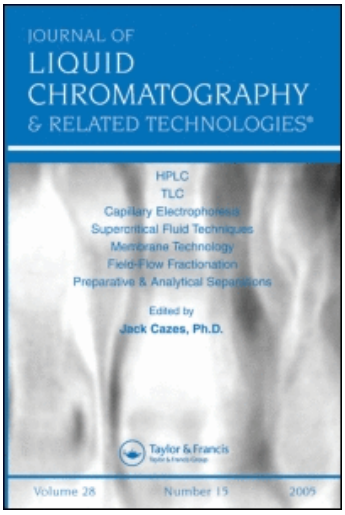


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Application of Thin-Layer Chromatography to the Investigation of Oscillatory Instability of Selected Profen Enantiomers in Physiological Salt

Mieczysław Sajewicz[a]; Robert Piętka[a]; Agnieszka Pieniak[a]; Teresa Kowalska[a]
[a] Institute of Chemistry, Silesian University, Katowice, Poland

To cite this Article Sajewicz, Mieczysław , Piętka, Robert , Pieniak, Agnieszka and Kowalska, Teresa(2006) 'Application of Thin-Layer Chromatography to the Investigation of Oscillatory Instability of Selected Profen Enantiomers in Physiological Salt', Journal of Liquid Chromatography & Related Technologies, 29: 14, 2059 — 2069
To link to this Article: DOI: 10.1080/10826070600759710
URL: http://dx.doi.org/10.1080/10826070600759710

Journal of Liquid Chromatography & Related Technologies®, 29: 2059–2069, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600759710

Application of Thin-Layer Chromatography to the Investigation of Oscillatory Instability of Selected Profen Enantiomers in Physiological Salt

Mieczysław Sajewicz, Robert Piętka, Agnieszka Pieniak, and Teresa Kowalska
Institute of Chemistry, Silesian University, Katowice, Poland

Abstract: In earlier investigations, we evaluated the performance of thin-layer chromatography as an analytical tool to study oscillatory instability of selected profens dissolved in 70% ethanol and dichloromethane. In this study, the solvent used to dissolve profens is changed to physiological salt. The purpose was to assess if the selected test profens [i.e., *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *R,S*-(±)-2-phenylpropionic acid] can undergo oscillatory transenantiomerization if dissolved in physiological salt. It is a commonly known fact that physiological salt is often drip-infused into the human (or animal) body with the addition of a drug. Therefore, the question arises whether profen drugs dispensed as physiological salt solutions can undergo oscillatory transenantiomerization in this particular medium. The results of our study confirm that such a transformation is in fact possible.

Keywords: Profens, TLC, Chirality, Oscillatory transenantiomerization

INTRODUCTION

Thin-layer chromatography (TLC) conditions that are the best suited for separation of profen enantiomers involve silica gel impregnated with L-arginine, which is kept in the cationic form at an appropriately fixed pH value (<4.8). The mechanism of such separations can best be summarized with aid of the stoichiometric equations given below,[1,2] which reflect

Address correspondence to Teresa Kowalska, Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland. E-mail: kowalska@us.edu.pl

ion-pair formation between the cationic impregnant and the profen enantiomers in the anionic form:

$$\text{L-arginine}^{+} + S\text{-(+)-profen}^{-} \longleftrightarrow \text{L-arginine}^{+}S\text{-(+)-profen}^{-};\ (K_1)$$
$$\text{L-arginine}^{+} + R\text{-(−)-profen}^{-} \longleftrightarrow \text{L-arginine}^{+}R\text{-(−)-profen}^{-};\ (K_2)$$

In our previous publications,[3,4] we reflected on the possible molecular mechanisms of the observed continuous oscillatory transenantiomerization of profens, and a conclusion was drawn, supported by the convincing data taken from the literature,[5] that the most probable mechanism involved keto-enol tautomerism as an important intermediate step, self-catalyzed by the proton originating from the electrolytic dissociation of a given acidic species (all the profens are the aryl-substituted propionic acids) in the alcohol-aqueous medium. It is particularly noteworthy that we managed to demonstrate oscillatory transenantiomerization of profens, not only by means of polarimetry (which is the routine measuring technique in chiral studies), but also with aid of TLC (which was a pioneering replacement of polarimetry by this particular separation technique). Schematic presentation of the assumed molecular mechanism of trans-enantiomerization is shown in Fig. 1.

