

Application of Thin-Layer Chromatography to Investigate Oscillatory Instability of the Selected Profen Enantiomers in Dichloromethane

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Abstract

The usefulness of thin-layer chromatography (TLC) as an efficient measuring technique in the studies of oscillatory *trans*-enantiomerization of profens from the *S* to the *R* configuration (and vice versa) during their storage as 70% ethanol solutions is demonstrated in the literature (1). *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S*, *R*-(±)-2-phenylpropionic acid are utilized as the test profens. It is proven possible to show oscillatory instability with the racemic *S*, *R*-(±)-2-phenylpropionic acid also. Correctness of the TLC assessment is successfully confirmed by means of polarimetry. Upon these preliminary results, it is concluded that the most probable mechanism might embrace the keto-enol tautomerism because of a convenient migration of the proton from one moiety of the profen molecule to another in an aqueous medium. To indirectly verify this hypothesis, profens are stored in dichloromethane, deliberately hampering their ability to dissociate and to re-structure. It is obvious though that the (much less pronounced) electrolytic dissociation can occur in the non-aqueous media as well. It is shown that the non-aqueous solvent considerably suppresses, although they do not completely eradicate, the oscillatory *trans*-enantiomerization of profens. In view of these findings, the reports which claim a predominant therapeutic potential of the respective *S*-profens become less convincing and certainly need reconsideration.

Introduction

Thin-layer chromatographic (TLC) conditions, best suited for the separation of the profen enantiomers, embrace silica gel impregnated with L-arginine, which is kept in the cationic form because of the properly fixed pH (< 4.8) value. The mechanism of such separations can best be summarized with the aid of the given stoichiometric equations (2,3), which reflect the ion-pair formation between the cationic impregnant and the profen enantiomers in the anionic form:



The success of the separation is, however, rather limited because a prolonged (e.g., lasting several hours) storage of the profens dissolved in 70% ethanol results in a disappearance of the two well-separated chromatographic bands and, instead, results in the steadily changing position of a single and skewed profen band. In fact, this single and skewed profen band attains quite different (yet clearly depending on the storage time) positions on the successive chromatograms, even when kept within the rigid range of the boundary (i.e., of the uppermost and the lowermost) retardation factor (R_f) values. In the other words, profens stored for a longer period of time in 70% ethanol undergo a continuous oscillatory *trans*-enantiomerization, shifting from the *S* to the *R* configuration and vice versa. Moreover, it is possible to demonstrate the oscillatory instability even in the case of a supposedly stable racemic mixture. In this experiment, the following test profens are utilized: *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S*, *R*-(±)-2-phenylpropionic acid (1).

In a previous publication (1), the possible molecular mechanisms of the continuous oscillatory *trans*-enantiomerization of profens were reflected on, and a conclusion was drawn [supported by the convincing data taken from literature (4)] that the most probable mechanism should embrace the keto-enol tautomer (as a short-living intermediate species), owing to the proton, which is easily transported from one moiety of the molecule to another in the alcohol-aqueous medium. A schematic presentation of the assumed molecular mechanism of *trans*-enantiomerization is shown in Figure 1.

In order to indirectly confirm the earlier assumption as to an easy transport of the proton in an aqueous medium, in the present study, the same three test profens [i.e., *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S*, *R*-(±)-2-phenylpropionic acid] were stored for longer periods of time as dichloromethane solutions.

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Evidently, the low-polar organic solvent (i.e., dichloromethane) provides incomparably less favorable conditions for the splitting of the proton from the molecule of each profen studied than the earlier employed alcohol–aqueous medium, and it should largely hamper the tendency of these compounds to undergo oscillatory *trans*-enantiomerization. In the present experiment, the same techniques of tracing the configuration changes were engaged as in the earlier study (1) and, namely, TLC and polarimetry.

Experimental

Profens

This experiment was performed with the following profens (their general chemical structures and optical rotation characteristics are given in Table I).

S-(+)-Ibuprofen was obtained from Sigma-Aldrich (cat. # I-106, St. Louis, MO). For TLC, solutions of *S*-(+)-ibuprofen were prepared at a concentration of 1 $\mu\text{g}/\mu\text{L}$ (ca. 5.8×10^{-3} mol/L) in

