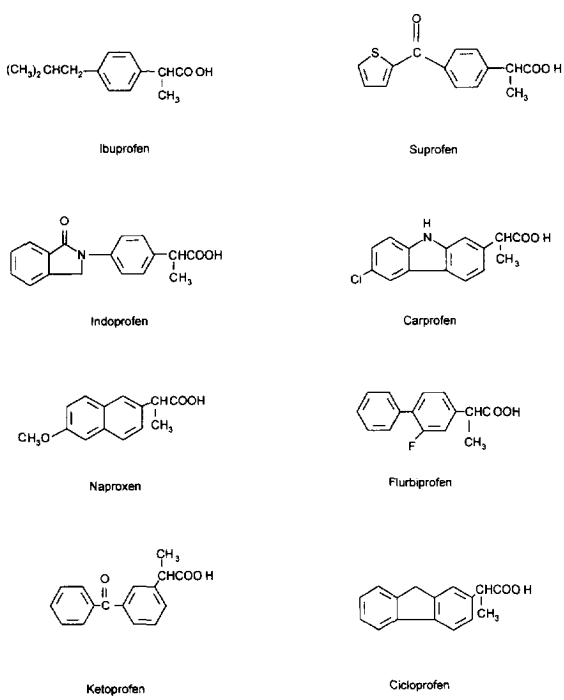

Fig. 1. Chemical structures of the arylpropionic acids studied.

present in the capillary as well as in the electrode compartments.

As previously discussed, the formation of labile diastereoisomers with charged CDs will increase the difference in mobility between the complex and the free analyte, increasing the chiral resolution [21].

Fig. 2 shows the dependence of enantiomeric resolution (R_s) of the eight APAs on three heptamethylamino-β-CD concentrations.

A general increase of resolution was recorded using higher concentrations of CD in the range 0–10 m*M* for all the studied compounds except for indoprofen and carprofen which showed a maximum of R_s at 5 m*M* CD. The resolution of ibuprofen enantiomers has not been observed because we could not detect any peaks (electrophoretic run 40 min) at the studied concentrations of CD and this is probably due either to the strong complexation analyte–chiral selector or to dispersion effect caused by the interactions with the modified CD. Thus indoprofen was analyzed using the same BGE containing 0.1 m*M* of heptamethylamino-β-CD and the two enantiomers were detected as broadened peaks in less than 10 min with very poor resolution.

The increase of CD concentration in the BGE caused also a general increase of migration time and reduction of electrophoretic mobility (results not shown).

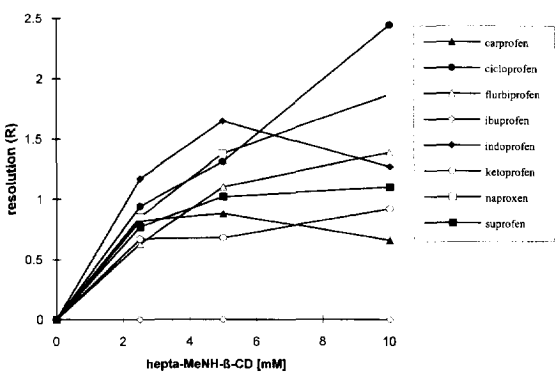

Fig. 2. Effect of heptamethylamino-β-cyclodextrin concentration on resolution of arylpropionic acids. Capillary coated with polyacrylamide 35 (30.5) cm×50 μm I.D.; background electrolyte, 75 m*M* Britton Robinson buffer (B.R.B.) (pH 5) and the appropriate amount of CD; −15 kV, 20.4–37.6 μA; injection 10 p.s.i.·s of cicloprofen, flurbiprofen, indoprofen ($5 \cdot 10^{-5}$ *M*) and carprofen, ibuprofen, ketoprofen, naproxen and suprofen ($1 \cdot 10^{-4}$ *M*).