In the first paper from this series,[3] we employed 70% ethanol as a solvent and, in that medium, the selected test profens were oscillating. In the second paper,[4] we purposely used dichloromethane as a nonaqueous solvent and oscillations were also noted, although somewhat less strongly pronounced than in 70% ethanol. Now we attempt to answer the question of whether

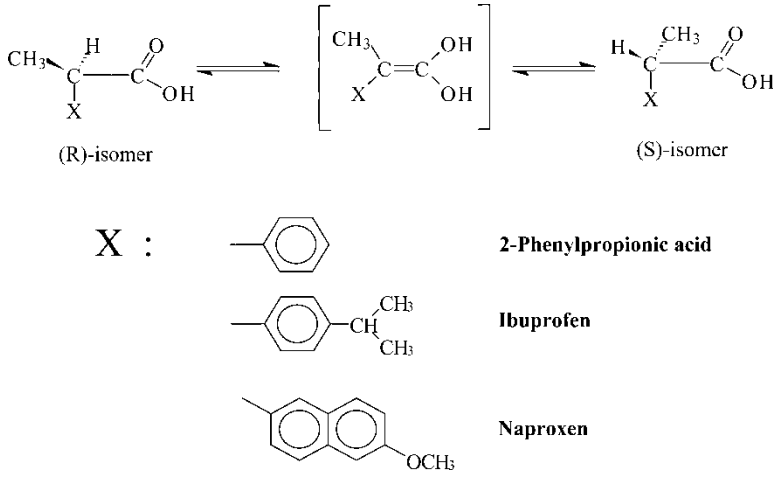


Figure 1. Schematic representation of transenantiomerization of profens by keto-enol tautomerism.

our test profens can oscillate if dissolved in physiological salt. This medium plays a key role in our studies, due to the fact that it is often used as a liquid in which various drugs are first dissolved and then dispensed as a drip infusion. Oscillatory transenantiomerization of a chiral species changes its steric arrangement to that of its chiral antipode, which can affect the efficiency of the medical treatment.

In the present part of our segmented study, we did not employ the parallel polarimetric confirmation of transenantiomerization, due to the low solubility of profens in physiological salt, which is far below the measuring sensitivity of our polarimetric equipment. Thus, the oscillatory transenantiomerization of the test profens was, for the first time, monitored by means of TLC alone.

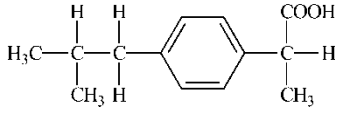
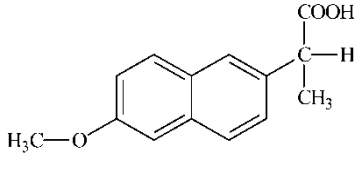
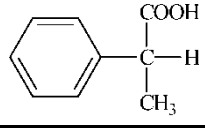
EXPERIMENTAL

Profens

Our experiments were performed with the following profens (their chemical structures are shown in Table 1):

- *S*-(+)-Ibuprofen (Sigma–Aldrich, St Louis, MO; # I-106). Solutions of *S*-(+)-ibuprofen were prepared at a concentration of 0.25 $\mu g\,\mu L^{-1}$ (ca. $1.4 \times 10^{-3}\,mol\,L^{-1}$) in physiological salt, and 10 μL volumes were applied to chromatographic plates with a micropipet.

Table 1. Schematic representation of the chemical structures of the three profens used in this study

Profen	Chemical structure
Ibuprofen	
Naproxen	
2-Phenylpropionic acid	

- *S*-(+)-Naproxen (Sigma–Aldrich; # 28,478-5). Solutions of *S*-(+)-naproxen were prepared at a concentration of 0.25 $\mu g\,\mu L^{-1}$ (ca. $1.1 \times 10^{-3}\,mol\,L^{-1}$) in physiological salt, and 10 μL volumes were applied to chromatographic plates.
- *S,R*-($\pm$)-2-Phenylpropionic acid (Merck KGaA, Darmstadt, Germany; 8.20651.0010). Solutions of *S,R*-($\pm$)-2-phenylpropionic acid were prepared at a concentration of 0.5 $\mu g\,\mu L^{-1}$ (ca. $3.2 \times 10^{-3}\,mol\,L^{-1}$) in physiological salt, and 10 μL volumes were applied to chromatographic plates.