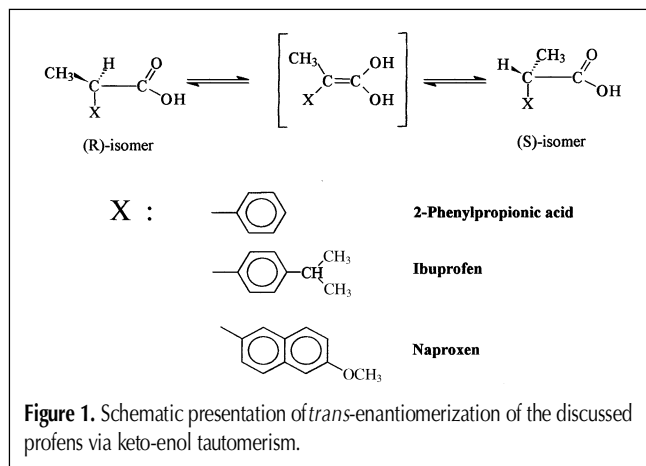


Table I. Schematic Presentation of the Chemical Structure of the Three Profens Discussed in This Study and the Specific Rotation ($[\alpha]_D$) of Their *S* and *R* Enantiomers at 25°C, as Taken from the Literature

Profen	Chemical structure	Specific rotation, $[\alpha]_D$ [angle degrees, °]	
		<i>S</i>	<i>R</i>
Ibuprofen		+53.2 (5)	-57.5 (6)
Naproxen		+64.9 (7) +66.3 (8)	-67.2 (8)
2-Phenylpropionic acid		69.2 (9) 79.2 (6)	-80.0 (6)

dichloromethane, and 10- μL volumes were applied to the plates with a micropipet. For polarimetry, solutions of *S*-(+)-ibuprofen were prepared at a concentration of 50 $\mu\text{g}/\mu\text{L}$ (ca. 0.25 mol/L) in dichloromethane.

S-(+)-Naproxen was obtained from Sigma-Aldrich (cat. # 28,478-5). For TLC, solutions of *S*-(+)-naproxen were prepared at a concentration of 1 $\mu\text{g}/\mu\text{L}$ (ca. 4.3×10^{-2} mol/L) in dichloromethane, and 5- μL volumes were applied to the plates with a micropipet. For polarimetry, solutions of *S*-(+)-naproxen were prepared at a concentration of 12.5 $\mu\text{g}/\mu\text{L}$ (ca. 5.4×10^{-2} mol/L) in dichloromethane.

S,R(\pm)-2-Phenylpropionic acid was obtained from Merck KGaA (cat. #8.20651.0010, Darmstadt, Germany). For TLC, solutions of *S,R*(\pm)-2-phenylpropionic acid were prepared at a concentration of 1 $\mu\text{g}/\mu\text{L}$ (ca. 6.5×10^{-3} mol/L) in dichloromethane, and 5- μL volumes were applied to the plates with a micropipet. For polarimetry, solutions of *S,R*(\pm)-2-phenylpropionic acid were prepared at a concentration of 500 $\mu\text{g}/\mu\text{L}$ (ca. 3.33 mol/L) in dichloromethane.

Storage of the test analytes

Samples of *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*(\pm)-2-phenylpropionic acid dissolved in CH_2Cl_2 were stored at the two different temperatures (i.e., at $6^\circ\text{C} \pm 2^\circ\text{C}$ and $22^\circ\text{C} \pm 2^\circ\text{C}$) for the period of 5 days each, and the R_f values were measured for each compound and each experimental series twice a day (in 5-h intervals). Each storage experiment was repeated twice, thus providing the two series of measured data.

Commercial TLC silica gel layers and their pretreatment

In this experiment, commercial glass TLC plates precoated with 0.25-mm layers of silica gel 60 F₂₅₄ (#1.05715, Merck) were used. Before use, the plates were carefully washed by predevelopment in methanol–water (9:1, v/v), then dried at ambient temperature for 3 h. Washing of the plates prior to initialization of the more “sensitive” separations is often recommended by the manufacturer.

The washed and dried plates were then impregnated with a 3×10^{-2} -mol/L solution of L-arginine in methanol by conventional dipping for 2 s. Concentration of the impregnating solution was purposely calculated to deposit on the adsorbent layer the amount of 0.5 g of L-arginine/50 g of the dry silica gel. Finally, the adsorbent layers (washed with methanol–water and fortified with L-arginine) were ready for chromatography.

Development of the chromatograms

S-(+)-Ibuprofen

Development of the ibuprofen samples was carried out at two different temperatures ($6^\circ\text{C} \pm 2^\circ\text{C}$ and $22^\circ\text{C} \pm 2^\circ\text{C}$). Chromatographic plates with the three adjacent spots from 10- μL volumes of the *S*-(+)-ibuprofen solution were developed to a distance of 15 cm using the ternary mobile phase [i.e., ACN–MeOH–H₂O, 5:1:1 (v/v) with several drops of acetic acid, in order to fix the pH < 4.8 level]. After development of the chromatograms, the plates were dried at ambient temperature for 3 h, and the three lanes for *S*-(+)-ibuprofen were scanned densitometrically. This experiment was repeated twice for each measuring temperature.

