The Influence of Mobile Phase Demixion on Thin-Layer Chromatographic Enantioseparation of Ibuprofen and Naproxen

Mieczysław Sajewicz[1], Krzysztof Kaczmarski[2], Monika Gontarska[1], Sylwia Kiszka[1], and **Teresa Kowalska[1,*]**

[1]Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland and [2]Department of Chemical and Process Engineering, Technical University of Rzeszów, Al. Powstanców Warszawy 6, 35-959 Rzeszów, Poland

Abstract

In our earlier article we presented the results of tracing the enantioseparation of the two test analytes (ibuprofen and naproxen) by means of video densitometry and scanning densitometry. In that way we demonstrated an excellent performance of this combined approach to the thin-layer chromatographic detection in the area of enantioseparation. In this paper we study an impact of the four different mobile phases on the enantioseparation of the scalemic mixtures of ibuprofen and naproxen on the silica gel layers impregnated with L-arginine as chiral selector. The main component of all the investigated mobile phases is 2-propanol. Mobile phase 1 consists of pure 2-propanol, while mobile phases 2–4 contain, respectively, ca. 0.66, 1.32, and 1.98 g/L of glacial acetic acid in 2-propanol. Acetic acid is used to protonate L-arginine, as the involved retention mechanism consists of the ion pair formation between L-arginine in the cationic form and the chiral 2-arylpropionic acids (2-APAs), ibuprofen and naproxen, in the anionic form. It is shown that in the absence of glacial acetic acid no enantioseparation can be obtained. Then with adding of 0.66 g/L glacial acetic acid partial enantioseparation of the naproxen and ibuprofen antimers is obtained, with a simultaneous effect of the mobile phase demixion. With the amount of acetic acid increasing, the effect of demixion becomes increasingly perceptible. In that case the displacement effect is observed (and mathematically modeled), which results in compressing of the antimer pairs by the second front of mobile phase. The obtained results allow a deeper insight into the mechanism of enantioseparation with the two test 2-APAs. A combined impact of the crystalline chirality of silica gel and the molecular chirality of L-arginine on the vertical and the horizontal enantioseparation of ibuprofen and naproxen is also discussed.

Introduction

Thin-layer chromatographic systems well suited for separation of the 2-APA antimers can include silica gel impregnated with L-arginine, which is kept in the cationic form by adding of a low amount of glacial acetic acid to the mobile phase. In that case, the mechanism of separation can be summarized with the aid of the two below given stoichiometric equations (1), which reflect the ion-pair formation between the cationic impregnant (L-arginine) and the two antimers of a given 2-APA in the anionic form:

$$\text{L-arginine}^+ + S\text{-(+)-2-APA}^- \leftrightarrow \text{L-arginine}^+ \cdot S\text{-(+)-2-APA}^-;\ (K_1)$$

$$\text{L-arginine}^+ + R\text{-(–)-2-APA}^- \leftrightarrow \text{L-arginine}^+ \cdot R\text{-(–)-2-APA}^-;\ (K_2) \quad \text{Eq. 1}$$

where $K_1 \neq K_2$.

The previous mechanism can successfully work even in the absence of water from mobile phase, as the trace amounts of humidity always present on the hygroscopic surface of the silica gel layer and also in the applied solvents are enough to transfer the proton (H^+) from the acetic acid molecule to the amino group of L-arginine, and a partial electrolytic dissociation of the analyte (2-APA) molecules can also take place.

In our earlier study on the enantioseparation of the ibuprofen and naproxen scalemates by means of thin-layer chromatography (TLC) (2), we explored the potential of the combined video and scanning densitometry to get a deep enough insight into the formation of the antimer bands. Unique comparisons were made between the pictures of the chromatographic spots taken by the video densitometer in UV light (λ = 254 nm) and the 3D concentration profiles of these spots. Convincing empirical evidence was gathered witnessing to the fact that the enantioseparation of the ibuprofen and naproxen antimers was induced by the two different mechanisms, namely by the crystalline chirality of the silica gel particles and by the molecular chirality of L-arginine. It was shown that the chirality of silica gel alone is responsible for the horizontal enantioseparation (i.e. for the deviation of the migration tracks of the two antimers to the left and to the right from the vertical direction of the mobile phase flow. The molecular chirality of L-arginine is responsible for the vertical enantioseparation and it can be expressed by the differentiated

*Author to whom correspondence should be addressed: email kowalska@us.edu.pl.

numerical values of the retardation parameter (R_f) valid for the two separated bands.

