STUDY OF THE OSCILLATORY IN-VITRO TRANSENANTIOMERIZATION OF THE ANTIMERS OF FLURBIPROFEN AND THEIR ENANTIOSEPARATION BY THIN-LAYER CHROMATOGRAPHY (TLC)

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SUMMARY

In our earlier studies on the spontaneous in-vitro oscillatory transenantiomerization of profens we investigated optically pure S-(+)-ibuprofen and S-(+)-naproxen and the racemic mixtures S,R-(±)-2-phenylpropionic acid and S,R-(±)-ketoprofen, which remained in a state of dynamic equilibrium between the two antimers yet also had the ability to transenantiomerize. In this study we have demonstrated, for the first time, the spontaneous oscillatory in-vitro transenantiomerization of S-(+)-flurbiprofen (an important non-steroidal anti-inflammatory drug, NSAID) and R-(−)-flurbiprofen, as monitored by polarimetry.

It is also noteworthy that – as far as we are aware – this is the first report of separation of the enantiomers of flurbiprofen by TLC. This separation was achieved by two-dimensional development using a simple chromatographic system comprising a commercial silica gel layer impregnated with L-arginine as stationary phase and ethanol containing a few drops of glacial acetic acid as mobile phase. Unfortunately, this chromatographic system resulted in catalysis of structural conversion of the optically pure flurbiprofen enantiomer, either S-(+), or R-(−), to the scalemic or racemic mixture of the two antimers. This is an interesting contribution to general knowledge about the reactivity of this particular profen, although the spontaneous and rapid conversion observed prevents use of this TLC system for identification and quantification of individual flurbiprofen enantiomers.

INTRODUCTION

We have previously [1–4] presented results from studies of the spontaneous oscillatory in-vitro transenantiomerization of the profens *S*-(+)-ibuprofen, *S*-(+)-naproxen, *S*,*R*-(±)-2-phenylpropionic acid, and *S*,*R*-(±)-ketoprofen when dissolved in low-molecular-weight solvents (e.g. 70% ethanol, dichloromethane, and physiological saline solution). The most probable conversion mechanism, with the keto–enol tautomer as intermediate, is shown in eq. (1):

$$S\text{-(+)-profen} \leftrightarrow \text{keto-enol tautomer} \leftrightarrow R\text{-(−)-profen} \qquad (1)$$

From our earlier considerations it was apparent that oscillatory transenantiomerization of the optically pure enantiomers of profen can be illustrated by the scheme given in Fig. 1, which shows the oscillatory decrease of the concentration with the starting *S*-(+) enantiomer and the simultaneous oscillatory increase in the concentration of its *R*-(−) antimer, the transenantiomerization product.
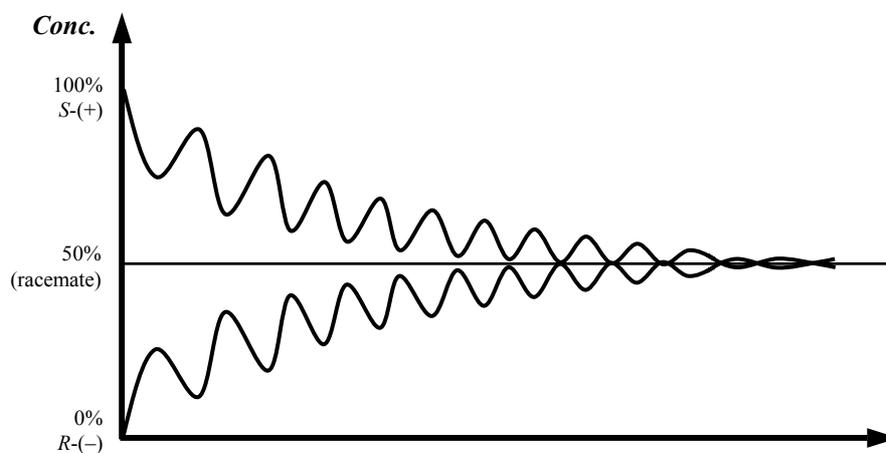
Fig. 1

Schematic representation of the oscillatory transenantiomerization of an *S*-(+) profen to its *R*-(−) antimer. The oscillatory plots mirror the oscillatory decrease of the concentration of the *S*-(+) species and the corresponding oscillatory increase of the concentration of the *R*-(−) species