The enantioresolution of the eight profens performed in methylamino-β-CD was compared with that achieved using an uncharged modified β-CD (tri-OMe-β-CD). The uncharged β-CD behaved as a quasi-stationary phase forming labile diastereoisomeric complexes with analytes, decreasing the charge/mass ratio and thus the electrophoretic mobility. Based on our previously published data [16] we tested the influence of CD concentration on the enantiomeric resolution at two different concentration levels, 15 and 30 m*M* (see Table 1). At the lowest concentration of CD the base line enantiomeric resolution was achieved only for naproxen, indoprofen, cicloprofen and carprofen. Increasing the concentration of the uncharged chiral selector to 30 m*M* the resolution increased for all the analytes studied except for indoprofen; baseline resolution was achieved in all instances. Comparing the results obtained in this study with those previously achieved by us for some of the racemic analytes at the same pH and concentration of CD but with different BGE (type and ionic strength) [16] revealed that a lower resolution is obtained with the present experimental conditions. This effect can be explained by considering the different experimental parameters, e.g., BGE counter-ion, ionic strength, current which modulate the effective mobility of the studied enantiomers.

Finally, considering the published data [17,18], vancomycin was selected as a chiral selector for the enantiomeric separation of the racemic arylpropionic acids.

Several experiments were carried out using an untreated fused-silica capillary rinsed with 75 m*M* of B.R.B. buffer at different pH values in the range 4–9 and injecting standard solution of vancomycin in order to measure the effective mobility of the chiral selector. Recently it has been shown that the effective mobility can be influenced by the type of BGE [22]. Increasing the pH of the BGE, the effective mobility of vancomycin decreased from $+6 \cdot 10^{-5}$ to $-8 \cdot 10^{-5}$ $cm^2 V^{-1} s^{-1}$ and zero mobility was obtained at pH 7.5 (results not shown).

Preliminary experiments were carried out in a BGE of pH 5 containing 5 m*M* of vancomycin for the enantiomeric resolution of racemic naproxen; at this pH vancomycin was positively charged. In the two different operational modes tested in order to find the optimal experimental conditions for the best

Table 1
Effect of 2,3,6-tri-O-methyl-β-cyclodextrin concentration on enantiomeric resolution and migration time of arylpropionic acid enantiomers

Analytes	2,3,6-Tri-O-methyl-β-cyclodextrin concentration (m*M*)					
	0		15		30	
	t_m	R_s	t_m	R_s	t_m	R_s
Carprofen	5.5	–	13.5	1.2	15.7	2.3
			14.1		17.0	
Cicloprofen	5.2	–	19.8	1.3	21.3	2.7
			20.2		22.8	
Flurbiprofen	5.0	–	13.8	<0.5	20.2	1.9
			14.0		21.2	
Ibuprofen	5.3	–	22.3	1.1	31.0	1.8
			23.4		34.9	
Indoprofen	5.6	–	9.7	1.9	13.4	1.2
			10.1		13.8	
Ketoprofen	5.3	–	8.3	<0.5	11.2	1.3
			8.4		11.6	
Naproxen	5.1	–	10.5	2.9	12.2	5.2
			11.5		13.9	
Suprofen	5.1	–	11.6	0.6	13.9	1.3
			11.9		14.4	

Capillary, 35 (31.5) cm×50 μm I.D. (coated); BGE, 75 m*M* B.R.B. (pH 5) and cyclodextrin; −20 kV, 32.8–33.6 μA; injection at 10 p.s.i.·s of cicloprofen, flurbiprofen, indoprofen ($5 \cdot 10^{-5}$ *M*) and carprofen, ibuprofen, ketoprofen, naproxen and suprofen ($1 \cdot 10^{-4}$ *M*)
t_m=migration time (min).

sensitivity, the chiral selector–buffer was present either in all the system (including the electrode compartments) or in the capillary only (entirely or partly filled).

Fig. 3a and b show the electropherograms of the enantiomeric separation of naproxen at 206 and 254 nm, respectively when the chiral selector was present in the capillary only (entirely filled). The enantiomeric separation of naproxen was achieved in <8 min and the detector signal, at the beginning of the electrophoretic run, was relatively high due to the presence of vancomycin in the detector cell. The signal later dropped below zero due to the movement of vancomycin, positively charged, in the opposite direction of the detector path. A constant value of absorbance was recorded after 5 and 3 min run at 206 and 254 nm, respectively. This approach gave a higher sensitivity than that achieved when the chiral selector was present in both capillary and electrode compartments (results not shown).