In this study we intend to demonstrate the influence of the mobile phase composition on the enantioseparation of the ibuprofen and naproxen antimers on the silica gel layers impregnated with L-arginine. As mobile phases, we used pure 2-propanol and also 2-propanol modified with different amounts of glacial acetic acid. Detection was carried out with use of the video and the scanning densitometry, and the results were presented as the video scans and also as classical densitograms. Demixion of the mobile phases composed of 2-propanol and glacial acetic acid was shown in the form of the densitograms, and the displacement effect was demonstrated and modeled (i.e., the case when the analyte molecules are pushed forward by the molecules of the displacer–in our case of acetic acid–creating the second front of the eluent).

Experimental

S-(+)-Ibuprofen and *S*-(+)-naproxen as the test analytes

In our study we used *S*-(+)-ibuprofen and *S*-(+)-naproxen, manufactured by Sigma-Aldrich (St Louis, MO; cat. nos I-106 and 28,478–5, respectively). As it has been shown in our earlier papers (3–5), solutions of the optically pure *S*-(+)-ibuprofen and *S*-(+)-naproxen undergo in the course of a prolonged storage a spontaneous in vitro structural inversion to form the scalemic mixtures of the two respective antimers. In our present experiments we used solutions of *S*-(+)-ibuprofen and *S*-(+)-naproxen in 70% ethanol after their storage for at least two weeks in the sealed vessels at ambient temperature, which was enough to obtain the scalemic mixtures of these two enantiomers. Concentration of ibuprofen in the solution was equal to 5 mg/mL (ca. 2.2×10^{-2} mol/L) and that of naproxen was equal to 1 mg/mL (ca. 4.3×10^{-3} mol/L). The 5-µL volumes of these solutions were then applied to the chromatographic plates.

Ethanol used for preparation of the ibuprofen and the naproxen solutions was of HPLC grade (Merck, Darmstadt, Germany), and water was double distilled in our laboratory.

Commercial TLC silica gel layers and their pretreatment

TLC was performed on commercial glass plates (20 × 20 cm) precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany; cat. no. 1.05715). Before use, the plates were carefully washed by predevelopment with methanol

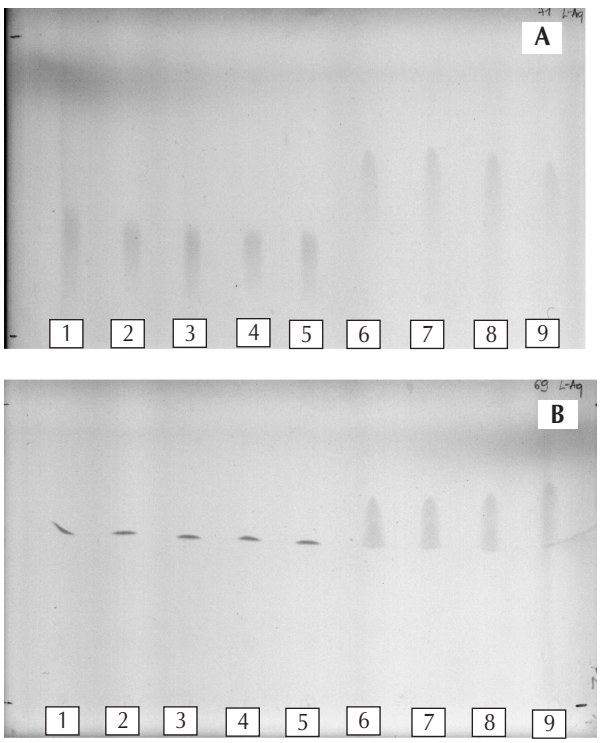

Figure 1. Video densitograms obtained from the L-arginine-impregnated chromatographic plates precoated with silica gel 60 F_{254} and developed with pure 2-propanol (A); and 2-propanol plus 1.98 g/L glacial acetic acid (spots 1–5: naproxen; spots 6–9: ibuprofen) (B).