We previously [5] offered an explanation of the molecular mechanism responsible for the oscillatory processes. There are two necessary preconditions for such oscillation. One is that the oscillatory processes con-

sist of more than one elementary step (at least one of which is, preferably, of a higher reaction order), so they must be chain reactions. The other necessary precondition is that the solutions in which oscillatory processes occur are anisotropic. For profens and their transenantiomerization both necessary preconditions seem to be fulfilled. From eq. (1) it is clearly apparent that transenantiomerization of the profens is not the one-step process. It was also shown [5] that the molecules of profens have the ability to self-organize within a solution (either by gelation or by formation of liquid crystal-like structures), which results in anisotropy of the solution. Thus the oscillatory mechanism of transenantiomerization in the multi-step process in the anisotropic solution ought to be kinetic–diffusive in nature.

The separate objectives of this study were to investigate the ability of the two flurbiprofen antimers to undergo oscillatory structural conversion and to elaborate working conditions for separation of the enantiomers of flurbiprofen by TLC.

EXPERIMENTAL

S-(+)-Flurbiprofen and *R*-(−)-Flurbiprofen

The structure of the flurbiprofen molecule is shown schematically in Fig. 2. It is noteworthy that − unlike ibuprofen, naproxen, ketoprofen,
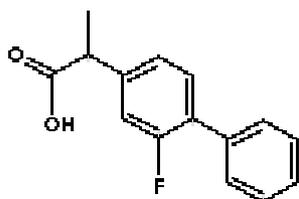
Fig. 2

Schematic representation of the molecular structure of flurbiprofen

or 2-phenylpropionic acid − flurbiprofen contains one strongly electronegative fluorine atom in its structure. In our study we used *S*-(+)-flurbiprofen and *R*-(−)-flurbiprofen manufactured by Sigma–Aldrich (St Louis, MO, USA; cat. # 482641-500MG and 545740-1G, respectively). In thin-layer chromatography we used solutions of each enantiomer in 70% ethanol at concentrations of 0.1, 0.5, and 1.0 mg mL^{-1} (i.e. ca 0.41×10^{-3}, 2.05×10^{-3}, and 4.10×10^{-3} mol L^{-1}). We also used a racemic mixture of flurbiprofen dis-

solved in 70% ethanol, containing 0.2 mg mL^{-1} (i.e. ca 0.82×10^{-3} mol L^{-1}) of each enantiomer. In polarimetry we used solutions of each enantiomer in 70% ethanol at a concentration 50 mg mL^{-1} (i.e. ca 2.05×10^{-1} mol L^{-1}).

Polarimetric Measurement of Specific Rotation, $[\alpha]_D$

Measurement of the specific rotation ($[\alpha]_D$) of solutions of S-(+) and R-(−)-flurbiprofen in 70% ethanol were performed at ambient temperature for eight days (the S-(+) species) or nine days (the R-(−) species), seven hours per day (at 10-min intervals), by use of a Polamat A polarimeter manufactured by Carl Zeiss (Jena, Germany). The optical path length of the measurement cell was 10 cm, and its volume was ca 1 mL. Specific rotation, $[\alpha]_D$, was calculated by use of the standard equation:

$$[\alpha]_D = 100\alpha/cd \tag{2}$$

where α is the measured rotation (in angle degrees), D is the wavelength used, $\lambda = 589$ nm, which corresponds to the sodium D line, c is the concentration of a given compound in g (100 mL)$^{-1}$ solution, and d is the measured sample thickness in dm.

From the literature [6] it is known that the specific rotation of S-(+) and R-(−)-flurbiprofen is ca +43° and –43°, respectively.

Commercial TLC Silica Gel Layers and Their Pretreatment

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

The washed and dried plates were then impregnated by conventional dipping for 2 s in a solution (3×10^{-2} mol L^{-1}) of L-arginine in methanol. The concentration of the impregnating solution was calculated as that depositing 0.5 g L-arginine per 50 g dry silica gel. The impregnated adsorbent layers were ready for chromatography.

