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Article

Polarimetric Detection in HPLC of *R*(−)-Naproxen: Features and Intrinsic Weakness

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Polarimetric detection in high-performance liquid chromatography (HPLC) might seem like a matter of course, especially in areas such as control of the optical purity of drugs or fingerprinting of herbal extracts. However, there are well founded reasons for the relatively low popularity of polarimetric detection in HPLC. Such reasons include, for example, the insufficient sensitivity of this type of detector when compared with photodiode array detection or evaporative light-scattering detection, or the economic factors. This paper, regarding an example of *R*(−)-naproxen, discusses physical phenomena (i.e., gelation of organic solvents by small organic molecules, the effect of molecular rotors and oscillatory interconversion of chiral analytes) that might obstruct the quantification of profen drugs (more generally, of chiral low molecular carboxylic acids) with the use of HPLC with polarimetric detection. The discussed (or analogous) phenomena are even more general, which hamper the widespread application of polarimetric detection in HPLC.

Introduction

Developing an efficient polarimetric detection for high-performance liquid chromatography (HPLC) may seem like a predominantly technical task for enhancing measuring sensitivity, which, if properly solved, can be a panacea for many practical problems related to the analysis of chiral compounds; and more specifically, for the assessment of optical purity and the enantioseparation thereof. To this end, a selection of articles have been published, mostly dating from the last decade of the past century and heralding a real breakthrough with the construction of a new generation laser-based polarimetric detectors and hence, an emerging bright era in liquid chromatography of optically active compounds (1, 2).

The last few decades have witnessed the development of a novel field of investigation, which is dynamic stereochemistry of chiral compounds. This mostly focuses on the stereochemical integrity of optically active compounds and on the reversible and irreversible interconversion of the enantiomers. A monograph summarizing the achievements in this field was recently published by Wolf (3). Although the predominant majority of papers on this subject originate from the last two decades, polarimetry does not appear to be the best performing technique for the investigation of isomerization reactions of chiral compounds, as presented in an informative summary given in Table 4.2 in the paper (3). The analogous conclusion can be drawn from the fact that in sophisticated HPLC studies on direct enantioseparation, polarimetric detectors are often avoided (4), or circular dichroism (CD) detectors in dual combination with other non-polarimetric detectors are used (5, 6).

In 2001, an interesting Ph.D. thesis was released by Schoonbeek (7), devoted to the phenomenon of physical gelation of organic liquids by small organic molecules. The author has brought to wider public attention the ubiquitous self-organization of organic solutions by small organic molecules to form three-dimensional (3D) networks, even if highly diluted. The molecular level interactions responsible for the phenomenon of physical gelation can be quite different, such as hydrogen bonding, aromatic (π−π), solvophobic and other interactions. Self-assembled supramolecular structures formed by small organic molecules are usually fibroid aggregates with occasional linking nodes that are able to embrace the entity of a solution with a 3D network.

Many chiral drugs (e.g., profens), amino acids and hydroxy acids are small organic molecules and hence, tend to physical gelation in organic or aqueous organic solutions. This ability was demonstrated in earlier studies on the examples of ibuprofen and naproxen (8, 9). In one paper by Marczak *et al.* (9), a considerable difference was revealed between naproxen (able to form multimolecular aggregates and to build a stiff 3D gelating network, even if almost infinitely diluted in acetonitrile) and propionic acid as its structural precursor (which seems to remain in dimers rather than to aggregate). The structural difference between propionic acid and naproxen consists of the presence of the 6-methoxynaphthalene substituent in the latter compound, which probably substantially contributes to physical gelation through aromatic (π−π) interactions. This finding allows the conclusion that the investigated naproxen solution is a physical gel with thixotropic properties. Moreover, the use of thin-layer chromatography (TLC) has demonstrated the ability of naproxen, ibuprofen and 2-phenylpropionic acid (and of some other small chiral molecules from the groups of amino acids and hydroxy acids) to undergo a spontaneous oscillatory chiral conversion, both in aqueous and non-aqueous solvents (10–14).

The aim of this study was to scrutinize the response of a standard polarimetric detector used in HPLC to an *R*(−)-naproxen solution in non-aqueous and aqueous organic solvents at different concentrations and different flow rates. However, the obtained results seem to have substantially wider implications than only with reference to *R*(−)-naproxen, and to reveal certain physical barriers that obstruct the widespread application of polarimetric detection in HPLC.