Therefore, when using an absorbing and charged (opposite to the analytes) chiral selector entirely filling the capillary but not the electrode compartments, the separation method can improve the sensitivity (relatively low wavelengths can be used). However, with the proposed system, optimum experimental conditions can only be achieved after 5 min which is a drawback of the method. In fact analytes migrating with times shorter than 5 min cannot be detected. This method has been used by Ward [23] for the enantiomeric separation of several racemic compounds including some arylpropionic acids with detection at 254 nm.

Fig. 3c shows the separation of naproxen enantiomers using a partial filling method where the chiral selector, dissolved in the BGE, was present only in part of the capillary. Such an electrophoretic system has been used by several authors for enantiomeric separations employing proteins, cyclodextrin derivatives and vancomycin [23–25].

Thus we decided to fill only part of the capillary using a low pressure verifying the exact time necessary for the BGE/chiral selector to reach the detector; this measurement was repeated each time the concentration of vancomycin was changed in order to account for the different viscosity. For the method optimization the buffer–vancomycin zone present in the capillary should be sufficient to reach the detec-

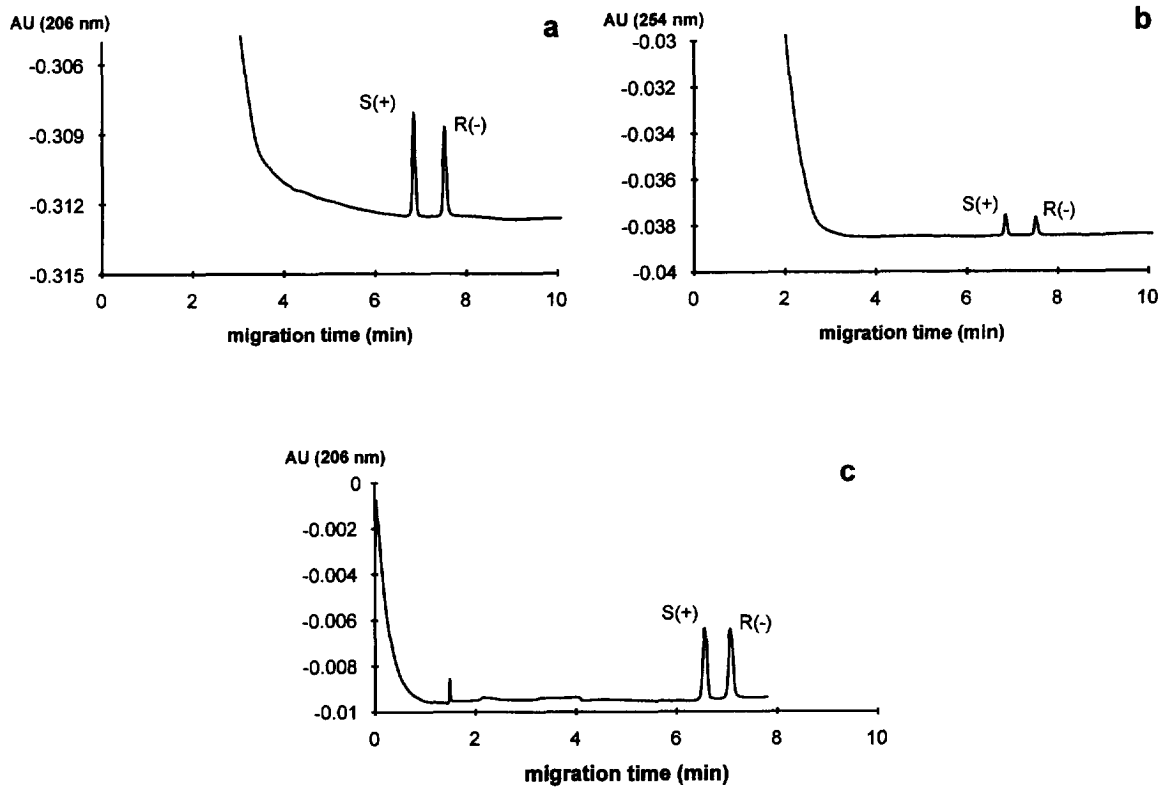

Fig. 3. (a), (b) and (c) Electropherograms of the enantiomeric separation of naproxen using vancomycin as a chiral selector. Capillary, 35 (31.5) cm×50 μm I.D. (coated); background electrolyte, (a) and (b) 75 m*M* B.R.B. (pH 5) and 5 m*M* of vancomycin (the electrode compartments contained the buffer without chiral selector); (c) the same BGE as (a) and (b) partial filling method (vancomycin zone injected at 175 p.s.i.·s); applied voltage, −18 kV, 34.6–38.5 μA; injection, pressure at 10 p.s.i.·s of naproxen $5 \cdot 10^{-5}$ *M*.

tor and should leave it as fast as possible in order to couple the best sensitivity and the widest window analysis. The optimum experimental conditions for the naproxen enantiomers separation allowed us to have the detector vancomycin free after about 1 min (see Fig. 3c). For automatic instrumentation the high precision of the partial filling method could be obtained by software that will automatically stop the injection of buffer–chiral selector zone at the exact time that the detector signal is increasing.