S-(+)-Naproxen

Development of the naproxen samples was carried out in basically the same way as that described for ibuprofen. The only difference was the quantitative composition of the employed mobile phase. In this case, the ternary mobile phase [i.e., ACN–MeOH–H₂O, 5:1:1.5 (v/v) with several drops of acetic acid, in order to fix the pH < 4.8 level] was used.

S,R-(±)-2-Phenylpropionic acid

Development of the 2-phenylpropionic acid samples was carried out basically in the same way as that described for ibuprofen and naproxen, and the only difference consisted in the quantitative composition of the employed mobile phase. In this case, the ternary mobile phase [i.e., ACN–MeOH–H₂O, 5:1:0.75 (v/v) with several drops of acetic acid, in order to fix the pH < 4.8 level] was used.

Densitometric assessment of the chromatograms

Densitograms were acquired with the Desaga (Heidelberg, Germany) model CD 60 densitometer equipped with Windows-compatible ProQuant software (Qivx Inc., Fort Collins, CO). Concentration profiles of the development lanes for the three investigated profens were recorded in UV light from the deuterium lamp (in reflectance mode) at 210 nm (at approximately this wavelength the stronger one of the two UV absorption maxima for ibuprofen appears, whereas the stronger maxima for naproxen and 2-phenylpropionic acid, respectively, are located in relative proximity to this position). The dimensions of the rectangular light beam were 0.02 × 0.4 mm. The maxima of the concentration profiles were used for calculation of R_f values.

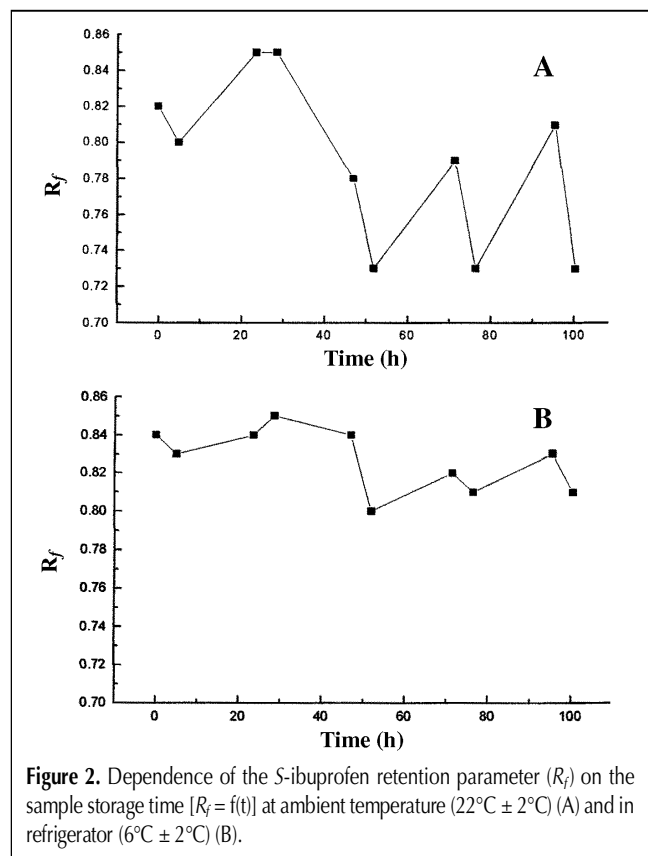


Figure 2. Dependence of the *S*-ibuprofen retention parameter (R_f) on the sample storage time [$R_f = f(t)$] at ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$) (A) and in refrigerator ($6^\circ\text{C} \pm 2^\circ\text{C}$) (B).

Polarimetric measurements of specific rotation of the investigated profens

Measurements of specific rotation $[\alpha]_D$ of the *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*-(±)-2-phenylpropionic acid samples (dissolved in dichloromethane) were carried out at $6^\circ\text{C} \pm 2^\circ\text{C}$ and $22^\circ\text{C} \pm 2^\circ\text{C}$ each day in the course of their 5-day storage time (each day for the 5-h period) with use of the 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 approximately 1 mL. $[\alpha]_D$ was calculated using the following standard equation:

$$[\alpha]_D = 100\alpha/cd \quad \text{Eq. 3}$$

where α is the measured rotation (in the angle degrees), D holds for the employed wavelength $\lambda = 589$ nm (which corresponds with the sodium D line), c is the concentration of a given compound in grams per 100 cm³ solution, and d is the measured sample thickness in decimeters (dm).

