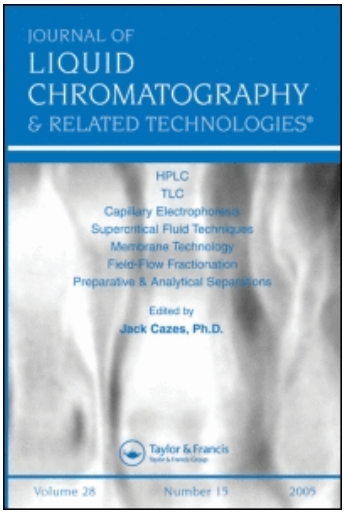

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On the Mechanism of Oscillatory Changes of the Retardation Factor (R_F) and the Specific Rotation $[\alpha]_D$ with Selected Solutions of *S*-(+)-Naproxen

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Abstract: In our earlier investigations, we demonstrated oscillatory instability of selected profens (*S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*-(±)-2-phenylpropionic acid), and their marked tendency to change chiral configuration, most probably *via* the keto-enol tautomerism. In our earlier papers oscillatory transenantiomerization of the profens had been demonstrated in a standard manner, i.e., by means of polarimetry, and also by means of thin-layer chromatography (TLC). The ability of profens to change chiral configuration is due to their gelating property (as the low-molecular-weight gelators) and a subsequent increase of solutions' viscosity. In this study, we attempt to provide sufficient experimental evidence in favor of keto-enol tautomerism as a cause of the oscillations observed. Keto-enol tautomerism is catalyzed in a basic environment and is hampered in acid. In our study focused on the molecular-level mechanism of the observed oscillations, we purposely stored samples of *S*-(+)-naproxen in an acidic and a basic solution. It was clearly shown that the acidic environment both hampers transenantiomeric oscillations of *S*-(+)-naproxen and stabilizes naproxen in its *S*-(+)-form. Conversely, a basic environment facilitates partial transformation of *S*-(+)-naproxen to the *R*-(−)-form, which is the best proof of keto-enol tautomerism as a mechanism of the oscillatory instability of profens. Culminating our experiments were the attempts to obtain two-dimensional chiral separations of *S*-(+)-naproxen from its *R*-(−)-antipode, generated in the course of storage of the *S*-(+)-sample in basic and acidic solutions. The results obtained provided direct confirmation as to the key role played by the basic environment in generating

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R-(−)-naproxen *via* keto-enol tautomerism. To our best knowledge, this is the first thin-layer chromatographic separation of the two chiral antipodes of naproxen.

Keywords: Naproxen, TLC separation of naproxen enantiomers, Oscillatory transenantiomerization, Keto-enol tauromerism

INTRODUCTION

In our earlier studies,[1−3] we demonstrated the striking effect with the three selected profens (*S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*-(±)-2-phenylpropionic acid), which consisted of an oscillatory change of their retardation factor (R_F) values when stored for a long enough period of time as solutions in an aqueous medium and/or chromatographed with aid of an aqueous eluent. The oscillations observed were characterized with periodic changes of the retardation parameter (R_F) values and with a quasi-regular amplitude. The amplitude of these oscillations clearly depended on the quantitative proportions between water and the organic solvent present in the stored solution and/or in the applied mixed eluent. From additional high performance liquid chromatography (HPLC) experiments,[4] it was quite obvious that these oscillations neither happened in an absolute absence of water nor in an absolute absence of an organic solvent.

A parallel investigation, carried out by means of polarimetry,[1−3] showed that oscillations of the retardation parameter (R_F) with solutions of the three investigated profens were accompanied by analogous oscillations of their specific rotation $[\alpha]_D$ values. Oscillations of the specific rotation $[\alpha]_D$ of the examined profen solutions were the first clue as to the their possible molecular-level mechanism. In the absence of any evident decomposition of the scrutinized profens, which had been convincingly confirmed by means of HPLC with a diode array detector (DAD) and thin layer chromatography (TLC)-densitometry with *in situ* ultraviolet (UV) detection, it seemed very likely to expect that the oscillations observed were due to transenantiomerization of the profen molecules *via* keto-enol tautomerism. Simultaneously, we encountered a convincingly documented pharmaceutical study on the mechanism and kinetics of the base-catalyzed racemization of ibuprofen enantiomers in the literature.[5] The authors suggested the molecular-level mechanism of this racemization *via* keto-enol tautomerism, as shown in Figure 1.