Physiological Salt

Physiological salt (solution for infusion, Natrii chloridum 9 $mg\,mL^{-1}$) was manufactured by the firm Fresenius Kabi (Kutno, Poland).

Storage of the Test Analytes

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

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 $3 \times 10^{-2}\,mol\,L^{-1}$ 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

S-(+)-Ibuprofen

Development of the ibuprofen samples was performed at two different temperatures, $6 \pm 2^{\circ}C$ and $22 \pm 2^{\circ}C$. Plates with three adjacent spots from the

10 μL volumes of *S*-(+)-ibuprofen solution were developed to a distance of 15 cm using the ternary mobile phase acetonitrile (ACN)-methanol (MeOH)-H_2O (5 : 1 : 1, *v*/*v*) containing several drops of 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 for each measurement temperature.

S-(+)-Naproxen

Development of the naproxen samples was performed as described for ibuprofen, except that the mobile phase was ACN-MeOH-H_2O (5 : 1 : 1.5, *v*/*v*), again containing several drops of acetic acid to fix the pH at <4.8.

S,*R*-(±)-2-Phenylpropionic Acid

Development of the 2-phenylpropionic acid samples was performed as described for ibuprofen and naproxen, except that the mobile phase was ACN-MeOH-H_2O (5 : 1 : 0.75, *v*/*v*) containing several drops of acetic acid to fix the pH at <4.8.

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 the three profens were recorded in UV light from the deuterium lamp (in the reflectance mode) at 210 nm. (This is the approximate wavelength of the stronger of the UV absorption maximum for ibuprofen; the stronger maximum for naproxen, and 2-phenylpropionic acid are close to this position). The dimensions of the rectangular light beam were 0.02 mm × 0.4 mm. The maxima of the concentration profiles were used for calculation of R_F values.

RESULTS AND DISCUSSION

The results presented in this paper clearly confirm an occurence of oscillations of the investigated profens when stored for a longer period of time as solutions in physiological salt. As has already been mentioned, in the present case, we could not apply polarimetry as a parallel measuring technique to assess transenantiomerization of profens, due to their low solubility in physiological salt. However, TLC performs excellently and allows tracing oscillatory transenantiomerization of profens in its own idependent way. This ablity of TLC had already been demonstrated in the preceding papers.[3,4]

As already mentioned, each experiment was repeated three times in each series, and two such series were run for each profen and each working temperature. The results originating from the same storage series were averaged; data originating from two different storage series were qualitatively almost identical. Thus, for reason of economy in this paper, we present the results from a single experimental series only.

There is an interesting observation which is valid for all of the plots shown in Figs. 2 and 3, namely that evident oscillations can be seen of R_F values at both measuring temperatures. For *S*-(+)-ibuprofen stored in physiological salt and chromatographed at ambient temperature ($22 \pm 2^{\circ}C$), the