Results and Discussion

The study began with an investigation of the numerical values of the R_f for the three investigated profens (each analyte dissolved in dichloromethane and then stored for 5 days either at $6^\circ\text{C} \pm 2^\circ\text{C}$ or $22^\circ\text{C} \pm 2^\circ\text{C}$). The experimental results, in the form of the

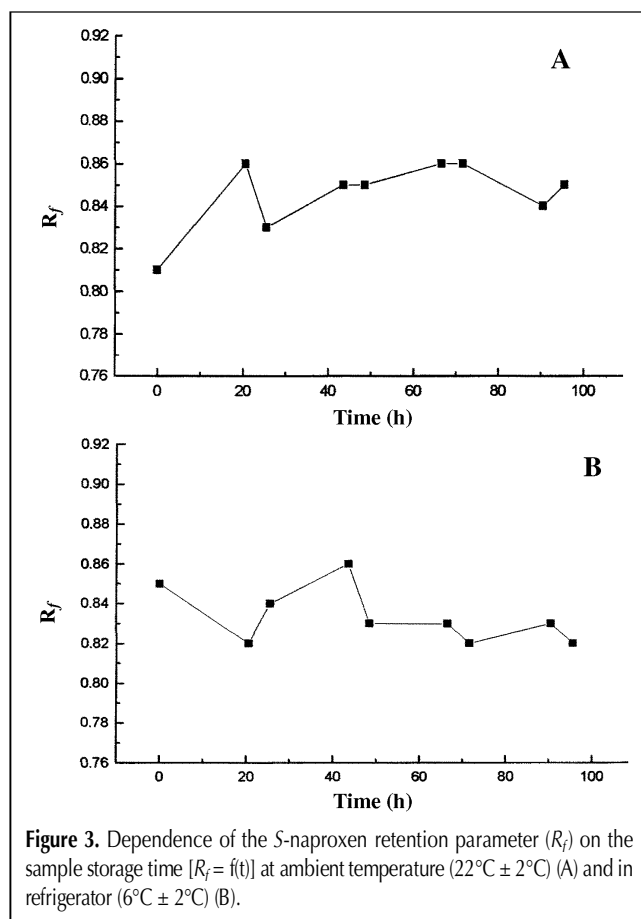


Figure 3. Dependence of the *S*-naproxen retention parameter (R_f) on the sample storage time [$R_f = f(t)$] at ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$) (A) and in refrigerator ($6^\circ\text{C} \pm 2^\circ\text{C}$) (B).

respective R_f value changes during the storage time, are shown in Figures 2 and 3, and they refer to *S*-(+)-ibuprofen and *S*-(+)-naproxen only. With the racemic *S,R*-(±)-2-phenylpropionic acid, measurements of the respective R_f values proved virtually impossible because in each case the chromatographic profiles appeared as unresolved, almost rectangular and relatively broad bands (see Figure 4), obviously representing nonseparated antipodes of the

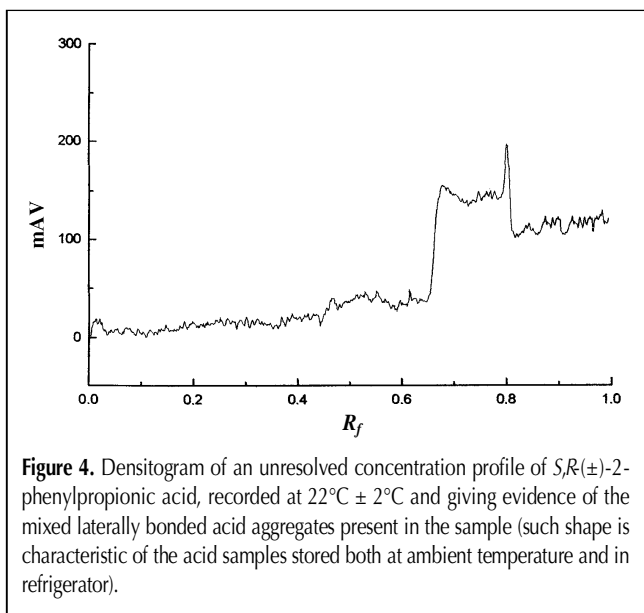


Figure 4. Densitogram of an unresolved concentration profile of *S,R*-(±)-2-phenylpropionic acid, recorded at $22^\circ\text{C} \pm 2^\circ\text{C}$ and giving evidence of the mixed laterally bonded acid aggregates present in the sample (such shape is characteristic of the acid samples stored both at ambient temperature and in refrigerator).

acid or the mixed laterally bonded aggregates thereof (or both) (i.e., most likely the cyclic dimers of the *SS*, *RR*, and *SR* type).

As mentioned in the Experimental section of this article, each experiment was repeated three times in one series, and two such series were run for each individual profen and each individual working temperature. The results originating from one and the same storage series were averaged, even though the data originating from the two different storage series were (in qualitative terms) practically identical. Thus for the reason of economy, in this paper only the results originating from a single experimental series are presented.