It was the aim of this study to confirm the importance of the environment (either its acidity or basicity) on the oscillatory transenantiomerization of the investigated profens. For this purpose, we selected *S*-(+)-naproxen as the best performing of our former three test species. In our earlier study,[1] we had demonstrated the impact of the sample storage time on the retardation factor (R_F) and the specific rotation ($[\alpha]_D$) with *S*-(+)-naproxen dissolved

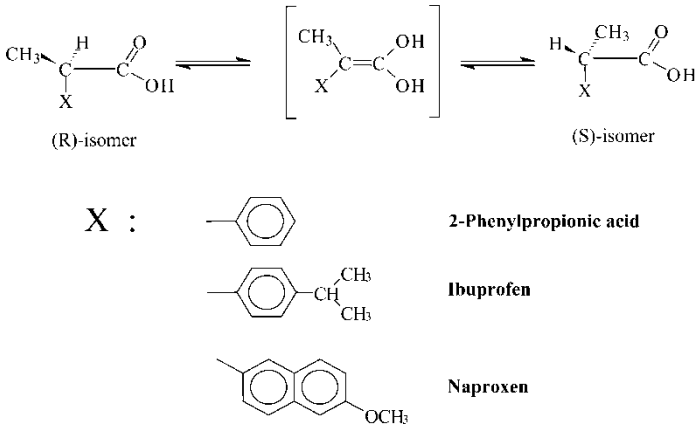

Figure 1. Schematic representation of transenantiomerization of profens by keto-enol tautomerism.

in 70% ethanol. In this study, we compared our previous findings with those obtained when storing *S*-(+)-naproxen in acidic and basic solutions.

EXPERIMENTAL

Solutions of *S*-(+)-Naproxen for Polarimetric Studies

Solutions of *S*-(+)-naproxen (Sigma–Aldrich; # 28,478-5) for polarimetric measurements were prepared at a concentration of 12.5 μg μL^{-1} (ca. 5.4×10^{-2} mol L^{-1}) in the following multicomponent liquid mixtures:

> EtOH (1) – H_2O (2) + glacial acetic acid or basic (pH = 9) buffer (3). In both cases we used the same volume proportions (v/v) of the solvents, as given below:

- 7 : 2.5 : 05;
- 7 : 2 : 1;
- 7 : 1.5 : 1.5;
- 7 : 1 : 2;
- 7 : 0.5 : 2.5; and
- 7 : 0 : 3.

Polarimetric Measurements of the Specific Rotation $[\alpha]_D$

Measurements of the specific rotation $[\alpha]_D$ of the *S*-(+)-naproxen solutions were carried out for each of the aforementioned proportions of the

multicomponent solvent at 22 ± 2°C for 120 min (in 10 min intervals) with use of a Polamat A model polarimeter (manufactured by Carl Zeiss, Jena, Germany). The optical path length of the employed measuring cell was exactly 10 cm (=1 dm), and its volume was ca. 1 mL. Specific rotation $[\alpha]_D$ was calculated using the following standard equation:

$$[\alpha]_D = 100\alpha/cd$$

where α is the measured rotation (degrees), D is the employed wavelength, 589 nm, which corresponds with the sodium D line, c is the concentration of a given compound in grams per 100 mL solution, and d is the measured sample thickness in dm.

Solutions of *S*-(+)-Naproxen for TLC Studies

Solutions of *S*-(+)-naproxen were prepared at a concentration of 1 μg μL^{-1} (ca. 4.3×10^{-3} mol L^{-1}) in the following mixed solvents:

(i) EtOH-H_2O-glacial acetic acid (7 : 0 : 3, v/v); and

(ii) EtOH-H_2O-basic buffer (pH = 9) (7 : 0 : 3, v/v),

and either 5 or 10 μL volumes were applied to chromatographic plates with a micropipet.

Commercial TLC Silica Gel Layers and Their Pretreatment

TLC was performed on commercial glass plates precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany; # 1.05715). Before use, the plates were carefully washed by predevelopment with methanol–water (9 : 1, v/v) and then dried at ambient temperature for 3 h. Washing of the plates before more sensitive separations is often recommended by the manufacturer.