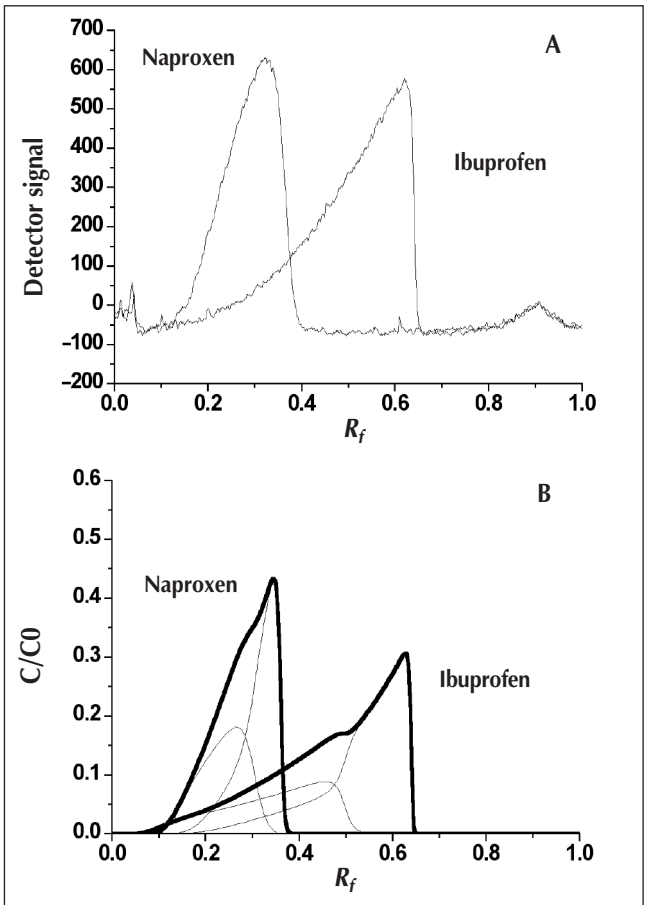

Figure 2. Densitograms of naproxen and ibuprofen chromatographed on the L-arginine-impregnated silica gel 60 F_{254} layer at 22 ± 2°C with a mobile phase of pure 2-propanol (A). Simulated concentration profiles of naproxen and ibuprofen with pure 2-propanol as mobile phase (B). Thick solid lines represent the sum of the two separated species and thin solid lines represent the single species, respectively.

and then dried at ambient temperature for 3 h. Methanol used for prewashing was of the HPLC grade (Merck).

Then the prewashed plates were impregnated with a 3×10^{-2} mol/L solution of L-arginine (analytical reagent grade, Merck) in methanol by conventional dipping for 2 s. The concentration of the impregnating solution was calculated as that depositing 0.5 g of L-arginine per 50 g of the dry silica gel layer. Finally, the washed and impregnated adsorbent layers were ready for chromatography.

Mobile phases and development of thin-layer chromatograms

Development of the ibuprofen and the naproxen samples was carried out at 22 ± 2°C for a distance of 8 cm, using the following four mobile phases: pure 2-propanol (A); 2-propanol plus 0.66 g/L glacial acetic acid (in order to protonate the amino group of L-arginine) (B); 2-propanol plus 1.32 g/L glacial acetic acid (C); and 2-propanol plus 1.98 g/L glacial acetic acid (D). 2-Propanol used as a constituent of mobile phase was of the HPLC grade (Merck) and glacial acetic acid was of the analytical reagent grade (manufactured by POCh, Gliwice, Poland).

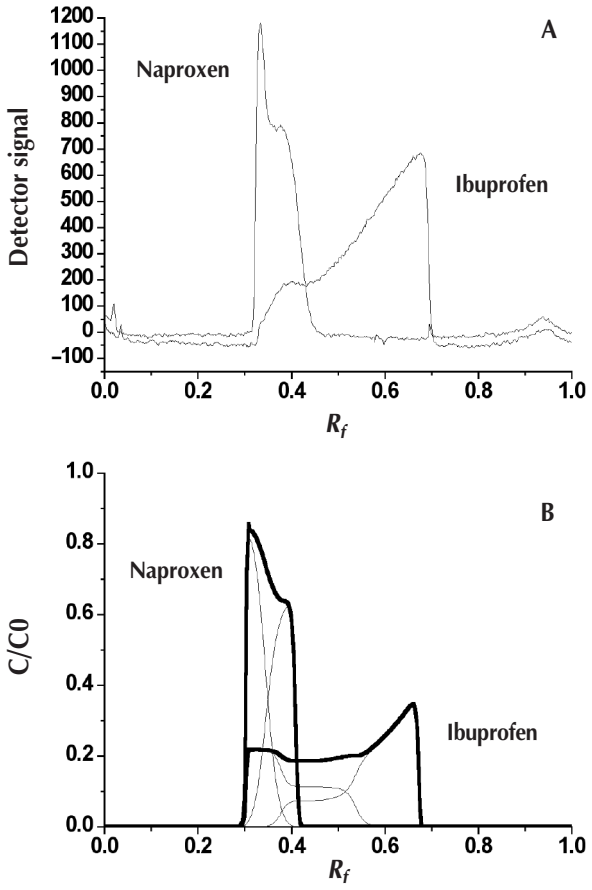

Figure 3. Densitograms of naproxen and ibuprofen chromatographed on the L-arginine-impregnated silica gel 60 F_{254} layer at 22 ± 2°C. Mobile phase: 2-propanol plus 0.66 g/L glacial acetic acid (A). Simulated concentration profiles of naproxen and ibuprofen for the 2-propanol plus 0.66 g/L glacial acetic acid mixture as mobile phase (B). Thick solid lines represent the sum of the two separated species and thin solid lines represent the single species, respectively.