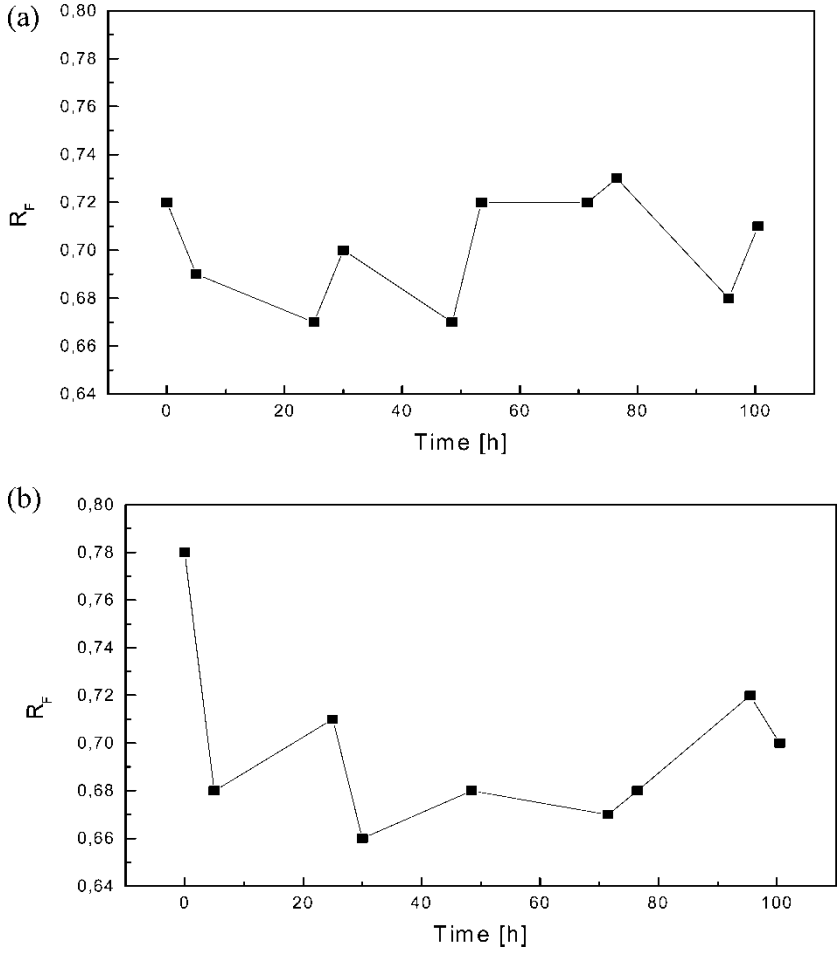


Figure 2. Dependence of retention, R_F, for *S*-(+)-ibuprofen on sample storage time [$R_F = f(t)$] (a) at ambient temperature ($22 \pm 2^{\circ}C$) and (b) under refrigeration ($6 \pm 2^{\circ}C$).

amplitude of R_F oscillations was equal to 0.06 R_F units. For the sake of comparison, the same compound stored in dichloromethane had an amplitude of 0.12 R_F units,[4] and in 70% ethanol the amplitude was 0.11 R_F units.[3]

For *S*-(+)-naproxen stored in physiological salt and developed at ambient temperature, the amplitude of R_F oscillations was equal to 0.04 R_F units. The same compound stored in dichloromethane and in 70% ethanol had the amplitudes of R_F oscillations equal to 0.05[4] and 0.07 R_F units,[3] respectively.

For *R,S*-(±)-phenylpropionic acid (which was a racemic mixture), it was virtually impossible to determine R_F values, either when stored at ambient temperature or under refrigeration (6 ± 2°C). The concentration profiles

