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Examination of the retention behavior of underivatized profen enantiomers on cyclodextrin silica stationary phases

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ABSTRACT

The separation of the enantiomers of five widely used profen-type non-steroidal anti-inflammatory agents: fenoprofen, flurbiprofen, ibuprofen, ketoprofen and naproxen was studied using four commercially available cyclodextrin silica stationary phases operated in the reversed-phase mode. The retention behavior of all profens was similar on all the cyclodextrin silica stationary phases studied, but the chiral selectivities differed significantly. Complete separation was achieved for the enantiomers of ibuprofen, flurbiprofen and naproxen.

INTRODUCTION

The 2-aryl propionic acids, or profens, belong to the family of non-steroidal anti-inflammatory drugs (NSAIDs). Once it became known that the different profen enantiomers have different pharmacokinetical properties, interest in their chromatographic separation has increased [1–20]. Some of the profen enantiomers were separated in both the derivatized form [2–9], and in the underivatized form [10–20]. According to the derivatization approach, the carboxyl group of the enantiomers was converted into an ester group [2,3,9], an anilide group [4,5,8], or an amide group [1,2,5–7,9] resulting in improved separation [l-3,5,7-9], and/or improved detection limits [4]. In some of the schemes, the profens were derivatized with an enantiomerically pure reagent leading to the formation of diastereomers [4,6], which then were separated either on a chiral (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine-based silica column [4] or on an achiral octadecyl silica column [6]. In other schemes, a non-chiral reagent was used to derivatize the profen enantiomers followed by their separation on one of the cellulose-based stationary phases, Chiralcel OC [1] or Chiralcel OJ [3], or on the (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine-based silica column [1,2,5,7,9], the (*R*)-N-(2-naphthyl)alanine-based silica column [8], or on the chiral stationary phase derived from (*S*)-N-(1-naphthyl)leucine [10]. Enantiomers of some of the underivatized profens have also been separated using this stationary phase [10,11], as well as the α_1-acid glycoprotein stationary phase [12,13], the human serum albumin stationary phase [14], the Cyclobond-I β-cyclodextrin silica phase [18], the Cyclobond-I-SN naphthylethylcarbamoylated cyclodextrin silica phase [15], and an acetylquinidine-silica column used in conjunction with quinidine as a mobile phase additive [16]. In our laboratory, we have been interested in the direct preparative-scale separation of the enantiomers of some of

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the profens, primarily using cyclodextrin silica stationary phases [17–20].

Cyclodextrins are toroidally shaped molecules containing 6, 7, or 8 glucose units (α-, β- or y-cyclodextrins) . Cyclodextrins have a hydrophobic cavity and two hydrophilic lips with secondary hydroxyl groups on the larger lip and primary hydroxyl groups on the smaller lip [21,22]. Cyclodextrins can include molecules into their cavities and form host-guest complexes [23]. The stability of the complex depends on the snugness of the fit between the molecule and the cavity and the strength of the polar intermolecular interactions between the host and the guest [24]. The secondary hydroxyl groups can be derivatized to extend the depth of the cavity and/or change the nature of the polar interaction sites.

The commercially available cyclodextrin silica HPLC phases contain cyclodextrin moieties which are chemically bonded to the silica support via the 3-glycidoxysilane [25] spacer. Hydroxypropylated cyclodextrin silicas of the desired chirality are produced by first reacting the cyclodextrin with the appropriate propylene oxide, followed by binding of the modified cyclodextrin to the glycidoxysilylated silica [26,27]. Naphthylethylcarbamoylated cyclodextrin silica stationary phases are produced by further reacting the cyclodextrin silica, *in situ*, with (*S*)-(+)- or (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate [28].