The washed and dried plates were then impregnated with a 3×10^{-2} mol L^{-1} solution of L-arginine 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 adsorbent layer. Finally, the washed, impregnated and dried adsorbent layers were ready for chromatography.

Development of the Chromatograms

One-Dimensional Development

Development of the *S*-(+)-naproxen samples was performed at 22 ± 2°C. Plates with three adjacent spots from the 5 μL volumes of *S*-(+)-naproxen solution

were developed to a distance of 15 cm using the ternary mobile phase acetonitrile (ACN)–methanol (MeOH)–H_2O (5 : 1 : 1.5, v/v) containing several drops of glacial acetic acid to fix the pH at <4.8. After development of the chromatograms, the plates were dried at ambient temperature for 3 h, and the three lanes were scanned densitometrically. The experiment was repeated twice.

Two-Dimensional Development

Plates with a single 10 μL spot of the *S*-(+)-naproxen solution [stored for five hours in the EtOH-basic buffer mixture, pH = 9 (7 : 3, v/v)] at the corner were developed to a distance of 15 cm in the first direction with ACN–MeOH–H_2O (5 : 1 : 1.5, v/v) plus several drops of acetic acid as mobile phase. The plates were first dried in an ambient atmosphere for 3 h and then developed to a distance of 15 cm in the second direction (perpendicular to the first one) with the same mobile phase. After development, the plates were again dried in an ambient atmosphere, and the track 35 mm wide, developed in the second direction, was scanned densitometrically in 1.5 mm intervals. This experiment was repeated three times.

Fully analogous two-dimensional development was carried out for *S*-(+)-naproxen solution stored for five hours in EtOH-glacial acetic acid (7 : 3, v/v).

Densitometric Assessment of the Chromatograms

Densitograms were acquired with a Desaga (Heidelberg, Germany) Model CD 60 densitometer equipped with Windows-compatible ProQuant software. Concentration profiles of the development lanes for *S*-(+)-naproxen were recorded in UV light from the deuterium lamp (in the reflectance mode) at 235 nm. (This wavelength is close to the first and stronger of the two UV absorption maxima for naproxen). 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.

RESULTS AND DISCUSSION

The starting point for the research presented in this study was our earlier findings.[1] They referred to the prolonged storage of *S*-(+)-naproxen in 70% ethanol [which, for the sake of our present study, can be described as the mixed EtOH-H_2O-acidic or basic component (7 : 3 : 0, v/v) solvent]. This was a neutral organic–aqueous solvent, without any acidic or basic additive. Oscillatory changes of the retardation factor (R_F) and the specific retention ($[\alpha]_D$) of the *S*-(+)-naproxen when dissolved and stored in the neutral organic–aqueous medium are shown in Figures 2a and 2b. From Figure 2a, it can easily be seen that *S*-(+)-naproxen shows a well pronounced tendency