The partial filling method was selected for further experiments in order to verify the effect of vancomycin concentration and pH of the BGE on the enantiomeric resolution of the eight arylpropionic acids listed in Fig. 1.

Electrophoretic experiments were carried out using the BGE at pH 5 in the absence or presence of 2.5 or 5 m*M* of vancomycin and the resolution was calculated. In the absence of the chiral selector all the studied compounds moved towards the detector in less than 6.25 min. The addition of 2.5 m*M* of vancomycin to the BGE caused a general increase of migration time as well as baseline enantiomeric resolution for all the studied analytes due to the complexing effect of the chiral selector. Increasing the vancomycin concentration to 5 m*M* caused an increase of both migration times and resolution for the eight couples of enantiomers, however the slowest migrating enantiomer was detected in less than 10 min. The results of the effect of vancomycin concentration on enantiomeric resolution of APAs are depicted in Table 2. Considering the resolution obtained at the highest level of vancomycin we can outline that the enantiorecognition of this chiral

Table 2
Effect of vancomycin concentration on enantiomeric resolution and migration time of arylpropionic acid enantiomers

Analytes	Vancomycin concentration (m*M*)					
	0		2.5		5	
	t_m	R_s	t_m	R_s	t_m	R_s
Carprofen	6.1	–	7.3	1.4	8.2	3.1
			7.5		8.8	
Cicloprofen	5.9	–	6.7	2.4	7.4	4.2
			7.0		8.3	
Flurbiprofen	5.7	–	6.2	3.2	6.8	6.0
			6.7		7.7	
Ketoprofen	6.1	–	6.8	4.3	7.4	5.0
			7.6		9.6	
Ibuprofen	6.2	–	6.8	1.4	7.3	2.4
			7.0		7.8	
Indoprofen	6.2	–	7.4	1.1	8.5	1.6
			7.6		8.8	
Naproxen	5.9	–	6.3	2.2	6.5	3.4
			6.6		7.0	
Suprofen	5.7	–	6.6	1.6	7.2	2.3
			6.8		7.6	

Capillary, 35 (31.5) cm×50 μm I.D. (coated); BGE, 75 m*M* B.R.B. (pH 5) and 2.5 or 5 m*M* vancomycin (partial filling zone at 175 p.s.i.·s); −18 kV, 31.6–35.6 μA; for other experimental conditions see Table 1.
t_m = migration time (min).

selector vs. the studied APAs was as follows: flurbiprofen>ketoprofen>cicloprofen>naproxen> carprofen>ibuprofen>suprofen>indoprofen.