EXPERIMENTAL

A custom-built liquid chromatograph consisting of an LC 2010 pump (Varian, Walnut Creek, CA, USA), a pneumatically activated Type 7000 injection valve (Rheodyne, Cotati, CA, USA), an LC 2050 variable-wavelength *W* detector (Varian) set at 254 nm, and a Series RI-3 differential refractive index detector (Varian) , was used for the experiments. The detector signals were recorded and analyzed by a Maxima Workstation (Millipore-Waters, Milford, MA, USA). Stainless-steel columns, 250 mm *x* 4.6 mm I.D. (BST, Budapest, Hungary), were custom packed with 5-μm cyclodextrin silicas (ASTEC, Whippany, NJ, USA) using a Model 53127-2 air amplifier pump (Haskel, Burbank, CA, USA). All columns were jacketed and thermostatted at 30°C by a Type UP3 circulating water bath (Science/Electronics, Dayton, OH, USA). All separations were completed at an eluent flow-rate of 1 ml/min.

The stationary phases used in this work are the native β- and y-cyclodextrin silicas, as well as the (*S*)- and racemic-2-hydroxypropyl-β-cyclodextrin silicas (average degree of substitution: 7.9 hydroxypropyl units per cyclodextrin) [27], and the (S)-naphthylethyl carbamoylated β-cyclodextrin [4] silica (average degree of substitution: 6.3 naphthylethyl units per cyclodextrin) [28]. These materials are all commercially available from ASTEC under the trade names of Cyclobond I, Cyclobond II, Cyclobond I-SP,

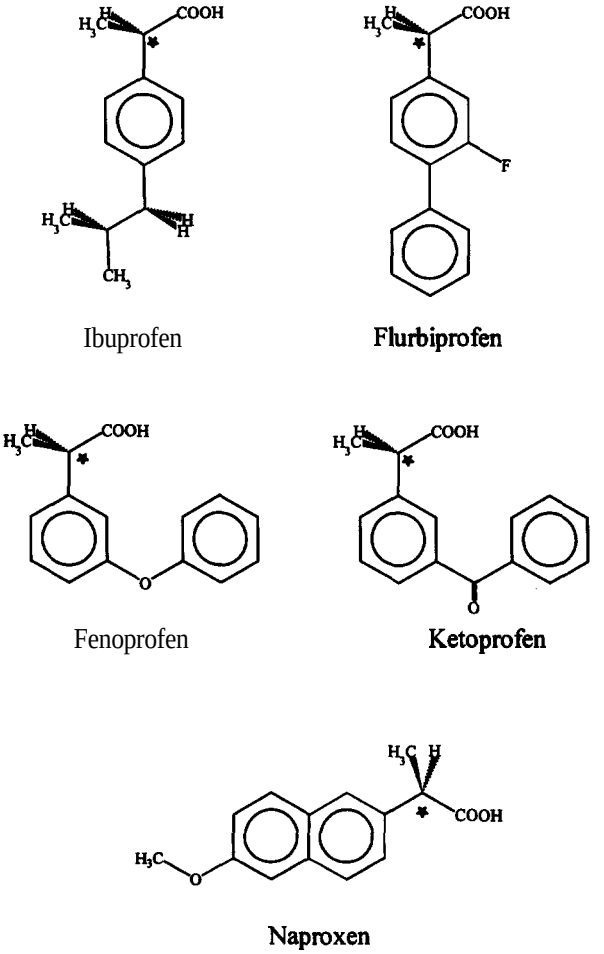

Fig. 1. Structures of the profens used in this study.

Cyclobond I-RSP, and Cyclobond I-SN, respectively.

The eluents were prepared as described before [29] from HPLC-grade acetonitrile (EM Science, Gibbstown, NJ, USA), water produced by a Milli-Q unit (Millipore, Bedford, MA, USA), and sodium citrate and citric acid, both from Aldrich (Milwaukee, WI, USA). The eluent pH values reported here are apparent pH values measured in the hydroorganic eluent using a glass electrode (Corning, Medfield, MA, USA) calibrated with standard aqueous buffers (Fisher Scientific, Fair Lawn, NJ, USA) as recommended in refs. 30 and 31. Racemic ibuprofen and racemic naproxen were gifts from ASTEC. Ketoprofen, flurbiprofen, and (*S*)-naproxen were obtained from Sigma (St. Louis, MO, USA). @)-Ibuprofen was obtained from Aldrich. Fenoprofen calcium was a gift from Eli Lilly (Indianapolis, IN, USA). (The solute structures are shown in Fig. 1.) The solutes were dissolved in the eluents immediately prior to use. The injected amounts were kept at minimum to approximate infinite dilution conditions and were adjusted to yield a signal-to-noise ratio of about 10 to 50.