Sample application to the plates was made with the use of an autosampler (the AS 30 model autosampler manufactured by Desaga, Heidelberg, Germany). The ibuprofen and the naproxen solutions were applied to the plate 1.0 cm above the lower edge of the plate. Nine samples (samples 1–5: naproxen; samples 6–9: ibuprofen) in the equal distance of 2 cm from one another were applied per one plate, and then the chromatogram was developed. After development, the plates were dried at ambient temperature for 3 h, and the chromatograms were evaluated by means of the flatbed video densitometry and the scanning densitometry.

Densitometric assessment of the chromatograms

The flatbed video densitometry

The chromatograms were scanned at the wavelength $\lambda = 254$ nm with use of the Chromimage flatbed scanner (manufactured by AR2i, Le Plessis Robinson, France), to save the pictures of the whole chromatograms. Each experiment was repeated at least three times.

The classical scanning densitometry

Densitograms were acquired with a Desaga (Heidelberg, Germany) Model CD 60 densitometer equipped with Windows-compatible ProQuant software. The track 20 mm wide was scanned densitometrically for each individual chromatographic spot in the 1-mm intervals. Concentration profiles of the scanned lines were recorded in UV light from the deuterium lamp (in the reflectance mode) at 210 nm. The dimensions of the rectangular light beam were 2.0 × 0.1 mm. The maxima of the 2-APA concentration profiles were used for calculation of the respective R_f values. The densitograms of the 21 lines taken from the 20 mm wide tracks were used to draw the concentration profiles of the examined chromatographic spots.

Circular dichroism of the enantioseparated chromatographic spots of ibuprofen

In the case of the spots of ibuprofen partially enantioseparated with aid of the 2-propanol–glacial acetic acid eluent (e.g., see Figure 1B, spots 6–9), we decided to instrumentally confirm this effect with aid of the spectroscopy of circular dichroism (CD). Thus we scraped out the upper and the lower part of the ibuprofen spots (shown in Figure 1B) to the separate vials and then run the CD spectroscopic measurements of these samples in the solid state.

The CD spectra of the upper and the lower part of the chromatographic spots were recorded between 188 and 400 nm at room temperature with a Jasco J-715 spectropolarimeter for solid-state samples (JASCO, Easton, MD). In accordance with the conventional procedure, the two chromatographic spot samples (the upper and the lower part thereof) in the aliquots between 1 and 4 mg were ground with nujol to prepare nujol mulls. To exclude artifacts, during CD measurement the samples were rotated around the optical axis into four positions differing by 90° and the resulting spectra were compared.

Results and Discussion

Video densitometry

The chromatograms obtained in this experiment were recorded with aid of the flatbed video densitometer. In Figure 1 we presented, as examples, two chromatograms, one obtained when developing the scalemic mixtures of ibuprofen and naproxen with pure 2-propanol (Figure 1A) and the other valid for the mobile phase composed of 2-propanol plus 1.98 g/L glacial acetic acid (Figure 1B). From Figure 1B it can be seen that the mixed mobile phase undergoes demixion and the chromatographic spots are arranged along the line of the second front. Evidently, it is glacial acetic acid that is so strongly retained as to form this line. The effect of demixion was additionally confirmed by spraying the chromatographic plates with the solution of ninhydrine in ethanol, as the plates were impregnated with L-arginine. After heating the sprayed plates for 5 min at 105°C, the two different shades of the pink color emerged, and the demixion line was exposed as the borderline between the two shades. It is noteworthy that demixion affected enantioseparation with each mixed mobile phase used in this experiment.

In Figure 1A, all chromatographic spots migrate on a relatively shorter distance than in Figure 1B (the mean R_F values for naproxen and ibuprofen are, respectively, 0.34 and 0.62), and they all show the back tailing. The back tail of each spot is rather untypical, as it is slightly widening with a weak split at the bottom, so the spots assume the V-shape (of the V character turned upside down). It seems clear that in the absence of acetic acid from mobile phase, L-arginine is not protonated and it does not act as chiral selector. In these circumstances, a slight enantioseparating effect is exerted by silica gel alone. Similar to the results shown in the literature (2,6,7), as well as in this study we observed a slight effect of horizontal enantioseparation of the two 2-APA antimers, with the migration routes of the two antimers deviating, respectively, to the left and to the right.