There is an interesting observation valid for all the plots shown in Figures 2 and 3, namely in the oscillations of the respective numerical values of the R_f at both measuring temperatures. For the most part, these oscillations are clearly evident, yet considerably weaker (in terms of their amplitude) than those reported earlier in the literature (1), which referred to the samples stored as 70% solutions in ethanol. For example, with *S*-(+)-ibuprofen dissolved in dichloromethane and then stored for 5 days at $6^\circ\text{C} \pm 2^\circ\text{C}$, the maximum numerical value of ΔR_f (which is the measure of the amplitude discussed) was equal to 0.05 and remained slightly above the experimental error, inherent of TLC (which is generally assumed as equal to ± 0.02 , in the unitless scale of the R_f values). In the case of *S*-(+)-naproxen dissolved in dichloromethane and stored at $6^\circ\text{C} \pm 2^\circ\text{C}$, the observed maximum numerical value of ΔR_f was equal to 0.04, which means that the amplitude of the R_f values remained within the experimental error.

In the case of the *S,R*-(±)-2-phenylpropionic acid racemate dissolved in dichloromethane and stored at $6^\circ\text{C} \pm 2^\circ\text{C}$, separation of the antipodes proved virtually impossible (see Figure 4), although oscillatory migrations of the densitometrically recorded samples' concentration profiles were clearly perceptible.

However, the amplitude of the discussed oscillations is somewhat higher for the samples of the three profens stored at ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$) than that it is for those stored in the refrigerator (i.e., at $6^\circ\text{C} \pm 2^\circ\text{C}$). This relation is in opposition to what had been observed earlier for the 70% ethanol solutions of the same profens (1). With *S*-(+)-ibuprofen dissolved in dichloromethane and then stored for five days at $22^\circ\text{C} \pm 2^\circ\text{C}$, the maximum numerical value of ΔR_f was equal to 0.12, although *S*-(+)-naproxen examined in the same conditions was equal to only 0.05.

In Figures 5 and 6, two sequences of the concentration profiles as a function of storage time are shown, one for the dichloromethane solution of the pure enantiomer (Figure 5; *S*-(+)-ibuprofen) and another one for that of the racemic mixture (Figure 6; *S,R*-(±)-2-phenylpropionic acid), which were meant as sequences of the consecutive "movie pictures". These figures illustrate the analytes' behavior at $22^\circ\text{C} \pm 2^\circ\text{C}$. Changes of the concentration profiles are characteristic of the two profens, examined as pure enantiomers [i.e., of *S*-(+)-ibuprofen and *S*-(+)-naproxen], and they

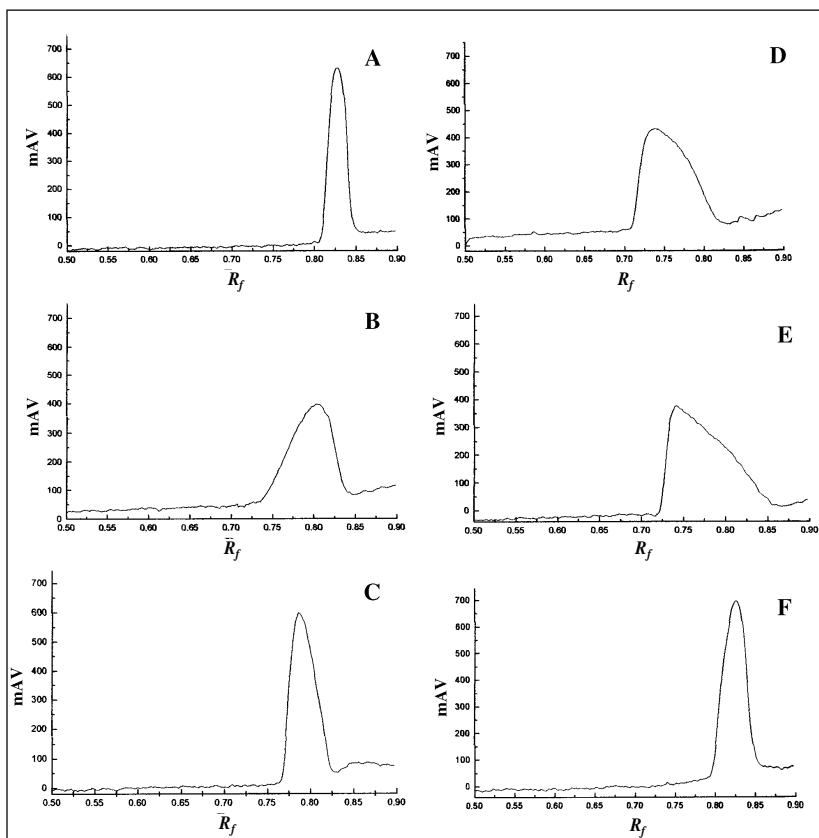


Figure 5. Sequence of the densitometric concentration profiles of *S*-ibuprofen after: 0 h (A), 5 h (B), 47 h (C), 52 h (D), 76.5 h (E), and 95.5 h (F) storage time at ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$). Changes of the concentration profiles are evidently accompanied by the changing R_f values..

correspond well with oscillations of the R_f values for the same two species. Envelopes of the examined concentration profiles visibly shift up and down the R_f scale of values, and (what is even more spectacular) they swell and then narrow in regular intervals, convincingly mirroring steric transformation of the analyte from one enantiomeric configuration to another.

