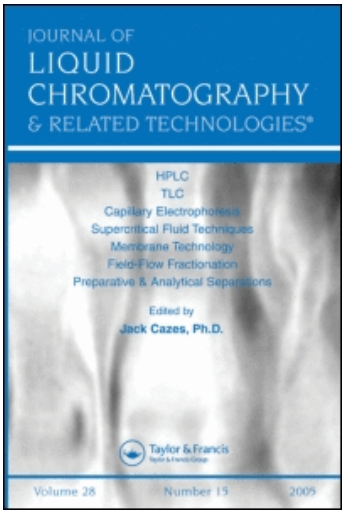

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Enantioseparation of *S,R*-(±)-Ketoprofen on Plain Silica Gel Layers with Achiral Mobile Phase

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Abstract: In an earlier study, we provided sufficient experimental evidence in favor of the hypothesis that—contrary to the conviction widespread among the practitioners of thin layer chromatography (TLC)—silica gel used for the coating of chromatographic plates is not amorphous, but microcrystalline and chiral. This evidence was procured both by the spectroscopic measurements of the circular dichroism of the binder-free silica gel samples (manufactured specially for coating of the TLC plates) and by TLC measurements with densitometric detection. From the chromatographic measurements, it was found that silica gel employed in the planar chromatographic mode enables two-dimensional enantioseparation of the racemic (or scalemic) mixtures of the selected test profens in the one-dimensional development mode, without using chiral mobile phases.

In our present study, this striking ability of the silica gel layers is further investigated with one more racemic mixture from the group of profens [i.e., *S,R*-(±)-ketoprofen]. Good separation was obtained of the racemic ketoprofen mixture on the chromatographic plates precoated with the plain silica gel, when using the achiral binary mobile phase acetonitrile-water (5:1, v/v) plus several drops of glacial acetic acid. In this case, three different components of the investigated mixture were found, similar to the case observed earlier, when using a silica gel layer impregnated with L-arginine. These three components were separated two-dimensionally in the one dimensional development mode, i.e., their positions differed in terms of the R_F values and, moreover, the respective migration tracks of these three species all deviated to the right. It seems justified to conclude that the two-dimensional enantioseparation in the one-dimensional planar chromatographic mode on the microcrystalline silica gel layers is a promising option, enhancing the enantioseparative potential

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of planar chromatography that cannot be challenged by the column liquid chromatography.

Keywords: *S,R*-(±)-Ketoprofen, TLC, Chirality, Chiral silica gel, Two-dimensional enantioseparation, Racemic mixtures

INTRODUCTION

In our earlier studies, we have reported on the chirality of microcrystalline silica gel used as a stationary phase in thin layer chromatography (TLC), as confirmed by the results of circular dichroism (CD) spectroscopy.[1] To our best knowledge, this was the first written report on this particular issue, available from a scientific source. Moreover, in publications[1,2] we have also reported on the deviations of the selected profens' migration tracks from the straight-line verticality (observed in the ascending development mode). With *S,R*-(±)-ibuprofen and *S,R*-(±)-naproxen, one enantiomer deviated to the left and the respective antimer deviated to the right.[1,2] This phenomenon has been observed both on the plain silica gel layers and on those impregnated with a chiral selector (i.e., L-arginine). In another paper[3] we presented the results of an analogous study with *S,R*-(±)-ketoprofen used as the chiral test analyte. In that case, the chiral chromatographic system was used with L-arginine as the chiral impregnant of the silica gel layer. As a result, we observed three (instead of the expected two) well separated species, with their migration tracks all deviating to the right. The ultraviolet (UV) spectra, run in situ for each individual separated band, were identical. Identification of these three species proved quite problematic.

In order to progress with our study on the chirality of the plain silica gel layers and to further explore their practical potential for enantioseparations, in this work, we examined the possibility to two-dimensionally separate the racemic ketoprofen mixture on plain silica gel layers in the one-dimensional TLC mode.

EXPERIMENTAL

S,R-(±)-Ketoprofen

In our study, we used *S,R*-(±)-ketoprofen, manufactured by Sigma-Aldrich (St Louis, MO, USA; cat. # K1751-5G). The manufacturer declared its purity at the ≥98% level. We additionally examined the *S,R*-(±)-ketoprofen sample originating from our batch for its purity by means of HPLC, ^{1}H NMR, and ^{13}C NMR, and its analytical grade was fully confirmed. The sample for the TLC study was prepared as a solution in 70% ethanol, its concentration being equal to 0.1 mg mL^{-1} (i.e., ca. 3×10^{-4} mol L^{-1}). This considerably low

concentration of the employed sample proved sufficient for densitometric detection at the UV wavelength of 252 nm, due to cummulation of the two phenyl groups and one carbonyl (═C═O) chromophore in the ketoprofen molecule.