Experimental

Reagents

R(−)-naproxen of analytical purity grade was purchased from Sigma-Aldrich (St. Louis, MO), and dichloromethane (DCM) and

methanol (MeOH) of HPLC purity grade were purchased from Merck (Darmstadt, Germany). Water used in the experiment was de-ionized and double distilled in the laboratory by means of Elix Advantage model Millipore system (Molsheim, France).

Operating system

For these investigations, an experimental setup was devised that consisted of the Knauer Maxi Star pump for HPLC (Knauer, Berlin, Germany) directly connected with the Perkin-Elmer precision polarimeter (Waltham, MA) equipped with the Perkin-Elmer HPLC flow cell (1 dm long) and the Windows-compatible PolWinLab software (BioLight Luminescence Systems, London, UK) for data acquisition and processing. In fact, the devised and assembled experimental setup imitated a proper HPLC system with polarimetric detection, although it was not equipped with a chromatographic column, because it was the authors' intent to emphasize the (largely unrepeatable) performance of the detector, and because *R*(−)-naproxen was used as a single test analyte, there was no separation task to accomplish. Measurements of the specific rotation ($[a]_D^{25}$) of the *R*(−)-naproxen solutions in DCM and 70% aqueous MeOH were conducted at 25°C, both in static and dynamic modes (in the latter case, at several different flow rates), and the polarimetric wavelength used was λ = 589 nm. In the dynamic mode, the solution of *R*(−)-naproxen was administered by the pump from the container directly to the polarimetric detector, then the effluent from the detector was returned to the container and the process was continuously repeated in a closed system for the assumed periods of time. A schematic diagram of the devised experimental setup is given in Figure 1.

The specific rotation ($[a]_D^{25}$) of the investigated samples was calculated from the following equation:

$$[\alpha]_D^{25} = 100\alpha/cd \qquad (1)$$

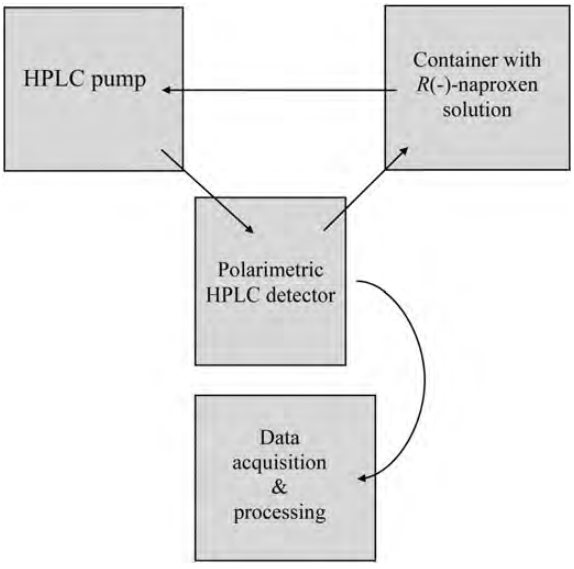

Figure 1. Block diagram of the operating system devised to monitor the influence of the forced flow on numerical values of specific rotation ($[a]_D^{25}$) measured at 25°C for *R*(−)-naproxen solutions in DCM and 70% aqueous MeOH.

where α represents the measured rotation angle (in degrees), c denotes the concentration of the investigated compounds in g/100/mL solvent, d is the optical path length in dm, and the employed working temperature was 25°C.

Results and Discussion

On the basis of earlier findings (8–14), it was anticipated that the pump of the HPLC system, which exerts certain pressure and also mixes the solutions of *R*(−)-naproxen in DCM and 70% aqueous MeOH, can easily disrupt the self-organization of the respective 3D supramolecular networks (Figure 1). Moreover, it was assumed that due to its specific chemical structure (basically, due to the bulk 6-methoxynaphthalene substituent), *R*(−)-naproxen can be considered to be a molecular propeller and its behavior in chromatographic systems can provide an analogy to macroscopic propeller-driven machines (e.g., windmills). Thus, the flowing mobile phase can blow the propeller blades, feeding them with rotational energy and thus substantially rearranging the 3D supramolecular network in the solution and falsifying the polarimetric quantification of the analyte. The concept of molecular propellers applied to the structural analog of naproxen (i.e., to 2-phenylpropionic acid) has already been utilized in an earlier paper by the authors (15). Spontaneous oscillatory chiral conversion of *R*(−)-naproxen is a complementary source of uncertainty when quantifying this compound with the use of an HPLC system with polarimetric detection.