In Figure 1B, the displacement effect (i.e., pushing forward of the analyte spots by the second front of the acetic acid mol-

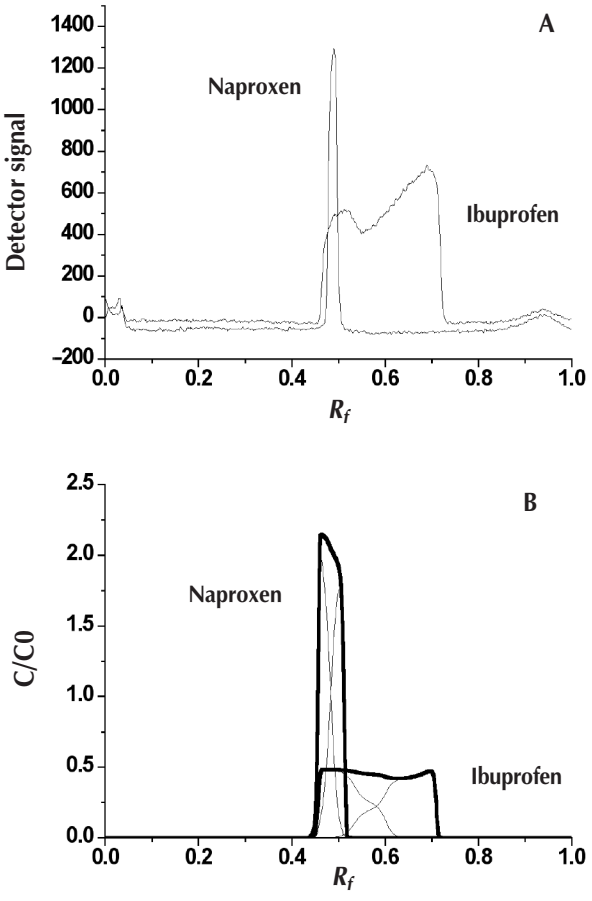

Figure 4. Densitograms of naproxen and ibuprofen chromatographed on the L-arginine-impregnated silica gel 60 F_{254} layer at 22 ± 2°C. Mobile phase: 2-propanol plus 1.32 g/L glacial acetic acid (A). Simulated concentration profiles of naproxen and ibuprofen for the 2-propanol plus 1.32 g/L glacial acetic acid mixture as mobile phase (B). Thick solid lines represent the sum of the two separated species and thin solid lines represent the single species, respectively.

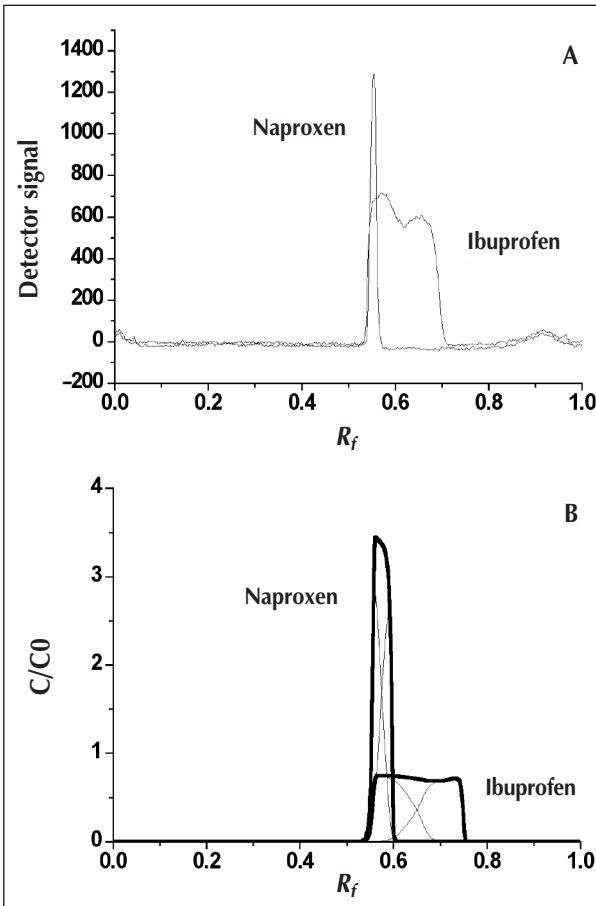

Figure 5. Densitograms of naproxen and ibuprofen chromatographed on the L-arginine-impregnated silica gel 60 F_{254} layer at 22 ± 2°C. Mobile phase: 2-propanol plus 1.98 g/L glacial acetic acid (A). Simulated concentration profiles of naproxen and ibuprofen for the 2-propanol plus 1.98 g/L glacial acetic acid mixture as mobile phase (B). Thick solid lines represent the sum of the two separated species and thin solid lines represent the single species, respectively.

ecules) evidently governs the retention of naproxen and ibuprofen. Under the influence of the displacement mechanism, the back tailing of the chromatographic spots disappears.