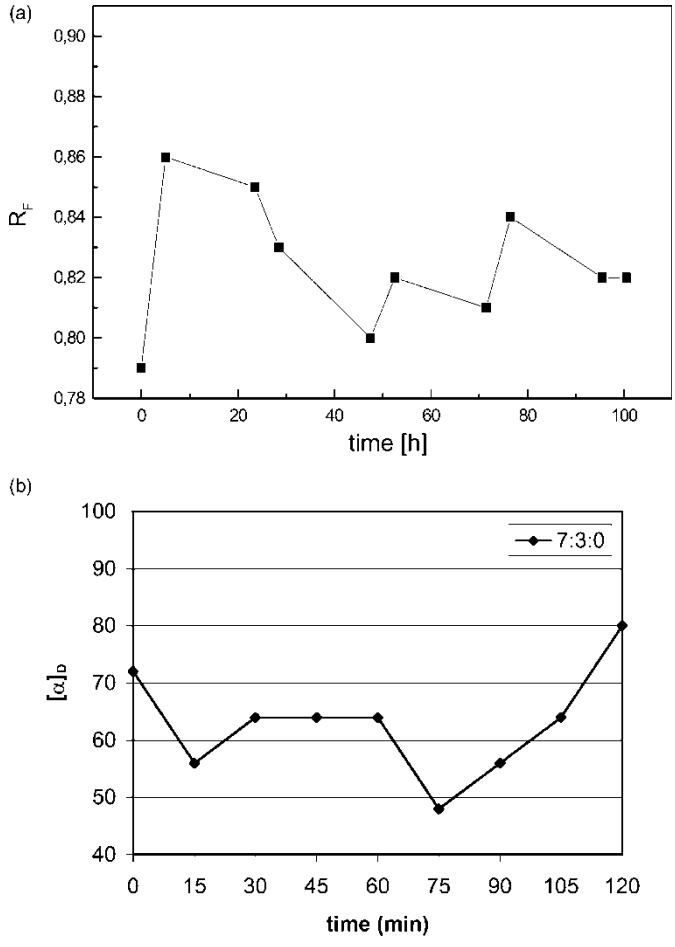

Figure 2. Dependence of (a) retention, R_F, and (b) specific rotation, $[\alpha]_D$, for *S*-(+)-naproxen dissolved in EtOH-H_2O (7 : 3, v/v) upon sample storage time at ambient temperature (22 $\pm$ 2°C).[1]

towards oscillation. This tendency is reflected in R_F changes as a function of time. The oscillatory changes of R_F considerably surpass the measurment error of the retardation factor's numerical values (equal to ± 0.02 R_F unit), thus furnishing a strong confirmation of the correctness of our observations and conclusions. The polarimetric data shown in Figure 2b are in excellent agreement with the results of the TLC investigations. Even with polarimetric and chromatographic measurements carried out within the different time ranges (which was basically due to the different measuring rates with each individual analytical technique), the results originating from each technique described the investigated phenomenon in a very similar manner.

The situation considerably changed when we replaced the neutral organic-aqueous solvent by a different one. In the experiment reported in this paper, we shifted from the ethanol-water mixture to the ethanol-glacial acetic acid mixture, and, due to this change the oscillations of the retention parameter, practically disappeared (see Figure 3a). Weak changes of the R_F value can basically be ascribed to an experimental error. In Figure 3b we show the specific rotation $[\alpha]_D$ values for *S*-(+)-naproxen dissolved in ethanol (its volume fraction was kept constant at 0.7) plus six different proportions of water and glacial acetic acid. Generally, it can be stated that the specific rotation $[\alpha]_D$ values were stable and characteristic of each individual