From the “movie pictures” presented in Figure 6 and referring to S,R -(\pm)-2-phenylpropionic acid, it easily comes out that even in the case of the racemic mixture dissolved in the nonaqueous and nonpolar solvent (i.e., dichloromethane), the storage-caused oscillation is also evident. However, hardly any swelling replaced in regular intervals by the narrowing of the concentration profiles is observed (except for the single case of narrowing, presented in Figure 6D). The characteristic quasi-rectangular shape of the S,R -(\pm)-2-phenylpropionic acid profiles seems to clearly suggest the presence of a series of the mixed self-associates (perhaps of the cyclic dimers of the acid's optical antipodes with three different compositions of the SS , RR , and SR type), coupled together by the lateral hydrogen bonds.

The TLC results show the phenomenon of oscillatory transitions from the profen's one enantiomeric configuration to the opposite one, equally taking place with S -(+)-ibuprofen, S -(+)-naproxen, and S,R -(\pm)-2-phenylpropionic acid, dissolved in dichloromethane [although incomparably weaker than the strongly pronounced oscillations occurring with the three investigated profens in the aqueous medium (1)]. In order to experimentally confirm these findings, the polarimetric measurements

were also performed. Namely, the $[\alpha]_D$ values for each individual profen discussed in this study and stored at $6^\circ\text{C} \pm 2^\circ\text{C}$ and $22^\circ\text{C} \pm 2^\circ\text{C}$, respectively, in the dichloromethane solution were recorded. The results obtained are given in Figures 7 and 8.

As can be seen from the results shown in Figures 7 and 8, the measuring periods in the polarimetric studies were 5-h units (to make the point, each day the 5-h measurement was carried out over the course of the 5-day storage period with each examined profen sample). Evidently, the polarimetric measurements embraced considerably shorter time units than the 5-day sample storage periods covered by our TLC experiments. This is because polarimetric measurements are incomparably faster than the TLC ones, and, moreover, they can practically be carried out either in a continuous manner or alternatively in very short time intervals, whereas collecting a single numerical R_f value takes approximately 1 h. Nevertheless, from the plots presented in Figures 7 and 8, it is clearly demonstrated that the $[\alpha]_D$ of S -(+)-ibuprofen and S -(+)-naproxen changes in a very weak manner in the function of time (and no measurable change of $[\alpha]_D$ was detected in the case of the racemic S,R -(\pm)-2-phenylpropionic acid). Thus, these direct polarimetric results seem to provide sufficient evidence of a seriously hampered, yet still observable change of configuration with a given enantiomeric species, which takes place with the profens examined in our study.

It is noteworthy that the molar amounts of the three investigated profens needed to monitor the oscillatory transenantiomerization by means of TLC were as low as 5.8×10^{-8} mol (S -(+)-ibuprofen), 4.3×10^{-8} mol (S -(+)-naproxen), and 6.5×10^{-8} mol (S,R -(\pm)-2-phenylpropionic acid). Even without purposely measuring the lower detectability limits for these three analytes, it seems quite obvious that in this experiment, TLC proved a very sensitive physicochemical tool, well suited for tracing of configuration changes with the examined enantiomeric profens.

Now let us reflect on the molecular mechanism of the aforementioned and relatively weak [especially, when compared with 70% ethanol solutions (1)] transenantiomerization of profens. In the literature (4), a report is available about a proven (and base-catalyzed) ibuprofen racemization mechanism through the keto-enol tautomerism. From the general knowledge about the keto-enol tautomerism, it is known that this process can best be carried out in an aqueous medium, enabling electrolytic dissociation. However, it is fully justified to expect that a very weak electrolytic dissociation can occur even in a nonaqueous medium (e.g., in dichloromethane), as is the case with most electrolytes. Thus, one can expect that the possible keto-enol transenantiomerization of profens in a nonaqueous medium (e.g., in dichloromethane) is also because of the transfer of protons from one molecular moiety to another. The phenomenon of slightly higher oscillation amplitudes with the $R_f = f(t)$ curves (see Figures 2 and 3) at 22°C (as compared with those