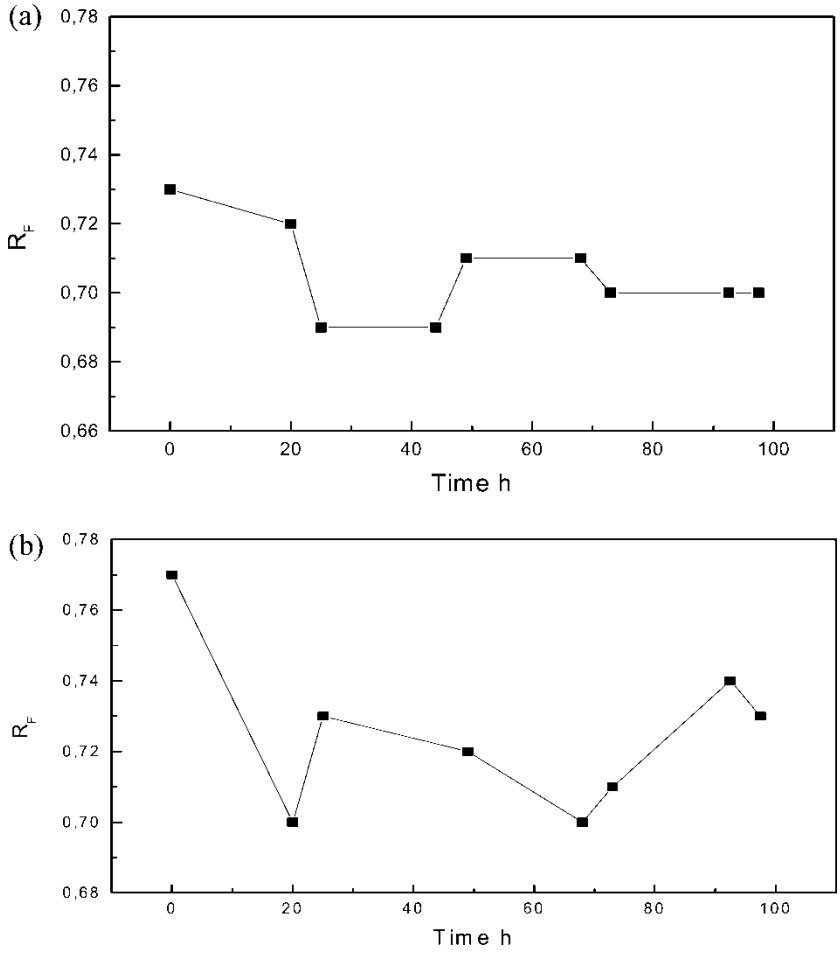


Figure 3. Dependence of retention, R_F, for *S*-(+)-naproxen on sample storage time [$R_F = f(t)$] (a) at ambient temperature (22 ± 2°C) and (b) under refrigeration (6 ± 2°C).

obtained for this profen were too broad and too skewed to allow reliable measurement of R_F values (see Fig. 5).

For *S*-(+)-ibuprofen stored in physiological salt and chromatographed at $6 \pm 2^{\circ}$C, the observed amplitude of R_F oscillations was equal to 0.12 R_F units. The same compound, when dissolved in dichloromethane had an amplitude of 0.05 R_F units,[4] and, when dissolved in 70% ethanol, the value of amplitude was 0.18 R_F units.[3]

For *S*-(+)-naproxen stored in physiological salt and developed at $6 \pm 2^{\circ}$C, the amplitude was equal to 0.07 R_F units. The same compound dissolved in dichloromethane and in 70% ethanol showed the amplitudes of R_F oscillations equal to 0.04[4] and to 0.06 R_F units,[3] respectively.