Mobile Phase and Development of Thin-Layer Chromatograms

Solutions of S-(+) or R-(−)-flurbiprofen (3-μL) or their racemic mixture (15 μL), in 70% ethanol, were applied to the lower left-hand corner of the plates, 1.5 cm from the edges, by use of a Desaga (Heidelberg, Germany) AS 30 autosampler. Two-dimensional development to a distance of 15 cm, in directions perpendicular to each other, was performed at 22 ± 2°C.

In both directions ethanol with addition of a few drops of glacial acetic acid was used as mobile phase. Between developments plates were dried at ambient temperature for 3 h. Each chromatographic experiment was repeated three times.

Scanning Densitometry and Flatbed Videodensitometry

After the second development the plates were again dried at ambient temperature. Tracks 30 mm wide, in the second direction of development, were then scanned densitometrically, at 1-mm intervals, with a Desaga CD 60 densitometer equipped with Windows-compatible ProQuant software. Concentration profiles of the development lanes of S-(+) and R-(−)-flurbiprofen were recorded in ultraviolet (UV) light from the deuterium lamp (in reflectance mode) at 245 nm. The dimensions of the rectangular light beam were 2.0 mm × 0.1 mm.

The chromatograms were also scanned at 254 nm and the Chromimage flatbed scanner (AR2i, Le Plessis Robinson, France) was used to record pictures taken in the UV light.

RESULTS AND DISCUSSION

Polarimetry

Polarimetric measurement of the specific rotation ($[\alpha]_D$) of solutions of S-(+) and R-(−)-flurbiprofen in 70% ethanol was performed for eight and nine days, respectively, seven hours per day (except at weekends, when no measurements were made for two consecutive days). The results obtained are shown in Figs 3 and 4, respectively.

It is apparent from Figs 3 and 4 that the general trend for both S-(+) and R-(−)-flurbiprofen was of the specific rotation drifting toward zero, which means that with the S-(+) species it was decreasing and with the R-(−) species it was increasing. It can be concluded that each flurbiprofen antimer tends, on dissolution in 70% ethanol, to racemize and that the oscillatory nature of this tendency is well depicted by the plots shown in Fig. 1.

To confirm directly that prolonged storage of flurbiprofen solutions results in structural conversion, a thin-layer chromatographic procedure for separation and quantification of the two enantiomers of flurbiprofen had to be established. The results obtained are presented in the next section.
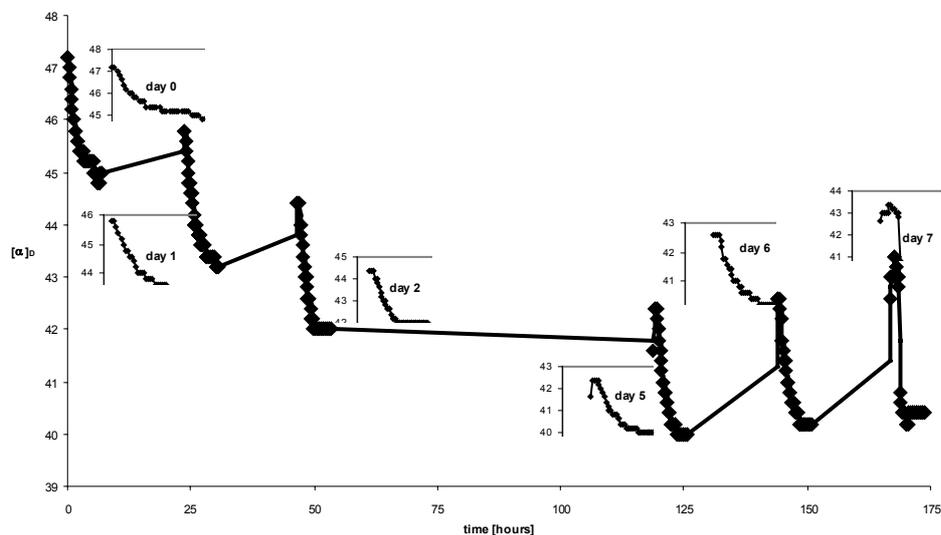
Fig. 3