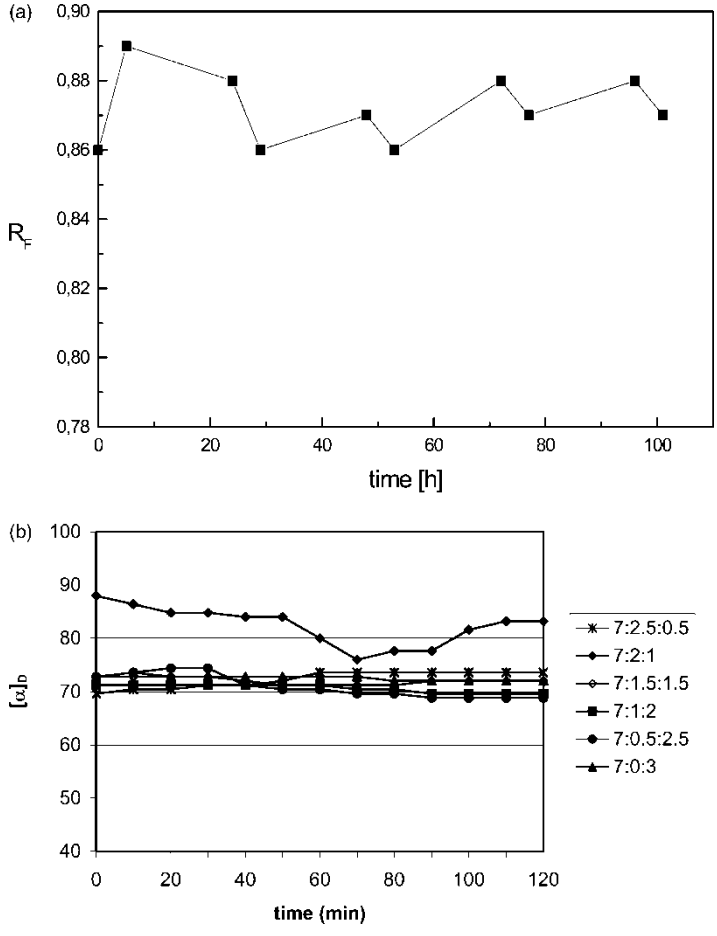

Figure 3. Dependence of (a) retention, R_F, for *S*-(+)-naproxen dissolved in EtOH-glacial acetic acid (7 : 3, v/v) and of (b) specific rotation, $[\alpha]_D$, for *S*-(+)-naproxen dissolved in EtOH-H_2O-glacial acetic acid (in six different volume proportions) upon sample storage time at ambient temperature (22 $\pm$ 2°C).

quantitative composition of the ternary solvent applied. In this sense, the polarimetric results given in Figure 3b coincide well with those originating from TLC and shown in Figure 3a.

In Figure 4a, we show the dependence of the numerical R_F values on the sample storage time for *S*-(+)-naproxen dissolved in the mixture of ethanol and the basic buffer (pH = 9). In this case, oscillations of the R_F values were clearly evident, and they definitely surpassed the experimental error level of TLC. The specific rotation $[\alpha]_D$ values for *S*-(+)-naproxen, stored in six different mixtures of ethanol, water, and the basic buffer, were again

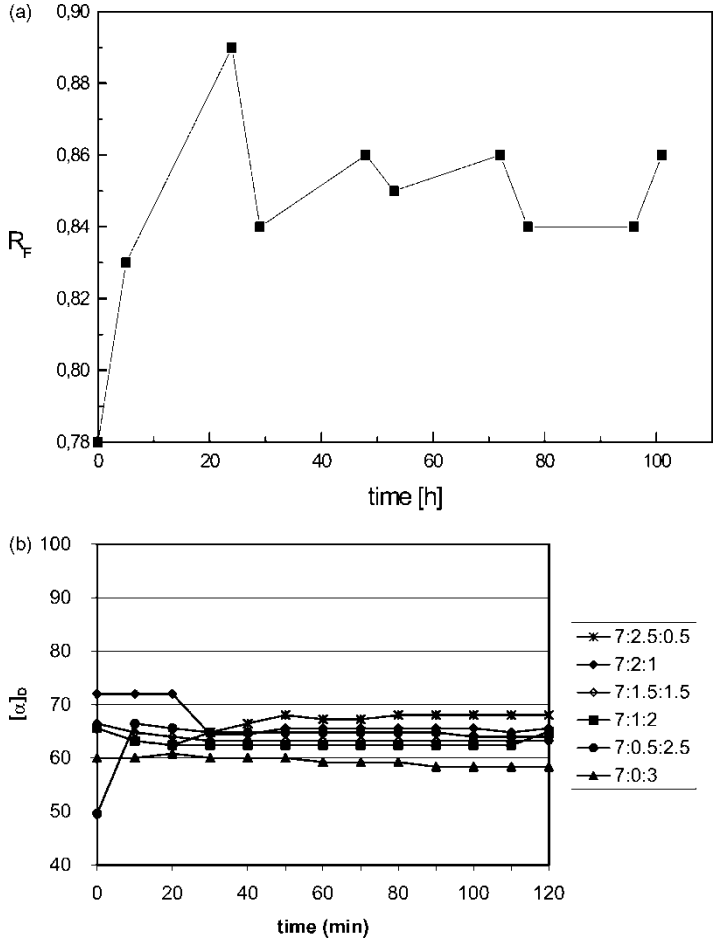

Figure 4. Dependence of (a) retention, R_F, for *S*-(+)-naproxen dissolved in EtOH-basic buffer (pH = 9) (7 : 3, v/v) and of (b) specific rotation, $[\alpha]_D$, for *S*-(+)-naproxen dissolved in EtOH-H_2O-basic buffer (pH = 9) (in six different volume proportions) upon sample storage time at ambient temperature (22 ± 2°C).

stable (see Figure 4b), i.e., they showed no evident oscillations and, in this sense, they resembled the results obtained for the samples stored in the ethanol-water-glacial acetic acid (see Figure 3b). However, the numerical values of the specific rotation $[\alpha]_D$ for the *S*-(+)-naproxen samples stored in the acidic and the basic media proved considerably different. *S*-(+)-Naproxen dissolved in the acidic mixtures (Figure 3b) showed higher levels of specific rotation than those dissolved in the basic mixtures (Figure 4b). At this moment, the physical meaning of these phenomena is not entirely clear to us, yet two optional explanations can seriously be taken into consideration.