Scanning densitometry

The scanning densitograms of naproxen and ibuprofen developed in the four different mobile phases are shown, respectively, in Figures 2A, 3A, 4A, and 5A (these densitograms were run for the spots No. 5 and 6, respectively, from all four chromatograms). From these plots we can trace the gradually growing impact of glacial acetic acid on the concentration profiles of naproxen and ibuprofen. Without adding acetic acid (Figure 2A), the mechanism of vertical enantioseparation (given by equation 1) simply cannot be perceived from the respective concentration profiles. Upon adding the lowest amount of acetic acid (0.66 g/L) to 2-propanol, an incomplete vertical separation of the two peaks is obtained both with naproxen and ibuprofen (Figure 3A). It is accompanied by the displacement effect (perceptible from an almost vertical line of the peak profiles' rear end, or "shock"). Upon adding the higher amounts of acetic acid (1.32 and 1.98 g/L), the displacement effect completely annihilates enantioseparation of naproxen, as follows from the shape of the densitometric peak profiles (Figures 4A and 5A). In the case of ibuprofen, independent of the amount of the added acetic acid, we observe vertical separation of the two peaks and an increasingly pronounced displacement effect (Figures 3A, 4A, and 5A).

Confirmation of enantioseparation by means of spectroscopy of CD

From the obtained CD results it comes out that the enantioseparation of the scalemic mixture of *S*-(+)- and *R*-(–)-ibuprofen with aid of 2-propanol plus 1.98 g/L glacial acetic acid was successfully performed. In Figures 6A and 6B we present, respectively, the CD spectra of the upper and the lower part of the scraped out chromatographic spots No. 6-9, each containing the separated ibuprofen enantiomer, silica gel, and L-arginine. The spectra of these mixed solid samples were run as nujol suspensions. In each case we observed two well pronounced Cotton bands showing the opposite maxima at ca. 212 and ca. 236 nm—the negative band for the upper part of the scraped out chromatographic spot (Figure 6A) and the positive one for the lower part of the spot (Figure 6B). From the general knowledge about enantioseparation of ibuprofen and of the most other profens (e.g., 1) it comes out that the upper part of the chromatographic spot can be ascribed to the *S*-(+) species, and the lower part to its *R*-(–) antimer.

Modeling of the displacement effect

Theory

In the case of TLC, it is impossible to perform a quantitative comparison of the experimental peak profiles with the theoretical simulations, as the adsorption isotherms and certain characteristic features of the adsorbents and the system hydrodynamics cannot precisely be measured. However, the qualitative considerations based on the appropriate simulations can help us better understand the obtained densitograms. As our chromatographic spots are roughly symmetrical along the direction of elution, in the qualitative consideration we ignored the perpendicular dispersion and instead, we used the simple equilibrium-dispersive (ED) model (8):

$$\frac{\partial c_i}{\partial t} + \mathrm{F}\frac{\partial q_i}{\partial t} + \mathrm{w}\frac{\partial c_i}{\partial x} = D_L\frac{\partial^2 c_i}{\partial x^2} \qquad \text{Eq. 2}$$

where c and q are, respectively, the analyte concentrations in liquid phase and on the adsorbent surface (g/L), F is the phase ratio, w is the linear velocity of the mobile phase flow (cm/min), D_L is the axial dispersion ($cm^2\ min^{-1}$), t is the time [min], and x is the spatial distance (cm).

After introducing the following dimensionless variables:

$$\tau = \frac{tw}{\mathrm{L}};\ \mathrm{X} = \frac{x}{L};\ Pe = 2N = \frac{wL}{D_L} \qquad \text{Eq. 3}$$

Equation 2 can be rewritten, as follows:

$$\frac{\partial c_i}{\partial \tau} + \mathrm{F}\frac{\partial q_i}{\partial \tau} + \frac{\partial c_i}{\partial X} = \frac{1}{2N}\frac{\partial^2 c_i}{\partial X^2} \qquad \text{Eq. 4}$$

where N is the number of theoretical plates and L is the plate length.

To solve equation 4, the boundary and the initial conditions should first be established. The model was solved assuming that the initial concentration of the analyte on the plate was equal to zero. It was also assumed that the concentration of the analyte at the end of the plate ($X = 1$) was equal to zero during the entire experiment.

Modeling of the boundary conditions at the starting line of the chromatogram ($X = 0$) was more complicated. In TLC, the analyte solution is normally spotted on the start line and the spot is dried. Then the chromatogram is run and at the first stage of the development the analyte is dissolved in mobile phase. The process of dissolution can mentally be replaced by the assumption that the analyte is introduced to the start line with an "inlet" concentration equal to $c0$ during the time equal to Δt. However, it is difficult to estimate the numerical values of $c0$ and Δt. In this study, we assumed that the time Δt is proportional to the dissolution time of the analyte. As our calculations were qualitative only, we ignored the different dissolution times for ibuprofen and naproxen, and for these two analytes we equally assumed Δt = 10 min. Now the "inlet" concentration could be evaluated keeping in mind that the initial diameter of the spot, d_s, was equal to ca. 0.3 cm, the thickness of the mobile phase layer, d_m, was equal to 0.033 cm, and the mobile phase velocity, w, was equal to 0.1 cm/min. Remembering that the mass, m, of ibuprofen spotted on to the chromatographic plate was equal to 25 µg and that of naproxen was equal to 5 µg, it was possible to estimate the average "inlet" concentration $c0 = \mathrm{m}/(d_m \times d_s \times w \times \Delta t)$ for ibuprofen as equal to 2.5 g/L and for naproxen as equal to 0.5 g/L. The other data needed for the simulation were as follows: L = 8 cm (the measured value) and F = 0.25 and N = 300 (the assumed values).