RESULTS

β-Cyclodextrin silica (Cyclobond Z) column

Because in a chiral separation it is the more retained enantiomer that experiences all the possible binding interactions to the fullest, the capacity factors of the more retained enantiomers (k_2') are used in this paper to discuss the retention behavior of the five profens. The k_2' obtained on the native β-cyclodextrin silica stationary phase (Cyclobond I) are plotted in Figs. 2 and 3 as a function of the acetonitrile concentration of the eluent at two different pH values: at pH 4, which is below, and at pH 6, which is above the pK_a values of all the profens studied here. On native cyclodextrin silicas operated in the reversed-phase mode, solute retention is attributed to the concerted action of the hydrophobic interactions between the hydrophobic parts of the solute and the hydrophobic interior of the cyclodextrin cavity (inclusion phenomenon), and the hydrogen bonding interactions between the polar functional groups of the solute and the hydroxyl groups of the cyclodextrin [22]. As can be seen in Figs. 2 and 3, the logarithm of the capacity factors of the profens decreases almost linearly as the concentration of acetonitrile in the eluent is increased, just as in a regular reversed-phase system consisting of an octadecyl silica stationary phase and a buffered hydroorganic eluent [32]. However, as long as there is more than 30% (v/v) acetonitrile in the eluent, the dissociated profen anions (predominant in the pH 6 eluents) are more

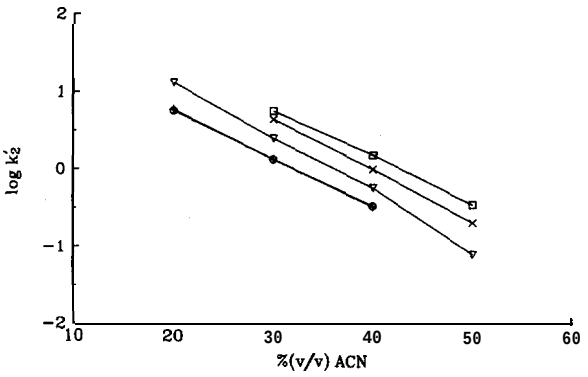

Fig. 2. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile (ACN) concentration in the pH 4.0, 5 m*M* citrate buffer eluents on the Cyclobond I β-cyclodextrin silica column. Flow-rate: 1 ml/min, temperature: 30°C, UV detection at 254 nm. Symbols: □ = ibuprofen; x = flurbiprofen; V = fenoprofen; + = naproxen, 0 = ketoprofen.

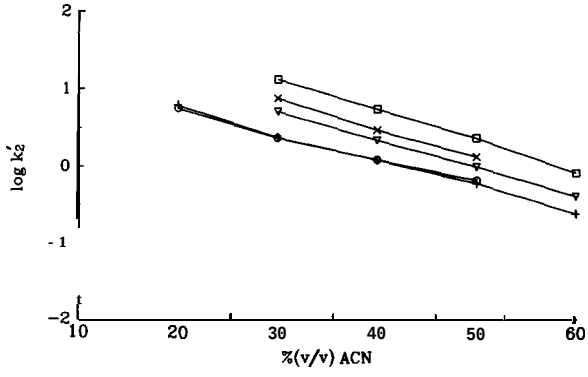

Fig. 3. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile concentration in the pH 6.0, 5 m*M* citrate buffer eluents on the Cyclobond I β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

strongly retained than the non-dissociated, free acid profens (predominant in the pH 4.0 eluents). This behavior is exactly opposite of what one can observe in a regular reversed-phase system [32]: it indicates that in the presence of an organic solvent the sum of the hydrophobic and the hydrogen bonding interactions between the anions and the cyclodextrin are stronger than those between the free acids and the cyclodextrins. As the acetonitrile concentration is decreased, the strength of the bonding interactions between the anions and the cyclodextrin increases only slightly, while the strength of the bonding interactions between the free acids and the cyclodextrins increases strongly. Eventually, in pure aqueous eluents, the free acids are bonded more strongly than the respective anions. This observation agrees qualitatively with our recent finding in capillary electrophoresis, that in a pure aqueous buffer the value of the formation constant of the cyclodextrin–profen acid complex is larger than that of the cyclodextrin–profen anion complex [33].