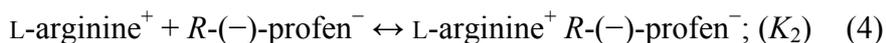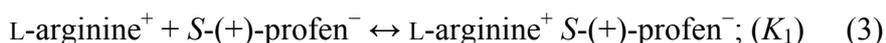The oscillatory change of the specific rotation, $[\alpha]_D$, of *S*-(+)-flurbiprofen as a function of time
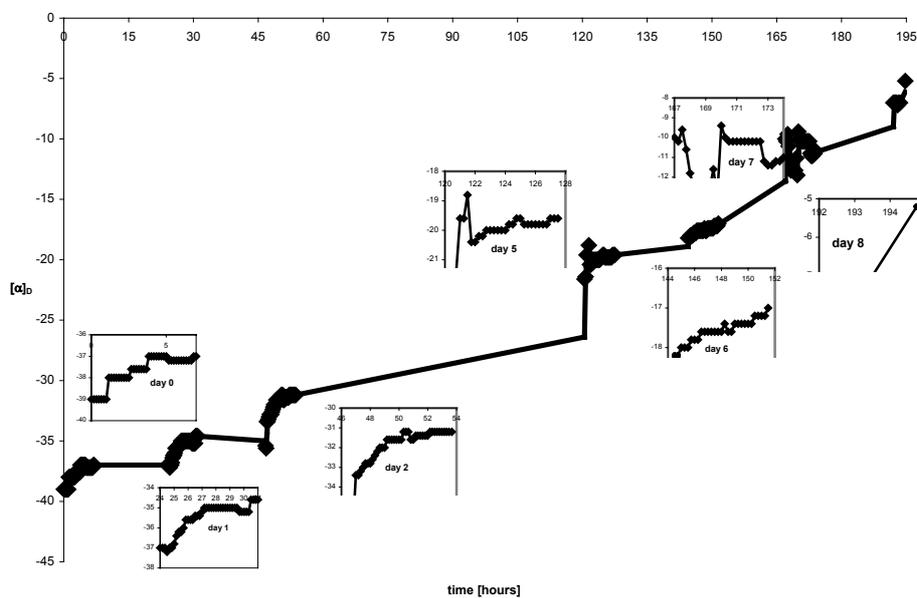
Fig. 4

The oscillatory change of the specific rotation, $[\alpha]_D$, of *R*-(−)-flurbiprofen as a function of time
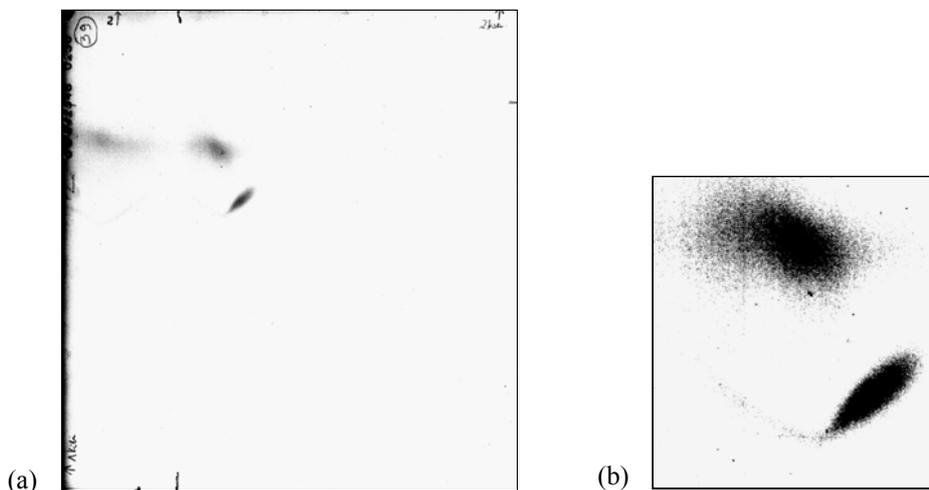
Thin-Layer Chromatographic Separation of the Enantiomers of Flurbiprofen