The more probable explanation is that, in the basic solvent, partial racemization of *S*-(+)-naproxen to its *R*-(−)-counterpart takes place, which results in lowering of the overall specific rotation value measured for the mixture of the two antipodes. Such an explanation strongly supports our hypothesis that keto-enol tautomerism is responsible for oscillations of the numerical R_F values for *S*-(+)-naproxen prolongedly stored in the basic solution. It is obvious that keto-enol tautomerism can only occur due to proton transfer from one site in the compound molecule to another and results in transformation of the original structure. Migration of the proton obviously has to depend on the reaction environment and the basic environment doubtlessly acts in its favor, whereas the acidic environment can only hamper such a migration.

An alternative (and less probable) explanation of the fact that the *S*-(+)-naproxen solutions stored in an acidic medium show considerably higher numerical values of the specific rotation ($[\alpha]_D$) than those stored in a basic medium can be given as follows. It is well recognized that the specific rotation ($[\alpha]_D$) of chiral compound solutions depends, to certain extent at least, on the solvent employed. Each of the six plots $[\alpha]_D = f(\text{time})$ shown in Figure 3b refer to *S*-(+)-naproxen dissolved in the acidic ternary mixtures of different quantitative proportions among their constituents; hence, from a formal point of view, each of these six plots refer to *S*-(+)-naproxen dissolved in a different solvent. Analogous reasoning has to be adopted also for the six plots given in Figure 4b and referring to *S*-(+)-naproxen dissolved in the basic ternary mixtures of different quantitative proportions among their constituents.

In order to support our hypothesis that the observed oscillations of the R_F and $[\alpha]_D$ values with *S*-(+)-naproxen are due to keto-enol tautomerism, and, hence, that they can either be stimulated in the basic environment or hampered in the acidic one, we studied the two-dimensional TLC separation of the *S*-(+)-naproxen from its *R*-(−)-antipode, generated in the *S*-(+)-naproxene solution when stored in the basic and the acidic solutions. In our investigations, we adopted the separation procedure first proposed for separation of the two enantiomers of ibuprofen by Bhushan and Parshad,[6] and then successfully adapted to commercial precoated plates and densitometric detection.[7]

The asymmetric concentration profile of *S*-(+)-naproxen, when stored in the basic solution for a few hours and then chromatographed in the first

direction, strongly suggested a possibility of attaining a complete separation of the *S*-(+)- and the *R*-(−)-antipode if the sample was chromatographed in the second direction as well. In fact, full separation of *S*-(+)-and *R*-(−)-naproxen was attained in the two-dimensional development of the chromatograms (Figure 5). The numerical values of R_F were 0.90($\pm$0.02) and 0.82($\pm$0.02) for the *S*-(+)-and the *R*-(−)-species, respectively, and the $\Delta R_F = 0.08$. The result presented in Figure 5 is a very persuasive proof of transenantiomerization of *S*-(+)-naproxen to the *R*-(−)-species in the course of a prolonged storage in the basic mixed solvent.

Contrary to the results obtained, when *S*-(+)-naproxen has been stored in the basic environment, the acidic mixed solvent causes no evident transisomerization of the profen considered. In Figure 6, we give the three-dimensional presentation of the chromatogram of *S*-(+)-naproxen dissolved and stored in the acidic medium (developement in the second direction). In