With regard to chiral selectivity, an interesting trend can be observed in Fig. 4. As long as the concentration of acetonitrile is higher than 20% (v/v), chiral separation is observed only in the high-pH eluent, and only for ibuprofen. As the acetonitrile concentration is decreased, the chiral selectivity coefficient for ibuprofen increases towards a limiting α value of about 1.06. In eluents with acetonitrile concentrations higher than 50% (v/v), chiral discrimination is lost, even in high-pH eluents. Though it would be interesting to compare the chiral recognition mechanisms operative in HPLC and capillary electrophoresis (where a limiting α value of about 1.04 has been observed for ibuprofen in low-pH buffers at a temperature of 37°C [33]), the HPLC α values for ibuprofen could not be determined in purely aqueous eluents, either at high pH or at low pH, because the k_2' values were excessively high (well over 100).

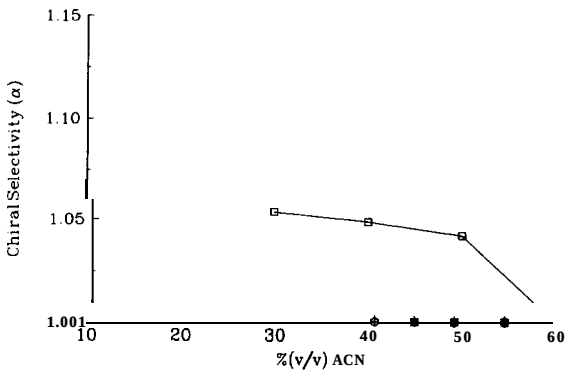

Fig. 4. Chiral selectivity as a function of the % (v/v) acetonitrile concentration in the pH 6.0, 5 m*M* citrate buffer eluents for profens on the Cyclobond I β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

Because good chiral separation was observed for the ionic form of ibuprofen, the buffer strength of the eluent was varied to see if solute retention and chiral selectivity could be controlled by this parameter, and/or chiral separation could be achieved for the other profens. While increased buffer strength greatly decreased the capacity factors of the profens both in high- and low-pH eluents, due to the increased competition of the buffer species for the available cyclodextrin binding sites [34], it did not lead to chiral recognition for any of the other profens.

The effect of column temperature on the chiral selectivity was also examined. For ibuprofen, a regular Van 't Hoff plot was obtained, similar to the ones reported earlier [19], and the chiral selectivity improved from α = 1.054 at 30°C to α = 1.075 at 2°C. However, chiral separation could not be achieved for the other profens, even at 2°C.

Naphthylethylcarbamoylated β-cyclodextrin silica (Cyclobond I-SN) column

On the naphthylethylcarbamoylated β-cyclodextrin silica column, the logarithms of the capacity factors decrease linearly with the increasing acetonitrile concentration: the rate of decrease is once again faster at pH 4 (Fig. 5) than at pH 6 (Fig. 6). When the acetonitrile concentration in the eluent is less than 20% (v/v), retention in the low pH eluents (non-dissociated profens) becomes stronger than in the high pH eluents (anionic profens). In the high-pH eluents, the k_2' values on the native and on the naphthylethylcarbamoylated β-cyclodextrin silica columns are identical within experimental error. However, in low-pH eluents,