First, it must be mentioned that as far as we are aware there is no literature report of successful separation of the enantiomers of flurbiprofen by TLC. It thus seemed an urgent task to establish satisfactory conditions for separation of these enantiomers. We decided to take advantage of the non-commercial chiral stationary phase proposed by Bhushan and Parshad [7] for separation of the enantiomers of ibuprofen – commercial silica gel impregnated with L-arginine. As mobile phase we chose pure ethanol containing a few drops of glacial acetic acid, to protonate the amino group of L-arginine. Thus separation of the enantiomers was achieved by the mechanism of ion-pair formation, as expressed by the equations:

$$\text{L-arginine}^+ + S\text{-}(+)\text{-profen}^- \leftrightarrow \text{L-arginine}^+ \, S\text{-}(+)\text{-profen}^-; (K_1) \quad (3)$$

$$\text{L-arginine}^+ + R\text{-}(-)\text{-profen}^- \leftrightarrow \text{L-arginine}^+ \, R\text{-}(-)\text{-profen}^-; (K_2) \quad (4)$$

where $K_1 \neq K_2$.

To achieve complete separation of the two flurbiprofen antimers, two-dimensional chromatography was used, as described in the Experimental section. Separation of the enantiomers of the test racemate comprising S-(+) and R-(−)-flurbiprofen standards is shown in Figs 5 and 6.

(a)　　　　　　　　　　　　　　　(b)

Fig. 5

Video densitograms of (a) the whole plate developed in the two-dimensional mode, and (b) the skewed chromatographic spots of the two separated flurbiprofen antimers. The chromatogram shows separation of a racemic mixture of S-(+) and R-(−)-flurbiprofen
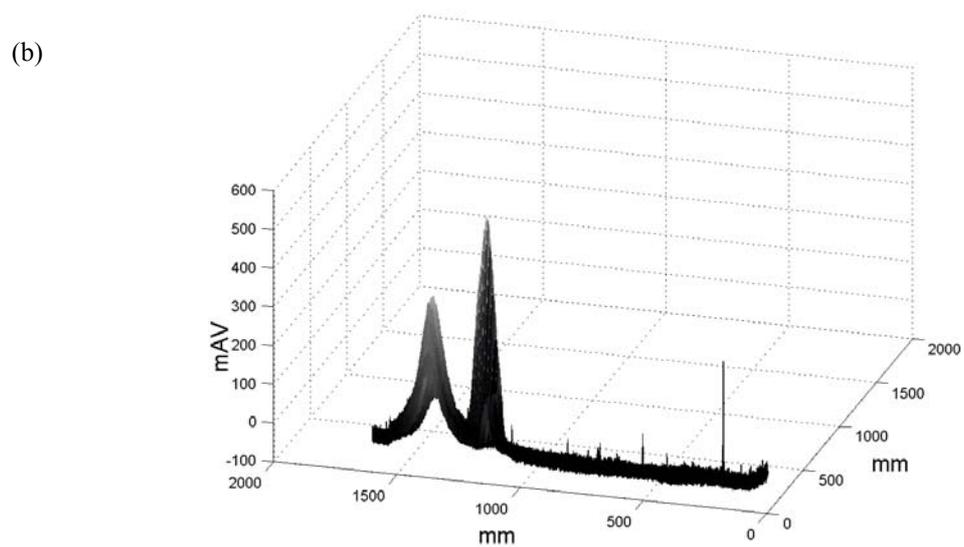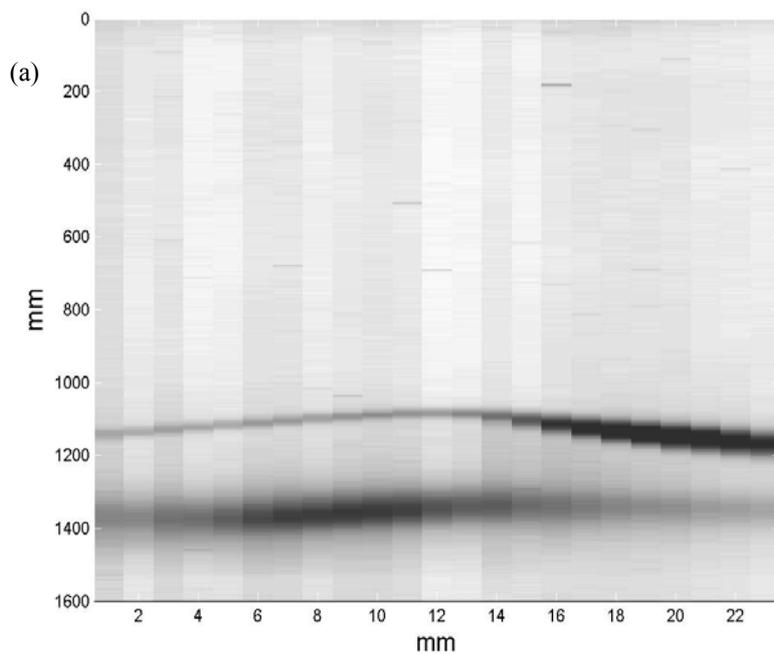
Fig. 6