The effect of the pH of the BGE on the enantiomeric resolution of the APAs was studied separately analyzing the eight racemic mixtures with 75 m*M* of B.R.B. at pH 5, 6 and 7 containing 5 m*M* of vancomycin using the partial filling method.

As can be seen in Fig. 4 the increase of the pH of the BGE caused a general decrease of migration times as well as resolution, especially for flurbiprofen, cicloprofen and ketoprofen. The decrease of migration time is probably due to the increase of current with the pH as well as the decrease of the positive charge of vancomycin. Both parameters (higher current and reduced positive charge of the chiral selector) also influence the resolution. In fact, the mobility difference between the complexed and free analyte is decreased. The best enantiomeric separations for the eight APAs were achieved at the lowest pH.

Migration order was verified for suprofen, naproxen and flurbiprofen analyzing their enantiomers in the BGE at pH 5 and containing 30 m*M* tri-OMe-β-CD or 5 m*M* heptamethylamino-β-CD or 5 m*M* vancomycin. In all instances the *S*(+) analyte mi-

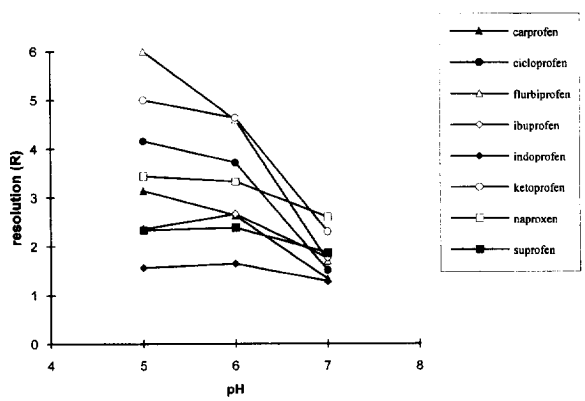

Fig. 4. Effect of the pH value of the background electrolyte on the enantiomeric resolution of arylpropionic acids using vancomycin as the chiral selector. Background electrolyte, 75 m*M* of B.R.B. and 5 m*M* of vancomycin, partial filling method at 175 p.s.i.·s (pH 5) and 168 p.s.i.·s (pH 6 and 7); applied voltage, −18 kV, 32.8–50.6 μA.

Table 3
Detection limits (LOD, S/N, 3:1) of some of the arylpropionic acids studied using β-CD derivatives and vancomycin

LOD (S/N=3:1)	Tri-OMe-β-CD (30 m*M*)	Hepta-MeNH-β-CD (5 m*M*)	Vancomycin (5 m*M*)
Indoprofen	$5 \cdot 10^{-6}$ *M*	$1 \cdot 10^{-5}$ *M*	$1 \cdot 10^{-6}$ *M*
Naproxen	$5 \cdot 10^{-6}$ *M*	$1 \cdot 10^{-5}$ *M*	$5 \cdot 10^{-6}$ *M*
Suprofen	$5 \cdot 10^{-6}$ *M*	$1 \cdot 10^{-5}$ *M*	$5 \cdot 10^{-6}$ *M*

For experimental conditions see Tables 1 and 2 and Fig. 2.
t_m=migration time.
A_t=corrected peak area.

grated faster than *R*(−) enantiomer indicating that the *R*(−) optical isomer forms more stable complexes with the three chiral selectors than the *S*(+) compounds.

Detection limit (LOD, signal-to-noise ratio S/N= 3:1) was evaluated for indoprofen, naproxen and suprofen. The racemic mixtures were analyzed in 75 m*M* B.R.B. (pH 5) in presence of 30 m*M* tri-OMe-β-CD or 5 m*M* heptamethylamino-β-CD or 5 m*M* vancomycin. In the last experiments the partial filling method was used. In all instances good detection limit was recorded and the results are shown in Table 3.

Table 4 shows the precision of migration time, corrected peak areas and the efficiency (*N*) obtained analyzing a racemic mixture of naproxen. Very good precision for migration time was achieved using the three chiral selectors separately, while for corrected peak areas satisfactory precision was obtained with all three chiral selectors. The highest efficiency was obtained when vancomycin was used.