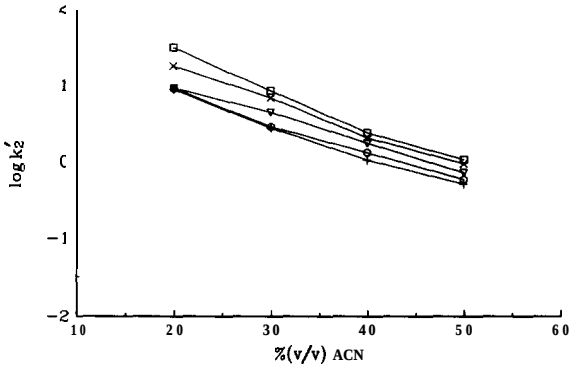

Fig. 5. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile concentration in the pH 4.0, 5 m*M* citrate buffer eluents on the Cyclobond I-SN naphthylethylcarbamoylated β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

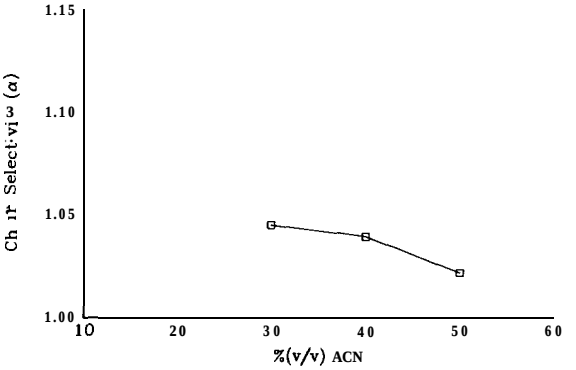

Fig. 7. Chiral selectivity as a function of the % (v/v) acetonitrile concentration in the pH 4.0, 5 m*M* citrate buffer eluents for profens on the Cyclobond I-SN naphthylethylcarbamoylated β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

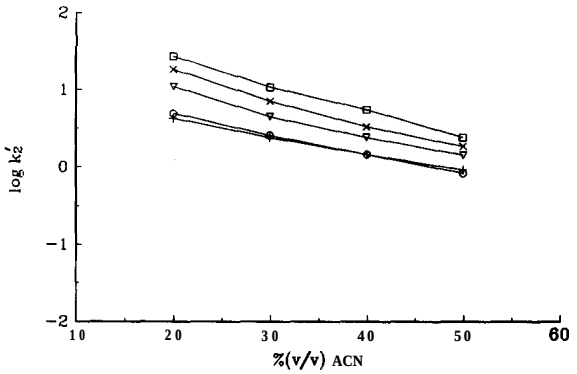

Fig. 6. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile concentration in the pH 6.0, 5 m*M* citrate buffer eluents on the Cyclobond I-SN naphthylethylcarbamoylated β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

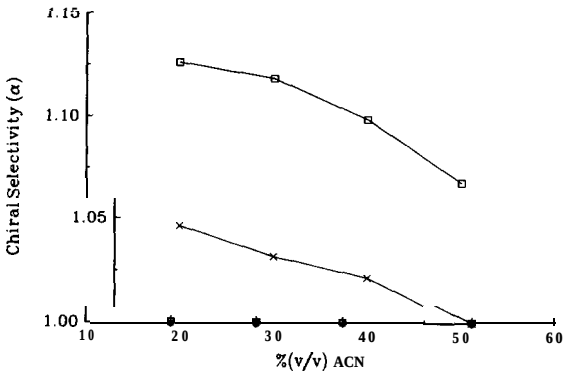

Fig. 8. Chiral selectivity as a function of the % (v/v) acetonitrile concentration in the pH 6.0, 5 m*M* citrate buffer eluents for profens on the Cyclobond I-SN naphthylethylcarbamoylated β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

the k_2' values are about 10–15% higher on the naphthylethylcarbamoylated β-cyclodextrin silica column than on the native β-cyclodextrin silica column.