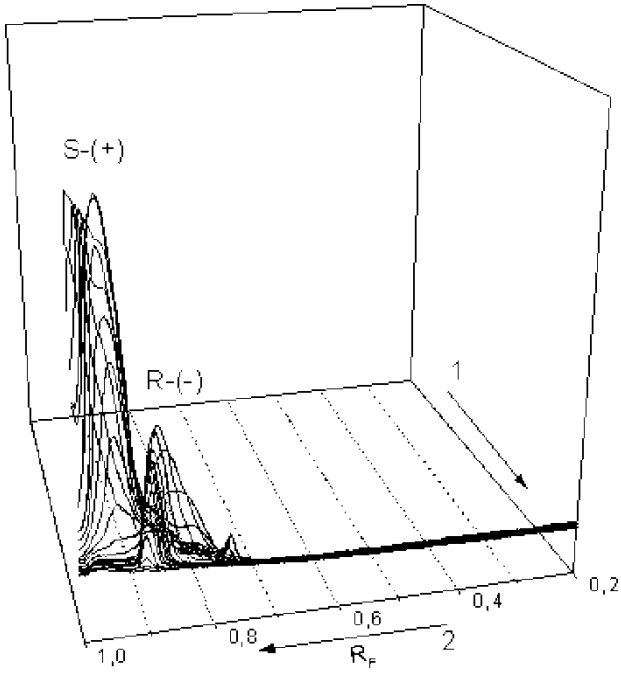

Figure 5. Three-dimensional presentation of the naproxen chromatogram with two development directions, 1 and 2, indicated. Densitometric scanning (at parallel 1.5-mm intervals) of the 35-mm wide track in the second direction of the development was performed to better illustrate the separation performance and the skewed arrangement of *S*-(+)-naproxen relative to its *R*-(−) counterpart for the *S*-(+)-naproxen sample stored for 5 hours in the EtOH-basic buffer mixture (pH = 9; 7 : 3, v/v).

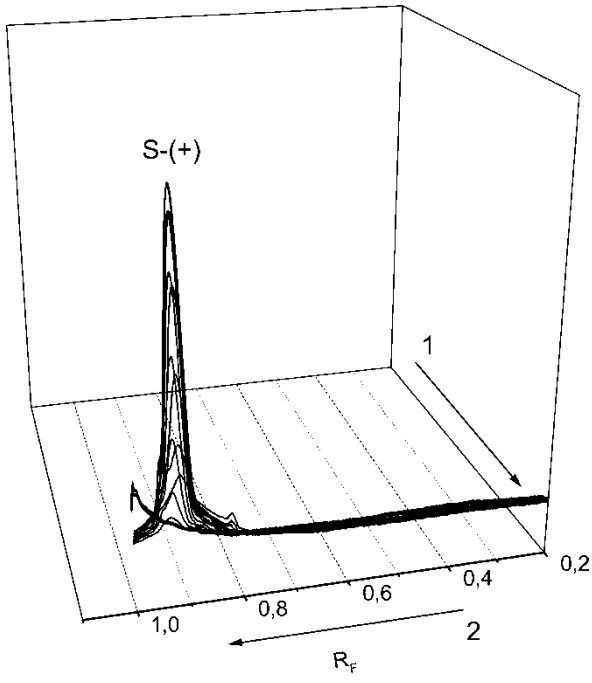

Figure 6. Three-dimensional presentation of the naproxen chromatogram with two development directions, 1 and 2, indicated. Densitometric scanning (at parallel 1.5-mm intervals) of the 35-mm wide track in the second direction of the development was performed to better illustrate an absolute lack of transenantiomerization for the *S*-(+)-naproxen sample stored for 5 hours in the EtOH-glacial acetic acid mixture (7 : 3, v/v).

this figure, we can see the single three-dimensional peak of naproxen only, and the numerical value of its retardation factor ($R_F = 0.90(\pm 0.02)$) corresponds well with the steric configuration of *S*-(+).

CONCLUSIONS

In this paper, we provide sufficient experimental evidence in favor of base-catalyzed keto-enol tautomerism as a driving force for the configurational change of chirally pure *S*-(+)-naproxen. This evidence was furnished with aid of the two measuring techniques, namely polarimetry and TLC. It seems fully justified to conclude that the analogous molecular mechanism is responsible for transenantiomerization of the other profens as well. In our earlier studies, we had managed to demonstrate a striking phenomenon of an

oscillatory chiral configurational change with the three selected profens (*S*-(+)-naproxen, *S*-(+)-ibuprofen, and *S,R*-(±)-2-phenylpropionic acid) when stored for a long enough period of time in certain solvents. We believe that the observed oscillatory changes of configuration with these three compounds occurred *via* keto-enol tautomerism.

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