Considering that vancomycin can offer several advantages over the other chiral selectors used for the enantiomeric resolution of the studied APAs, e.g., the best detection limit, the shortest analysis time, the highest number of theoretical plates and the highest stereoselective effect we further investigated another parameter to be controlled for the validation of the separation method, namely detector linearity for the two enantiomers of naproxen. The linearity was determined for naproxen enantiomers by injecting the racemic mixtures solutions in the presence of 1-naphthalenesulfonic acid sodium salt at a constant concentration as an internal standard (I.S.). The detector linearity for the analysis was studied in the concentration range $1-10 \cdot 10^{-5}$ *M* (ten measure-

Table 4
Precision of migration time, corrected peak areas and efficiency obtained for the enantiomeric separation of naproxen using the three chiral selectors

Chiral selector	S.T.D. (%) (*n*=6)				Efficiency $N \times 1000$(×meter)	
	t_{m1}	t_{m2}	A_{t1}	A_{t2}	*S*(+)	*R*(−)
Tri-OMe-β-CD	0.66 (8.3 min)	0.37 (10.3 min)	0.86	2.33	113	68
Hepta-MeNH-β-CD	0.79 (10.5 min)	1.01 (11.1 min)	1.67	1.87	84	55
Vancomycin	0.86 (6.46 min)	0.83 (6.98 min)	1.60	1.79	269	249

Chiral selector concentration, 30 m*M* tri-OMe-β-CD, 5 m*M* heptamethylamino-β-CD and 5 m*M* vancomycin dissolved in 75 m*M* of B.R.B. (pH 5). For other experimental conditions see Tables 1 and 2 and Fig. 2.

ments) and the correlation coefficient was found to be 0.9984 and 0.9988 for *S*(+) and *R*(−)-naproxen, respectively. The linear regression was found to be $y=-0.0337+0.0807x$ and $y=-0.0172+0.0689x$ for *S*(+) and *R*(−)-naproxen, respectively. An attempt to verify the detection linearity when the injected mixture contained an enantiomeric excess of either *S*(+) or *R*(−)-naproxen failed due to the presence of enantiomeric impurities in the samples (1–1.5%).

Fig. 5a and b show the electropherograms of the enantiomeric separation of indoprofen and flurbiprofen, respectively, in BGEs at pH 5 containing the three chiral selectors separately. We can observe that in the case of flurbiprofen the use of heptamethylamino-β-CD should be avoided because a relatively low UV signal is obtained. A similar problem was recorded with the same chiral selector for the ibuprofen enantiomeric separation.

4. Conclusions

Comparing the data of resolution and migration times obtained when analysing racemic APAs in the BGE at pH 5 for three different chiral selectors, the shortest analysis time and the highest efficiency were achieved when vancomycin was used as the chiral selector for the enantiomeric resolution of arylpropionic acids (see also Table 5). Concerning the enantiomeric resolution, vancomycin proved to be more effective than tri-OMe-β-CD and heptamethyl-β-CD except for naproxen and indoprofen (the two compounds were better resolved into their enantiomers using tri-OMe-β-CD and heptamethylamino-β-CD, respectively). The precision of migration time and corrected peak areas are similar for the three chiral selectors while vancomycin exhibited the highest detection limit.