As shown in Figs. 7 and 8, chiral selectivity on the naphthylethylcarbamoylated β-cyclodextrin silica (Cyclobond I-SN) column is better than on the native β-cyclodextrin silica column. In the pH 6.0 eluents, the highest selectivity factor value found for ibuprofen is α = 1.126, much higher than the 1.054 value on the native cyclodextrin silica (Cyclobond I) column. Additionally, chiral separation is observed for flurbiprofen as well: the highest selectivity value is α = 1.046 (Fig. 8). At pH 4.0, chiral separation is observed only for ibuprofen (Fig. 7), and the chiral selectivity is worse (α = 1.044) than at pH 6.0. When the acetonitrile concentration becomes higher than 50% (v/v), the chiral separation is lost, both at high pH and low pH, for both ibuprofen and flurbiprofen, following the trend that was observed on the Cyclobond I column (Figs. 7 and 8).

Hydroxypropylated β-cyclodextrin silica (Cyclobond I-SP) columns

The retention and the selectivity values observed on the (*S*)-hydroxypropylated β-cyclo-

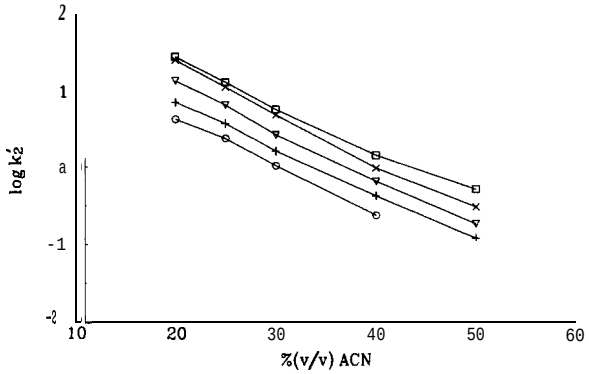

Fig. 9. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile concentration in the pH 4.0, 5 m*M* citrate buffer eluents on the Cyclobond I-SP (*S*)-hydroxypropylated β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

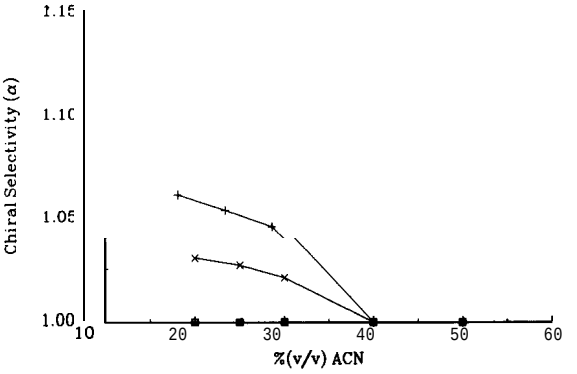

Fig. 11. Chiral selectivity as a function of the % (v/v) acetonittile concentration in the pH 4.0, 5 m*M* citrate buffer eluents for profens on the Cyclobond I-SP (*S*)-hydroxypropylated β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

dextrin silica stationary phase (Figs. 9-11) follow the trends observed with the native and the naphthylethylcarbamoylated β-cyclodextrin silicas: the k'_2 values are lower in the acetonitrile-rich eluents at pH 4 than at pH 6.5, the k'_2 values are higher in the purely aqueous eluents at pH 4 than at pH 6.5, the slopes of the k'_2 *vs. %* ACN curves are steeper at pH 4 than at pH 6.5. In the pH 4 eluents, solute retention on the native β-cyclodextrin and the (S)-hydroxypropylated cyclodextrin silica is also identical within experimental error. However, when it comes to chiral selectivity, an important difference is observed. Enantiomeric separation is achieved solely at pH 4.0, and only for flurbiprofen and naproxen.

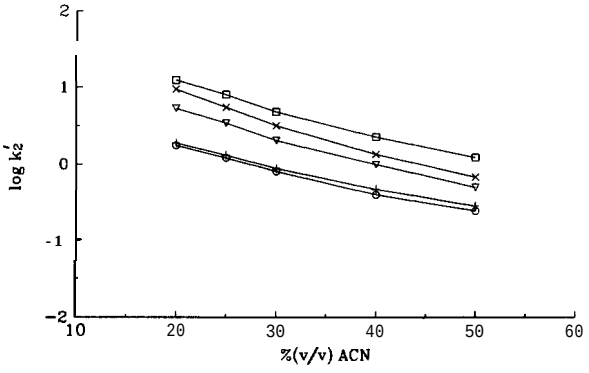

Fig. 10. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile concentration in the pH 6.5, 5 m*M* citrate buffer eluents on the Cyclobond I-SP (Qhydroxypropylated β-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