Sample application to the plates was automatic, with use of a Model AS 30 autosampler manufactured by Desaga, Heidelberg, Germany. The *S,R*-(±)-ketoprofen solutions were applied to the plate 1.5 cm above the lower edge in the aliquots of 5 mL spot^{-1}. Nine samples in the equal distance of 2 cm from one another and from the side edges of the plate were applied, and then the chromatogram was developed in the one-dimensional mode.

TLC Silica Gel Layers

TLC was performed on commercial glass plates (20 cm × 20 cm) precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck, Darmstadt, Germany, cat. # 1.05715). Before use, the plates were washed by predevelopment with methanol-water (9:1, v/v), and then dried at ambient temperature for 3 h.

Mobile Phase and Development of the Chromatograms

Development of the *S,R*-(±)-ketoprofen samples was carried in the normal (after Stahl) chromatographic tanks in the ascending mode, after their saturation for 0.5 h. It was carried out to a distance of 15 cm using the binary mobile phase acetonitrile (ACN)-water (H_2O) (5:1, v/v) plus several drops of glacial acetic acid to fix the pH < 4.8.

After development of the chromatograms, the plates were dried at ambient temperature for 3 h, and the surface of each plate was densitometrically scanned in the 1-mm intervals in the direction of developing the chromatograms.

The chromatographic experiment was carried out on three plates, with nine equal aliquots of the *S,R*-(±)-ketoprofen sample spotted per plate. Thus, the numerical results given in this paper originate from 27 individual development lanes, as it was our aim to most reliably assess the deviation from verticality of the migration tracks with the constituents of the investigated racemic mixture.

Densitometric Assessment of the Chromatograms

Densitograms were acquired for each chromatogram in the 1-mm intervals with a Desaga Model CD 60 densitometer equipped with Windows-compatible Pro Quant software. Concentration profiles were recorded in UV light from the deuterium lamp (in the reflectance mode) at 252 nm. The dimensions of

the rectangular light beam were 2.0 mm × 0.1 mm. The maxima of the concentration profiles were used for calculation of R_F values.

Moreover, the UV spectra were recorded in situ of the three chromatographic spots derived from the *S,R*-(±)-ketoprofen sample in the course of its separation on the plain silica gel layer.

RESULTS AND DISCUSSION

At the preliminary stage of our experiments, we checked the evenness and strict horizontality of the laboratory bench top with a spirit level, in order to eliminate the non-chromatographic external factors that might negatively affect our results. We also eliminated a possibility of cool air drafts in our laboratory, which might also contribute to the deviation of the enantiomers' migration tracks from verticality. In that way, we believe to have entirely excluded the influence of the non-chirality-based factors on the ultimate measuring results.

Similar to our earlier experimental evidence,[3] we again found three well separated spots originating from the *S,R*-(±)-ketoprofen sample; their respective migration tracks deviated from the strict verticality to the right, and these deviations were not random, but systematic (see Figure 1). The