Simulations

In Figures 2A, 3A, 4A, and 5A we can see the densitograms, showing the experimental concentration profiles of naproxen and ibuprofen obtained for the acetic acid concentrations equal to 0, 0.66, 1.32, and 1.98 g/L, respectively. The analysis of the peak profiles raises difficulties because each densitometrically acquired peak represents the total signal from the two antimers. However, all characteristic features of the changes observed with the analytes' concentration profiles and induced by the increasing concentration of the mobile phase modifier are clearly visible.

It can be seen that with an absence of acetic acid as the mobile phase modifier, the concentration profiles of the two 2-APAs are triangular, with the front shock and the long tail. The end of each tail is located close to the start line. With the growth of the acetic acid concentration, both peaks become increasingly narrower, the diffusional part of the peaks disappears and the second shock in the back part of the peaks emerges. Moreover, the positions of the rear fronts for naproxen and ibuprofen, respectively, remain unchanged and the peak height is increasing with the growing concentration of the modifier. The observed phenomena are very similar to the displacement effect resulting in the isotachic train, well known from the column chromatography (8). With an increasing concentration of the displacer, the analytes are increasingly stronger excluded from the adsorbent and finally the so-called train effect is observed. As a result, all species move with the same velocity, the concentration band of each component is rectangular, and it is separated from the other components.

It seems that the analogous mechanism is responsible for the observed phenomenon that occurs in the TLC mode. Demixion of the binary mobile phase is due to the strong adsorption of acetic acid. Acid is moving along the plate with its own characteristic velocity and excludes the analytes from active sites. To perform qualitative simulation of the experimental concentration profiles, the following assumptions were applied:

Acetic acid and the enantiomers of naproxen and ibuprofen adsorb according to the competitive Langmuir isotherm:

$$q_i = q_s \frac{K_i C_i}{1 + \sum_{j=1}^{3} K_i C_i} \qquad \text{Eq. 5}$$

The saturation capacity is the same for all the species considered.

The isotherm parameter values are given in Table I. These values were established as follows:

Firstly, the q_s and K values were chosen in such way as to obtain a good agreement between the experimental and the calculated position of the acetic acid demixion line for all the concentrations of this compound. Position of the demixion line coincides with the positions of the rear shock of the analytes (see Figures 3A, 4A, and 5A). Concentrations of the analytes were assumed as equal to zero.

Then the equilibrium constants for the ibuprofen antimers were estimated in such a way as to obtain a good agreement between the densitogram presented in Figure 2A on the one hand, and the calculated positions and the profile of the total concentration of the ibuprofen antimers on the other.

Table I. The Assumed Values of the Isotherm Model Parameters

Species	q_s (–)	K (L/g)
Acetic acid		20
Ibuprofen (first isomer)		3
Ibuprofen (second isomer)	6.5	6
Naproxen (first isomer)		2.5
Naproxen (second isomer)		3.5