The racemic hydroxypropylated p-cyclodextrin stationary phase (Cyclobond I-RSP column) was also evaluated. Using the same eluents, similar chiral selectivity was observed for the naproxen and the flurbiprofen on both the (*S*)-hydroxypropylated (Cyclobond I-SP) and the racemic hydroxypropylated p-cyclodextrin silicas (Cyclobond I-RSP). Retention was slightly lower, though, on the racemic hydroxypropylated β-cyclodextrin. No separation was achieved for the other profens either at low pH or at high pH. Thus, for the separation of the profen enantiomers, the use of the more expensive (*S*)-hydroxypropylated cyclodextrin column offers no advantage.

γ-Cyclodextrin silica (Cyclobond II) columns

Again, the same type of retention behavior was observed; as the concentration of acetonitrile increases, the logarithms of the k'_2 values decrease faster at pH 4.0 than at pH 6.0. However, regardless of the acetonitrile concentration, the retention times of the profens are very similar to each other, with the exception of flurbiprofen which is more retained. This indicates a lack of differentiation by the γ-cyclodextrin phase for the profens (Figs. 12 and 13). No separation of the enantiomers of any of the profens was observed using y-cyclodextrin silica.

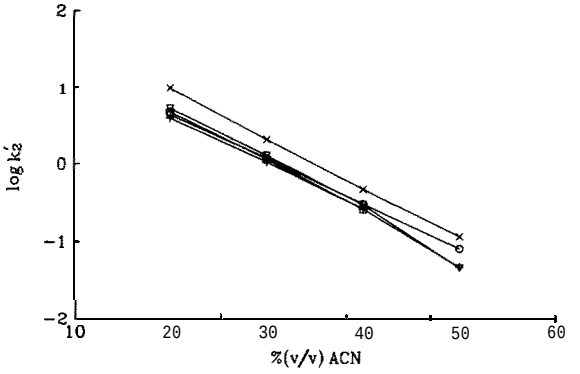

Fig. 12. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile concentration in the pH 4.0, 5 m*M* citrate buffer eluents on the Cyclobond II y-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

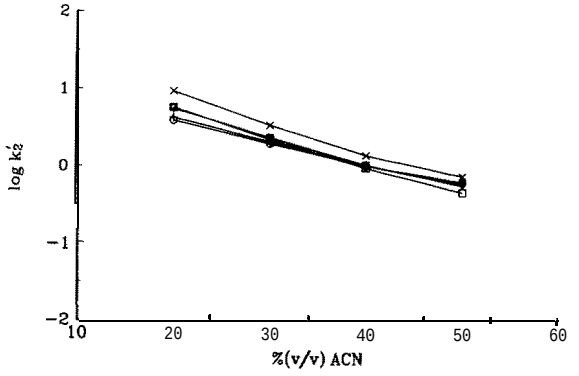

Fig. 13. Capacity factors of the more retained enantiomers of profens as a function of the % (v/v) acetonitrile concentration in the pH 6.0, 5 m*M* citrate buffer eluents on the Cyclobond II y-cyclodextrin silica column. Other conditions and symbols as in Fig. 2.

CONCLUSIONS

All the cyclodextrin silica columns investigated display similar retention behavior. Chiral separation was observed on the Cyclobond-I β-cyclodextrin silica column for ibuprofen at high pH, on the Cyclobond I-SN naphthylethylcarbamoylated β-cyclodextrin silica column for ibuprofen and flurbiprofen at high pH, and for ibuprofen at low pH, and on the Cyclobond I-SP and Cyclobond I-RSP hydroxypropylated β-cyclodextrin silica columns for flurbiprofen and naproxen at low pH. The Cyclobond-II y-cyclodextrin silica column could not differentiate either between the various profens, or their enantiomers.

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