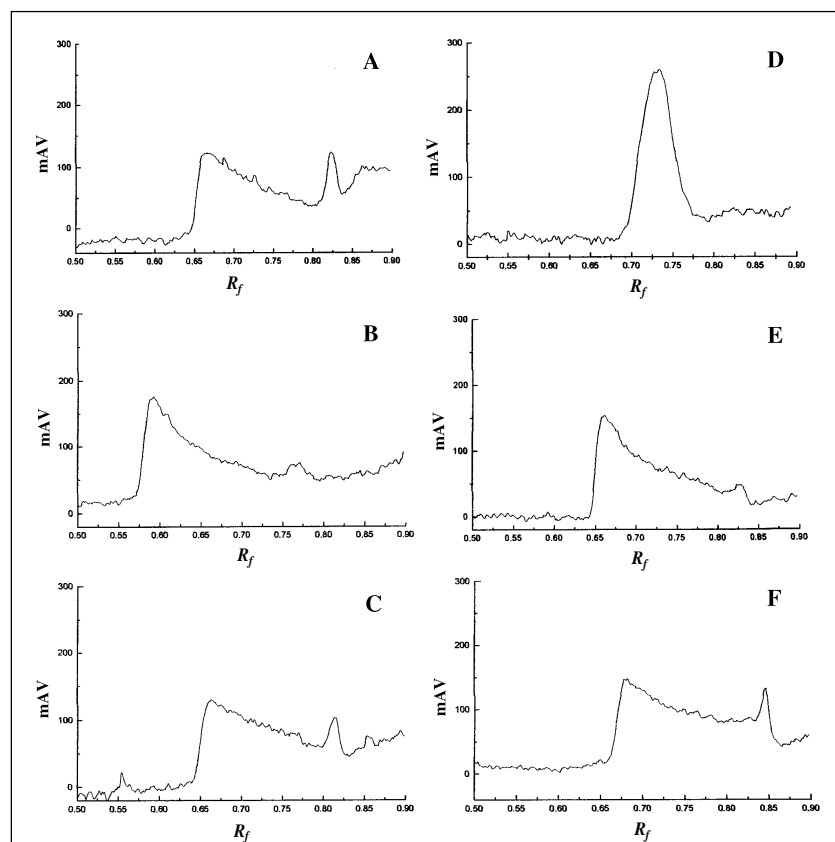


Figure 6. Sequence of the densitometric concentration profiles of S,R -(\pm)-2-phenylpropionic acid after: 0 h (A), 5 h (B), 24 h (C), 47.5 h (D), 72 h (E), and 95.5 h (F) storage time at ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$). Changes of the profiles' positions on the scale of the R_f values are evident.