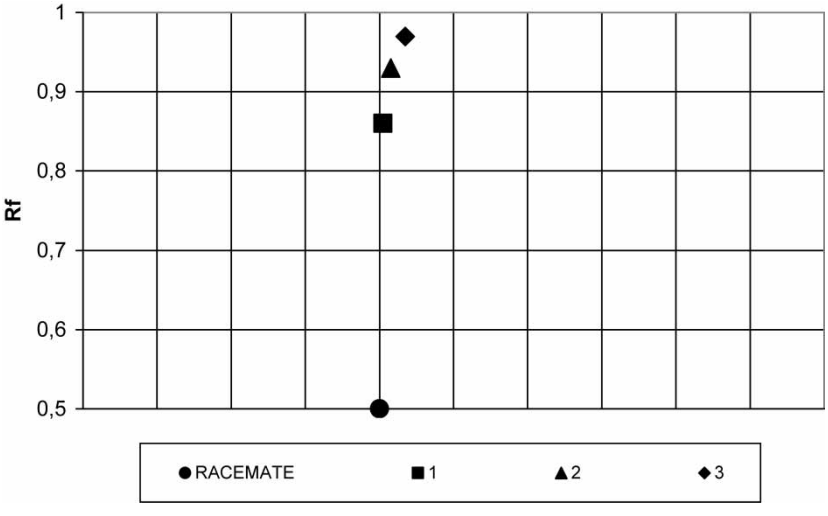

Figure 1. Schematic presentation of the right-handed deviation from verticality of the migration tracks of the three chromatographic spots derived from the same *S,R*-(±)-ketoprofen sample. The respective deviation values were: 1(±1) mm ($R_F = 0.86$), 3(±1) mm ($R_F = 0.93$), and 7(±1) mm ($R_F = 0.97$). Stationary phase: silica gel 60 F_{254} (precoated plates, Merck, cat. # 1.05715). Mobile phase: ACN – H_2O (5:1, *v*/*v*) plus several drops of glacial acetic acid.

magnitude of the deviations was, in the two out of the three cases, considerably higher than is normal in random cases (then it hardly surpasses ±1 mm), and for the separated spots it was equal to 1 (±1) mm ($R_F = 0.86 \pm 0.02$), 3 (±1) mm ($R_F = 0.93 \pm 0.02$), and 7 (±1) mm ($R_F = 0.97 \pm 0.02$). This result is comparable to the phenomenon observed on the L-arginine-impregnated silica gel layers in terms of direction and more pronounced in terms of magnitude.[3] Positions of the three spots on the chromatogram were perceptibly different from one another and higher than the maximum error of the technique (usually assumed as not higher than ±0.02 R_F units).

A comparison of separation of the three species on the impregnated[3] and the non-impregnated layers allows the following conclusions. The R_F values observed on the L-arginine-impregnated silica gel layers were equal to 0.58 ± 0.02, 0.69 ± 0.02, and 0.75 ± 0.02, respectively, and hence considerably lower than in the case of the plain silica gel. Also, the R_F differences (ΔR_F) between the spots with the lowest and the highest R_F value were, in the former case, greater than in the latter one. Thus, it can be deduced that the chiral selector (L-arginine) and the chiral microcrystalline silica gel contribute jointly to the vertical separation of the spots, whereas the chirality of the silica gel layer is the predominant factor responsible for the horizontal separation.

In order to find out about the nature of these three separated spots, we recorded their in situ respective UV spectra, as shown in Figure 2. In each case, the observed maximum was at the wavelength $\lambda_{max} \approx 252$ nm, which remains in conformity with the data taken from the literature and to the ketoprofen spectra recorded in the aqueous, acetonitrile, or alcohol solutions (λ_{max} was in these cases equal to 254 or 255 nm).[4,5] Both the shapes of the three recorded in situ spectra and the position of their maximum were identical, the only difference being their relative intensities (most evidently due to the different aliquots of the species per separated spot). The analogous UV spectra are presented in an earlier paper,[3] as recorded for the three constituents of the *S,R*-(±)-ketoprofen sample separated in the chiral TLC system with the L-arginine-impregnated silica gel stationary phase. We believe that, in both cases—in that of the silica gel layers impregnated with L-arginine[3] and in the present one of the plain silica gel layers—the chemical nature of these three components is the same. It is hard to judge their chemical nature, and some cautious speculations on this subject were offered in publication,[3] so we are not going to extensively repeat them now. It will only be said that two alternative options were suggested. According to option 1, the following three fractions of the cyclic dimers could have been separated: *RR* (the lowest), *SR* (the medium), and *SS* (the highest). According to option 2, the following chemical nature might be alternatively attributed to each of the three fractions: keto-enol tautomer derived from ketoprofen (the lowest), *SR* (the medium), and *SS* (the highest). At the moment, it is virtually not possible to experimentally verify these