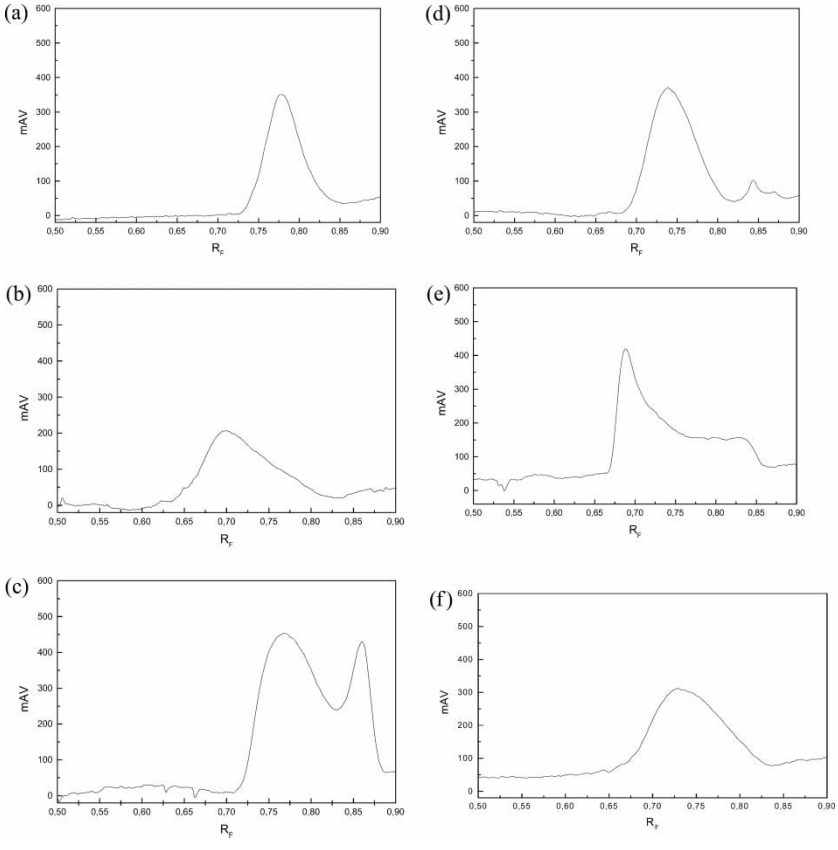


Figure 4. Sequence of densitometric concentration profiles for *S*-(+)-naproxen after (a) 0 h, (b) 20 h, (c) 44 h, (d) 68 h, (e) 73 h, and (f) 97.5 h during storage at ambient temperature ($22 \pm 2^{\circ}$C). Changes of peak concentration profiles were accompanied by changing R_F values.

Our measurements had an experimental error of ± 0.02 R_F units. Thus, the observed amplitudes of R_F oscillations were clearly above the experimental error and prove oscillatory transenantiomerization of the test profens dissolved in physiological salt and stored at $22 \pm 2^{\circ}$C and $6 \pm 2^{\circ}$C.

In Figs. 4 and 5, we show two sequences of the changing concentration profiles as a function of storage time, one for the solution of *S*-(+)-naproxen in physiological salt (Fig. 4) and the other for *S,R*-($\pm$)-2-phenylpropionic acid (Fig. 5). These figures are meant as two sequences of consecutive "movie pictures," illustrating the analytes' transenantiomerization at $22 \pm 2^{\circ}$C. Changes of the concentration profiles shown in Fig. 4 are characteristic of the two profens, examined as pure enantiomers [*S*-(+)-ibuprofen and *S*-(+)-naproxen], and they correspond well with the respective

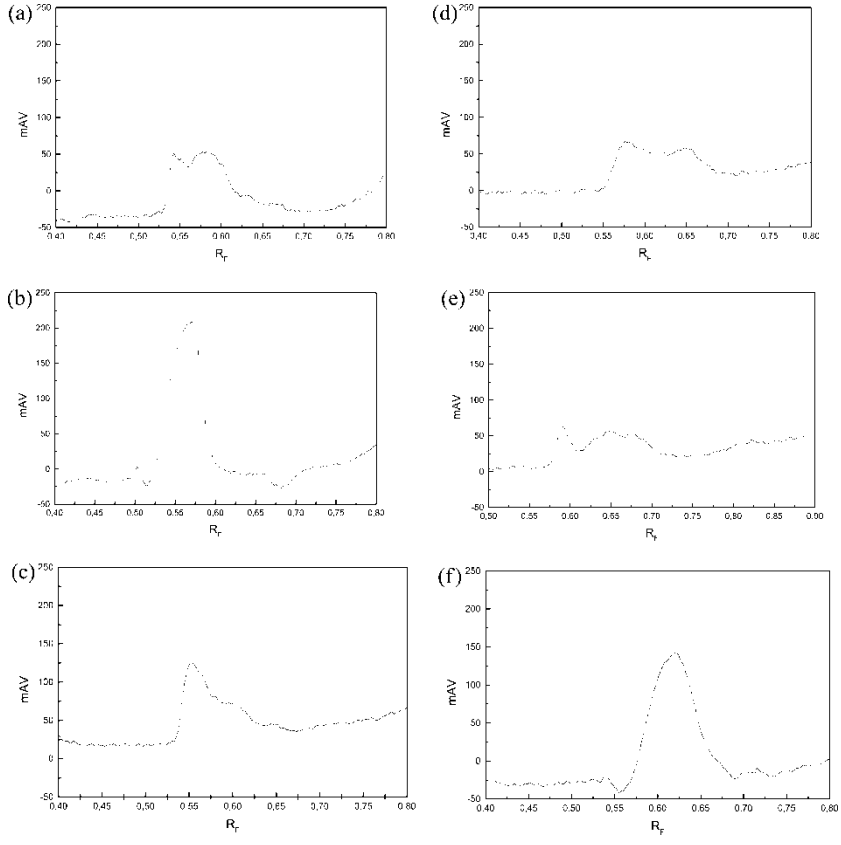


Figure 5. Sequence of densitometric concentration profiles for *S,R*-($\pm$)-2-phenylpropionic acid after (a) 25 h, (b) 43.5 h, (c) 72 h, (d) 91 h, (e) 96 h, and (f) 164.5 h during storage at ambient temperature ($22 \pm 2^{\circ}$C). Changes of peak concentration profiles were accompanied by changing R_F values.