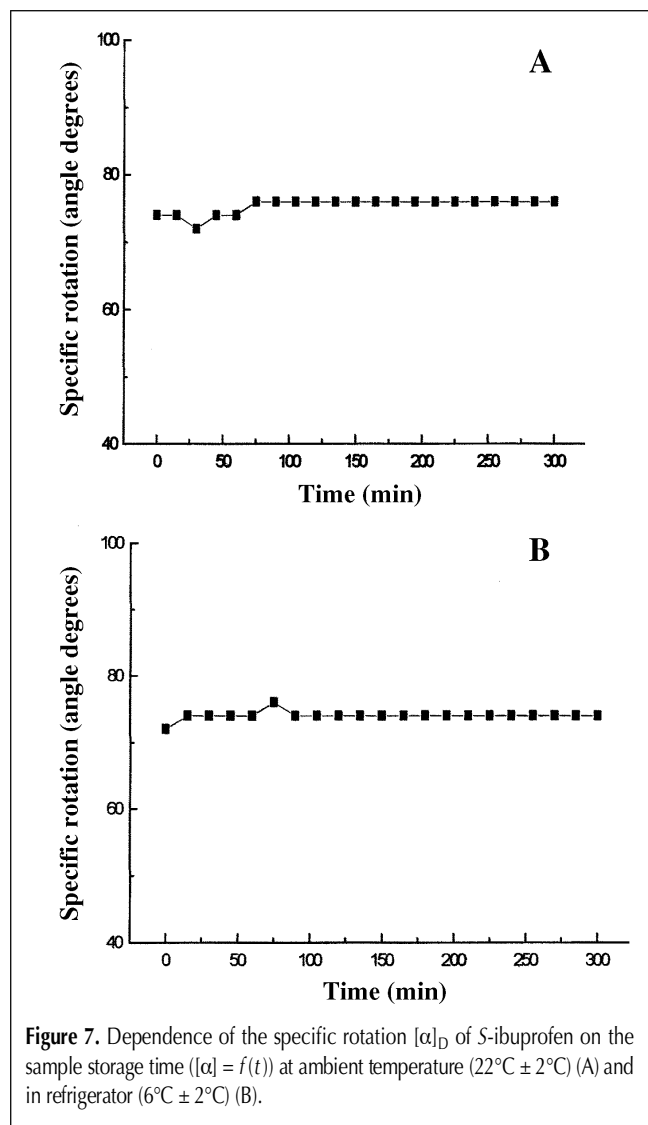


Figure 7. Dependence of the specific rotation $[\alpha]_D$ of *S*-ibuprofen on the sample storage time ($[\alpha] = f(t)$) at ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$) (A) and in refrigerator ($6^\circ\text{C} \pm 2^\circ\text{C}$) (B).

at 6°C) seems to originate from the fact that an elevated temperature (*i*) enhances splitting and transfer of the proton from one profen moiety to another and, consequently, (*ii*) contributes to acceleration of the keto-enol tautomerism. This assumption does not, however, explain the oscillatory nature of the phenomenon itself, which is going to be extensively discussed in one of the next papers in this series.

Conclusion

Strong oscillatory changes of the profens' enantiomeric configuration, observed in the aqueous medium (1), and weaker oscillatory changes, observed in the nonaqueous medium (and reported in this paper), shed a new light on pharmaceutical findings regarding an incomparably higher biological activity and hence a supposedly higher therapeutic potential of the pure enantiomeric *S* form (when compared with a respective *R* antipode) with the individual profens and reported in the numerous articles [e.g., in papers (10–14)]. It seems rather obvious to expect that the oscillatory changes of the profens' enantiomeric configuration are

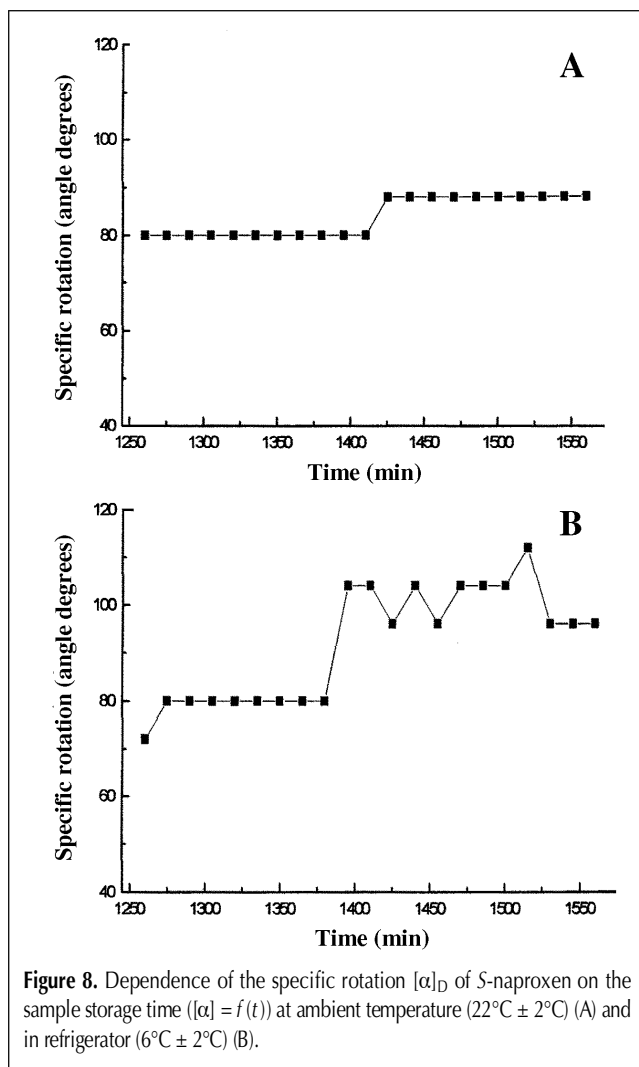


Figure 8. Dependence of the specific rotation $[\alpha]_D$ of *S*-naproxen on the sample storage time ($[\alpha] = f(t)$) at ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$) (A) and in refrigerator ($6^\circ\text{C} \pm 2^\circ\text{C}$) (B).

very likely to occur not only in laboratory conditions (i.e., *in vitro*) but also at the pharmacokinetic and the drug interaction stage in living organisms (i.e., *in vivo*). In view of our recent experimental findings, the scientific reports claiming a clearly predominant therapeutic potential of the *S*-profens simply become less convincing and, for this particular reason, certainly need to be carefully re-examined and reconsidered.

Acknowledgments

The authors wish to thank Merck KGaA for supplying the TLC plates and the sample of *S,R*-(±)-2-phenylpropionic acid used in this experiment.

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Manuscript received March 8, 2005;