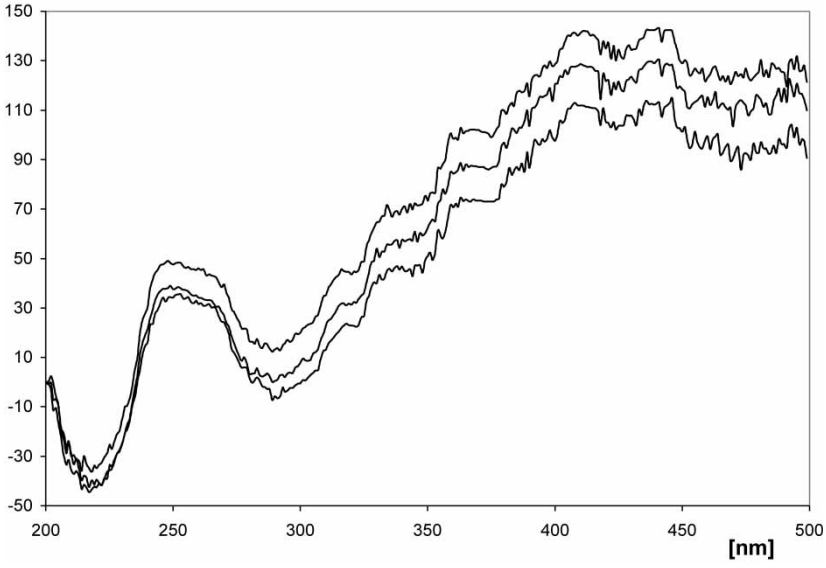

Figure 2. The UV spectra (recorded in situ on the chromatographic plate) of the three chromatographic spots derived from the same *S,R*-(±)-ketoprofen sample and all showing the right-handed deviation from verticality of their respective migration tracks (see Figure 1).

speculations, and it also has to be assumed that still another possibility might prove correct.

Finally, a comparison can be made of the results presented in papers[1–3] and in this one. In papers,[2,3] we enantioseparated the selected profens (i.e., ibuprofen, naproxen, ketoprofen, and 2-phenylpropionic acid) in the chiral thin layer chromatographic systems with silica gel layers impregnated with a chiral selector (i.e., L-arginine). In all those cases enantioseparation was two-dimensional, which means that the two antimers showed both different R_F values (enantioseparation in the vertical direction) and deviation of their respective migration tracks from verticality (enantioseparation in the horizontal direction).

In paper[1] and in the present paper we studied retention of the selected profens (i.e., *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*-(±)-ketoprofen) when developed in TLC systems with plain silica gel layers and an achiral mobile phase. In the case of *S*-(+)-ibuprofen and *S*-(+)-naproxen, the well pronounced deviation from verticality of their respective migration tracks was observed. In the case of *S,R*-(±)-ketoprofen (discussed in this paper), we again observed the well pronounced deviation from verticality of the three ketoprofen sample constituents and their two-dimensional separation. Using the example of *S,R*-(±)-ketoprofen, we can firmly conclude that the plain silica gel layer can provide a successful two-dimensional separation of certain racemic (or scalemic) mixtures in the one-dimensional development mode.

CONCLUSIONS

Based upon the results with respect to the profens enantioseparated on the silica gel layers impregnated and not impregnated with the chiral selector, the following general conclusions can be drawn:

- In the case of the silica gel layers impregnated with the chiral selector (i.e., L-arginine), the two-dimensional enantioseparation in the one-dimensional development mode was observed. Namely, the antipodes were simultaneously undergoing vertical separation (manifested by the difference of the R_F values) and horizontal separation (manifested by the deviation from the verticality of their migration tracks).
- In the case of the plain silica gel layers (i.e., not impregnated with the chiral selector) and the achiral mobile phase, the two-dimensional separation of the racemic mixture in the one-dimensional development mode was also observed. However, the extent of the vertical separation was, in the case of the plain silica gel layers, lower and the respective R_F values were higher than in the case of the silica gel layers with the chiral selector (L-arginine) deposited on its surface. The extent of the horizontal separation was, in the case of the plain silica gel layer, perceptibly higher than on the L-arginine-impregnated silica gel plates.
- Vertical enantioseparation in the TLC mode seems due to the joint action of the microcrystalline silica gel layer and of the chiral selector deposited on its surface, whereas horizontal enantioseparation seems predominantly due to the action of the microcrystalline silica gel layer.
- It is evident that the benefit of the two-dimensional enantioseparation in the one-dimensional development mode is inherent exclusively of planar chromatography. Even if intermolecular interactions between the chiral analyte and the chiral stationary phase take place in column chromatography also, it is due to the effective diffusion perpendicular to the direction of the mobile phase flow that the analyte molecules migrate sidewise (and the effective diffusion perpendicular to the direction of the mobile phase flow is absent from the column chromatography).

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