oscillations of the R_F values. Envelopes of the examined concentration profiles shift from one extreme position to the other and they swell and then narrow in rather regular intervals, convincingly mirroring steric transformation of the analytes from one enantiomeric configuration to another.

CONCLUSIONS

The results presented in this paper confirm the ability of profens to undergo oscillatory transenantiomerization when dissolved in physiological salt. Moreover, from the results of these and previous studies, it can be deduced that profens stored both in the aqueous and the nonaqueous media oscillate; the only difference is the frequency and the amplitude of these oscillations, which depends on the solvent and the storage temperature applied.

It seems justified to assume that oscillations of profens occur not only in laboratory conditions (*in vitro*), but also at the pharmacodynamic stage in the living organisms (*in vivo*). In view of our recent experimental results, the scientific reports claiming clearly predominant curing potential of the profen *S* enantiomers become less convincing and, for this reason, certainly need to be carefully reexamined and reconsidered.[6–10]

In present study, TLC once again proved to be a versatile experimental tool, well suited for tracing transenantiomerization of the profens studied.

ACKNOWLEDGMENT

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

REFERENCES

1. Bhushan, R.; Parshad, V. Resolution of (±) ibuprofen using L-arginine-impregnated thin layer chromatography. J. Chromatogr. A **1996**, *721*, 369–372.
2. Sajewicz, M.; Piętka, R.; Kowalska, T. Chiral separation of *S*-(+)- and *R*-(−)-ibuprofen by thin-layer chromatography. An improved analytical procedure. J. Planar Chromatogr.-Mod. TLC **2004**, *17*, 173–176.
3. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography (TLC) to investigating oscillatory instability of the selected profen enantiomers. Acta Chromatogr. **2005**, *15*, 131–149.
4. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography (TLC) to investigating oscillatory instability of the selected profen enantiomers in dichloromethane. J. Chromatogr. Sci. **2005**, *43*, 542–548.
5. Xie, Y.; Liu, H.; Chen, J. Kinetics of base catalyzed racemization of ibuprofen enantiomers. Int. J. Pharm. **2000**, *196*, 21–26.

6. Yamamoto, K.; Ueno, Y.; Otsubo, K.; Kawakami, K.; Komatsu, K. Production of *S*-(+)-ibuprofen from a nitrile compound by *Acinetobacter sp*. strain AK226. Appl. Environ. Microbiol. **1990**, *56*, 3125–3129.
7. Neupert, W.; Brugger, R.; Euchenhofer, C.; Brune, K.; Geisslinger, G. Effects of ibuprofen enantiomers and its coenzyme A thioesters on human prostaglandin endoperoxide synthases. Brit. J. Pharmacol. **1997**, *122*, 487–492.
8. Landoni, M.F.; Soraci, S. Pharmacology of chiral compounds: 2-Arylpropionic acid derivatives. Curr. Drug Metab. **2001**, *2*, 37–51.
9. Davies, N.M.; Wright, M.R.; A.S.Russell, A.S.; Jamali, F. Effect of the enantiomers of flurbiprofen, ibuprofen, and ketoprofen on intestinal permeability. J. Pharm. Sci. **1996**, *85*, 1170–1173.
10. Janjikhel, R.K.; Bricker, J.D.; Borochovitz, D.; Adeyeye, C.M. Stereoselective disposition of sustained release microspheres of ibuprofen enantiomers in rats: II. Acute gastrointestinal toxicity. Drug Deliv. **1999**, *6*, 163–170.

Received July 6, 2005
Accepted July 6, 2005
Manuscript 6864B