Figure 1 shows the specially devised operating system, which consists of the HPLC pump, the HPLC polarimetric cell and the software for the HPLC data acquisition and processing. However a chromatographic column was not used because it was a a superfluous element (because there was no real separation task in this study) that might obscure the relationship between an action of the HPLC pump and the *R*(−)-naproxen response in terms of its specific rotation ($[a]_D^{25}$) values. The obtained results are illustrated in Figures 2–6.

Figures 2–4 show the dependence of the specific rotation ($[a]_D^{25}$) on *R*(−)-naproxen dissolved in DCM and 70% aqueous MeOH, respectively, in the static conditions (i.e., without pumping these solutions through all of the elements of the assembled system).

The obtained results for the *R*(−)-naproxen solutions, freshly prepared at different concentrations in both solvents, demonstrate the strongly pronounced dependence of the measured $[a]_D^{25}$ values on the applied solvent and the analyte concentration. In the case of the freshly prepared DCM solution, the specific rotation difference ($\Delta[a]_D^{25}$) calculated for the two extreme concentrations of 0.42 and 3.00 g/100/mL is equal to approximately 30° (i.e., from approximately −52 to −82°), as shown in Figure 2A. The analogous difference observed for the two extreme concentrations of 0.10 and 0.80 g/100/mL in the freshly prepared 70% aqueous MeOH solution is not very significant and only equal approximately 0.5° (i.e., from approximately −54.5° to −54.0°), as shown in Figure 2B. However, the highest $\Delta[a]_D^{25}$ differences held for the concentrations of 0.10 and 0.60 g/100/mL, and equaled approximately 5° (i.e., from approximately −54.5° to −59.5°, respectively). The results presented in Figure 2 show evidence of the significant impact of

R(−)-naproxen concentration on its specific rotation in the freshly prepared samples at the same temperature, and in the same solvent. Apparently, at each concentration, the 3D supramolecular network of *R*(−)-naproxen is arranged differently, and the measured specific rotation is the net value for a given arrangement.

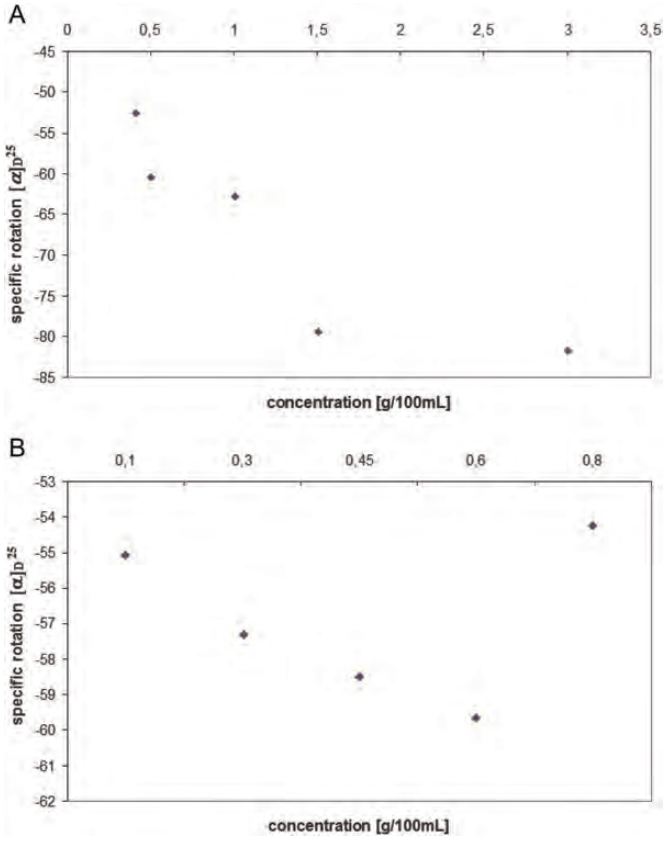

Figure 2. Specific rotation ($[a]_D^{25}$) of *R*(−)-naproxen measured at 25°C for the freshly prepared DCM solutions at concentrations of 0.42, 0.50, 1.00, 1.50 and 3.00 g/100/mL of solvent (A); specific rotation for the freshly prepared 70% aqueous MeOH solutions at concentrations of 0.10, 0.30, 0.45, 0.60 and 0.80 g/100/mL solvent (B).