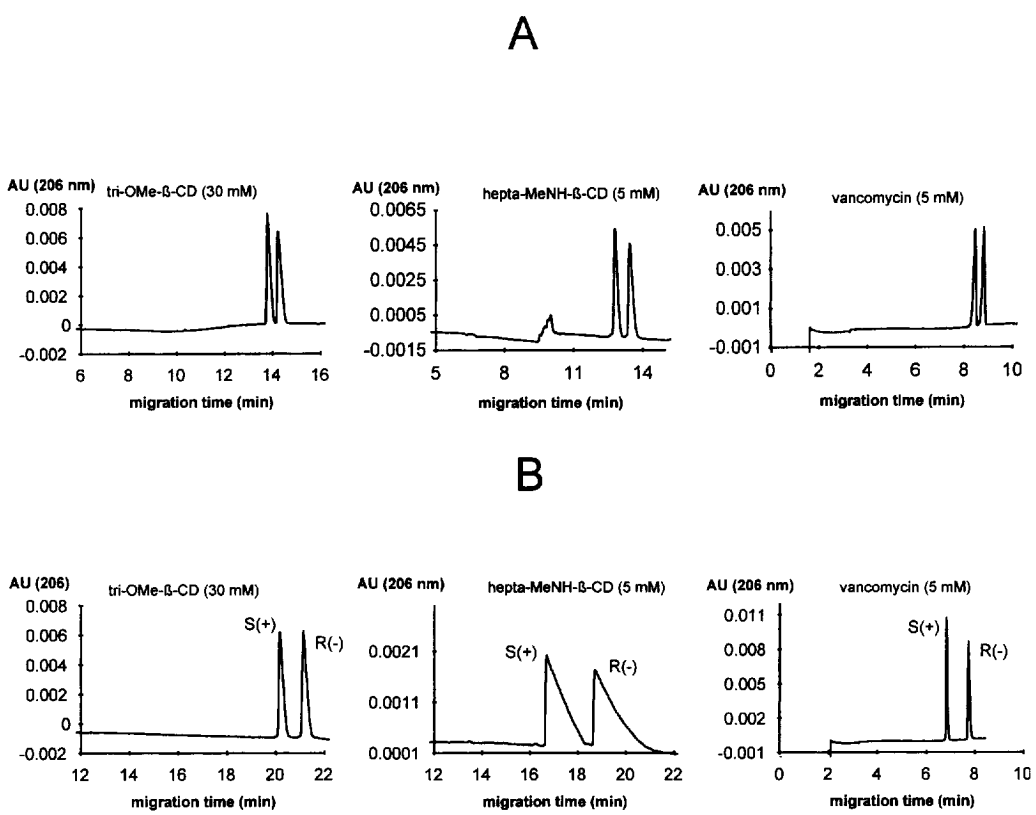

Fig. 5. Electropherograms of the enantiomeric separation of indoprofen (panel A) and flurbiprofen (panel B) using 75 m*M* B.R.B. (pH 5) and different chiral selectors. For other experimental conditions see Fig. 2 and Tables 1 and 2.

Table 5
Comparison of the enantiomeric resolution and migration time obtained using tri-O-methyl-β-CD, heptamethylamino-β-CD and vancomycin using the optimum experimental conditions

Samples	30 m*M* Tri-OMe-β-CD			5 m*M* Hepta-MeNH-Gb-CD			5 m*M* Vancomycin		
	t_{m1}	t_{m2}	R_s	t_{m1}	t_{m2}	R_s	t_{m1}	t_{m2}	R_s
Carprofen	15.7	17.0	2.3	21.5	22.7	0.9	8.2	8.8	3.1
Cicloprofen	21.3	22.8	2.7	13.8	14.9	1.3	7.4	8.3	4.2
Flurbiprofen	20.2	21.2	1.9	16.7	18.8	1.1	6.8	7.7	6.0
Ibuprofen	31.0	34.9	1.8	–	–	–	7.3	7.8	2.3
Indoprofen	13.4	13.8	1.2	12.8	13.5	1.6	8.5	8.8	1.6
Ketoprofen	11.2	11.6	1.3	12.1	12.4	0.7	7.4	9.6	5.0
Naproxen	12.2	13.9	5.2	10.6	11.0	1.4	6.5	7.0	3.4
Suprofen	13.9	14.4	1.3	13.3	14.2	1.0	7.2	7.6	2.3

For experimental conditions see Tables 1 and 2 and Fig. 2.
t_m=migration time (min).

Among the advantages of using vancomycin as a chiral selector are that an automatic instrumentation is necessary for the control of the injected zone containing BGE–chiral selector.

Acknowledgments

This work was supported by Consiglio Nazionale delle Ricerche (C.N.R.) Strategic Project 'Tecnologie Chimiche Innovative'.

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