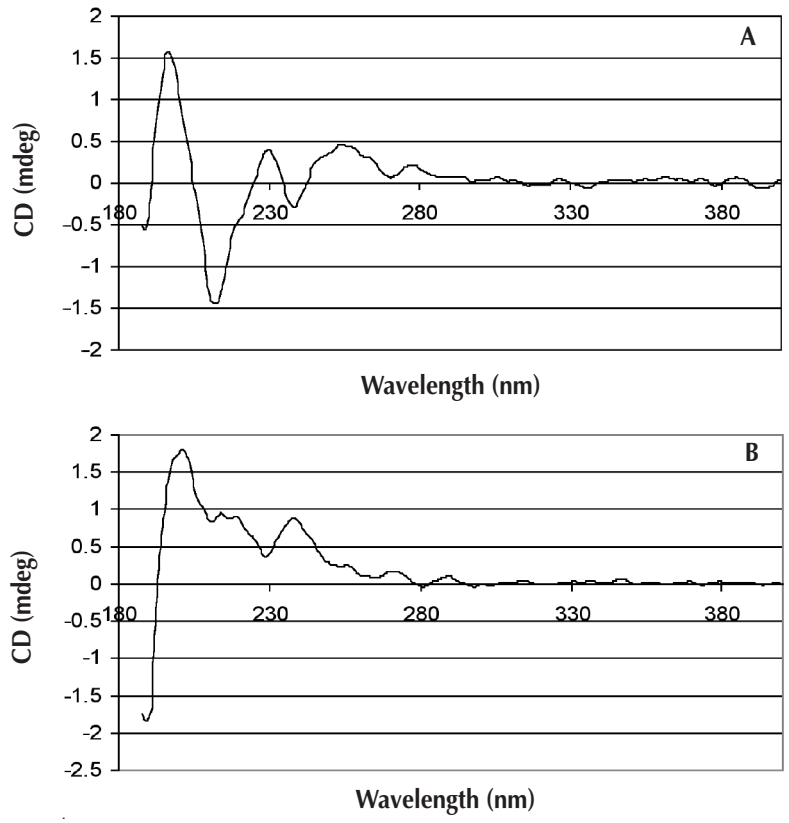

Figure 6. CD spectra run for the nujol suspensions of the upper part (A) and the lower part (B) of the scraped out chromatographic spots No. 6–9 (e.g., see Figure 1B), containing ibuprofen (plus silica gel as stationary phase and L-arginine as chiral selector). The respective chromatogram was developed with 2-propanol plus 1.98 g/L glacial acetic acid).

In a similar way, the equilibrium constants were estimated for naproxen.The numerical values of the Langmuir model isotherm estimated with aid of the previously described method were used for simulation of the chromatographic band profiles. The obtained results are shown in Figures 2B, 3B, 4B, and 5B. As it can be seen from these figures, the positions, widths and shapes of the simulated concentration profiles are similar to the experimental ones. This outcome confirms the correctness of our presumption that the observed shapes of the spots' concentration profiles are determined by the strong displacement effect.

Conclusions

In this study, we focused our attention on the demixion of mobile phase composed of 2-propanol and glacial acetic acid, and on the resulting displacement effect that affects the enantioseparation of ibuprofen and naproxen. Due to the demixion, the second front was formed by the acetic acid molecules stronger retained on the silica gel surface than those of 2-propanol. The displacement effect induced by acetic acid was clearly visible both from the video densitograms and from the classical scanning densitograms. It was also successfully modeled with use of the equilibrium-dispersive (ED) model, with a satisfactory qualitative effect.

Moreover, we directly confirmed the success of enantioseparations carried out by means of the 2-propanol–glacial acetic acid mobile phases by means of the spectroscopy of circular dichroism (CD).

Acknowledgment

The authors wish to thank Merck KGaA (Darmstadt, Germany) for supplying the TLC plates used in our experiments. The authors wish to thank the AR2i Company (Le Plessis Robinson, France) for supplying the Chromimage video densitometer and the Galaxie-TLC software used in this study.

References

1. R. Bhushan and V. Parshad. Resolution of (±) ibuprofen using L-arginine-impregnated thin layer chromatography. *J. Chromatogr. A* **721:** 369–72 (1996).
2. M. Sajewicz, M. Gontarska, A. Dąbrowa, and T. Kowalska. Use of video densitometry and scanning densitometry to study an impact of silica gel and L-arginine on the retention of ibuprofen and naproxen in the thin layer chromatographic (TLC) systems. *J. Liq. Chromatogr. Relat. Technol.* **30:** 2369–83 (2007).
3. M. Sajewicz, R. Pięktka, A. Pieniak, and T. Kowalska. Application of thin-layer chromatography (TLC) to investigating oscillatory instability of the selected profen enantiomers. *Acta Chromatogr.* **15:** 131–49 (2005).
4. M. Sajewicz, R. Pięktka, A. Pieniak, and T. Kowalska. Application of thin-layer chromatography (TLC) to investigate oscillatory instability of the selected profen enantiomers in dichloromethane. *J. Chromatogr. Sci.* **43:** 542–48 (2005).
5. M. Sajewicz, R. Pi tka, A. Pieniak, and T. Kowalska. Application of thin-layer chromatography to the investigation of oscillatory instability of selected profen enantiomers in physiological salt. *J. Liq. Chromatogr. Relat. Technol.,* **29:** 2059–2069 (2006).
6. M. Sajewicz, H.-E Hauck, G. Drabik, E. Namysło, B. Głód, and T. Kowalska. Tracing possible structural asymmetry of silica gel used for precoating thin-layer chromatography plates. J. *Planar Chromatogr.–Modern TLC* **19:** 278–81 (2006).
7. M. Sajewicz, R. Piętka, G. Drabik, E. Namysło, and T. Kowalska. On the stereochemically peculiar two-dimensional separation of 2-arylpropionic acids by chiral TLC. *J. Planar Chromatogr.–Modern TLC* **19:** 273–77 (2006).
8. G. Guiochon, S. Golshan-Shirazi, and A.M. Katti. Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 1994.

Manuscript received March 3, 2007.