Two-dimensional (a) and three-dimensional (b) representation of the chromatographic spots of the two separated flurbiprofen antimers from Fig. 5, reconstructed from scanning densitograms of a track 30 mm wide taken at 1-mm intervals

This separation was confirmed in two different and independent ways.

 i. The two separated bands of the *S*-(+) and the *R*-(−) species were identified directly on the chromatogram, by acquiring their respective UV spectra in situ. Identical UV spectra were obtained from both bands.

 ii. We also scraped the separated bands from the chromatogram, extracted the compounds of interest from the silica gel, with ethanol, and analyzed each extract separately by non-chiral HPLC with diode-array detection (DAD). From the two extracts we obtained chromatographic peaks with the same retention time and, again, the same UV spectrum of flurbiprofen.

After having established a TLC method for separation of the enantiomers of flurbiprofen, we chromatographed freshly prepared solutions of pure *S*-(+) and *R*-(−)-flurbiprofen in 70% ethanol. In that way we tried to ascribe the upper and the lower chromatographic band to the respective antimers. We did not succeed, however, because − to our utter surprise −

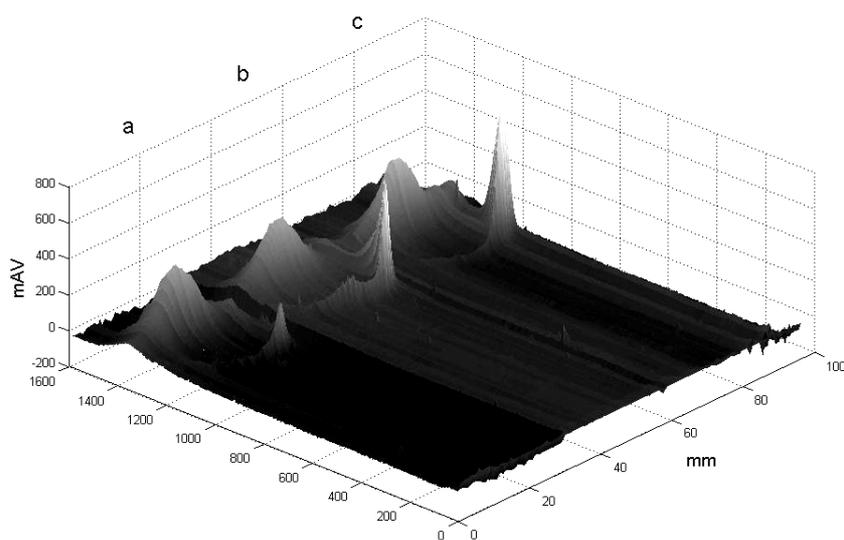(a)　　　　　　　　　　(b)　　　　　　　　　　(c)
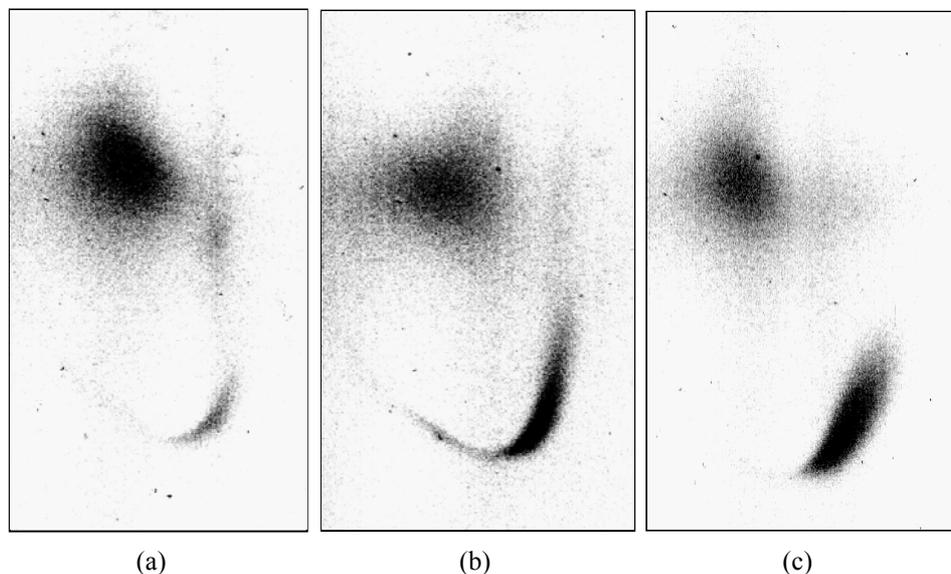
Fig. 7

Video densitograms of the spots obtained from the separated flurbiprofen antimers. The chromatograms show separation of the racemic mixtures obtained by chromatography of samples of pure *R*-(−)-flurbiprofen. The amount spotted was 3 μL and the concentrations of the solutions spotted were (a) 0.1 mg mL^{-1}, (b) 0.5 mg mL^{-1}, and (c) 1.0 mg mL^{-1}

the chromatograms obtained from the pure flurbiprofen antimers looked exactly the same and identical with that obtained from the racemic mixture. Pictures of the spots obtained for the three different concentrations of R-(−)-flurbiprofen dissolved in 70% ethanol and developed in the two-dimensional mode are shown in Fig. 7. In purely qualitative terms, Figs 7a–7c look identical with Fig. 5b, obtained for the racemic mixture of flurbiprofen. For the sake of brevity, we do not present the two-dimensional chromatograms obtained from different concentrations of the freshly prepared sample of optically pure S-(+)-flurbiprofen; for this isomer also we obtained chromatograms identical with that from the racemic mixture.

An even more persuasive picture of this most unexpected chromatographic result is shown in Fig. 8, which shows the three-dimensional densitograms obtained from the separated chromatographic spots shown in Figs 7a–7c. From this figure it is clearly apparent that with increasing amounts of R-(−)-flurbiprofen in the sample spotted, the chromatographic profiles of the separated bands also increase.

Fig. 8

Three-dimensional representation of the spots of the two separated flurbiprofen antimers from Figs 7a–7c, reconstructed from the scanning densitograms of tracks 30 mm wide taken at 1-mm intervals. The chromatograms show separation of the racemic mixtures obtained by chromatography of samples of pure R-(−)-flurbiprofen. The amount spotted was 3 μL and the concentrations of the solutions spotted were (a) 0.1 mg mL^{-1}, (b) 0.5 mg mL^{-1}, and (c) 1.0 mg mL^{-1}

This result leads to the conclusion that during chromatography R-(−) and S-(+)-flurbiprofen undergo racemization induced by the thin-layer chromatographic system. This is an interesting contribution to our general knowledge about the ease with which this particular profen spontaneously racemizes, if dissolved in a suitable medium. At the same time the rapid conversion observed prevents use of this TLC system for identification and quantification of the individual flurbiprofen enantiomers in any sample.

CONCLUSIONS

In this study the oscillatory transenantiomerization of S-(+) and R-(−)-flurbiprofen over one week was clearly demonstrated for the first time. We have previous reported the oscillatory transenantiomerization of single optically pure enantiomers of S-(+)-ibuprofen and S-(+)-naproxen only. As measurement tool we used polarimetry, and the change of the specific rotation, $[\alpha]_D$, was used as indicator of the oscillatory structural inversion of the two enantiomers of flurbiprofen.

In this paper the first separation of the two flurbiprofen enantiomers by two-dimensional TLC is also reported. The separation was achieved by TLC on silica gel impregnated with L-arginine, with ethanol containing a few drops of glacial acetic acid as mobile phase. Simultaneously, however, it was proved experimentally that during chromatography R-(−) and S-(+)-flurbiprofen undergo vigorous racemization which, despite good separation performance, prevents use of this TLC method for identification and quantification of the individual flurbiprofen enantiomers.

ACKNOWLEDGEMENT

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