If one considers *R*(−)-naproxen dissolved in DCM and stored under static conditions for 2 h (Figure 3), the respective concentration-dependent differences of the specific rotation are again evident. However, the numerical $[a]_D^{25}$ values for individual concentrations do not remain constant, but additionally undergo perceptible time changes, which can be attributed to the rearrangement of the 3D supramolecular networks and/or to spontaneous chiral conversion. Plots 1 and 2 in Figure 3 are remarkably similar to one another, as are plots 4 and 5. Plot 3 shows an intermediate profile type between these two plot groups. For each tested concentration, the changes of the numerical $[a]_D^{25}$ values were observed as equal to several degrees (i.e., up to approximately 10% of the starting value).

If one considers *R*(−)-naproxen dissolved in 70% aqueous MeOH and stored under static conditions for 2 h, the dependence between sample concentration and specific rotation ($[a]_D^{25}$) values is even more pronounced (Figure 4). Unlike DCM with its limited ability to build 3D H-bonded networks with *R*(−)-naproxen, both water and MeOH can satisfactorily participate in such structures. Thus, the observed general trend is as follows: with the lowest concentration of 0.10 g/100/mL solvent, the $[a]_D^{25}$ values vigorously oscillate within the range of approximately 14° (i.e., from approximately −55° to −69°), as shown by plot 1. The higher the concentration of *R*(−)-naproxen in 70% aqueous MeOH, the less vigorously the respective $[a]_D^{25}$ values oscillate (plots 2–5), and the narrower the range of these oscillations becomes. For the most concentrated sample (0.80 g/100/mL), the highest $[a]_D^{25}$ value of approximately 54° is observed, which in the course of 2 h storage period only changes by 1° (plot 5). The interpretation of these observations can be as follows. With the lowest concentration, the *R*(−)-naproxen molecule has greater freedom to relocate in the 3D supramolecular network than at higher concentrations. Combined relocation and chiral conversion contribute to the oscillatory changes of the net specific rotation value of the sample. At the highest concentration, the 3D H-bonded

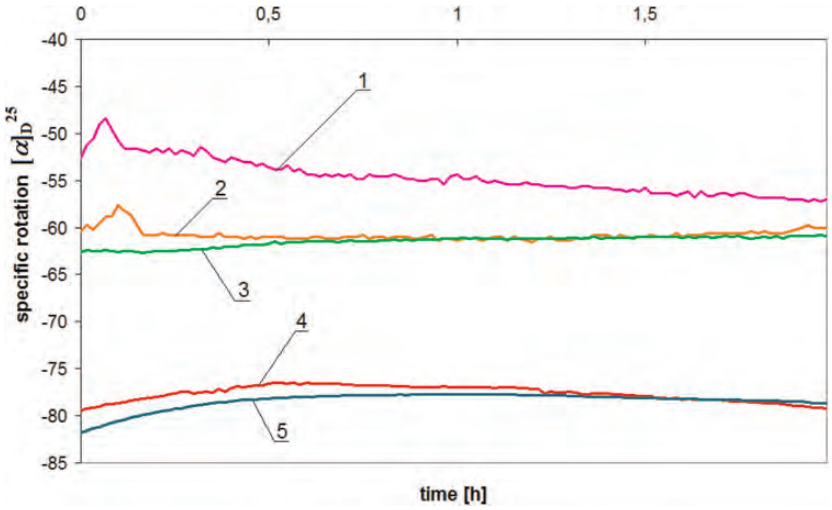

Figure 3. Changes of specific rotation ($[a]_D^{25}$) for the stagnant *R*(−)-naproxen solutions in DCM measured in the function of time at 25°C. Concentrations equal to 0.42 (plot 1), 0.50 (plot 2), 1.00 (plot 3), 1.50 (plot 4), and 3.00 g/100/mL solvent (plot 5).

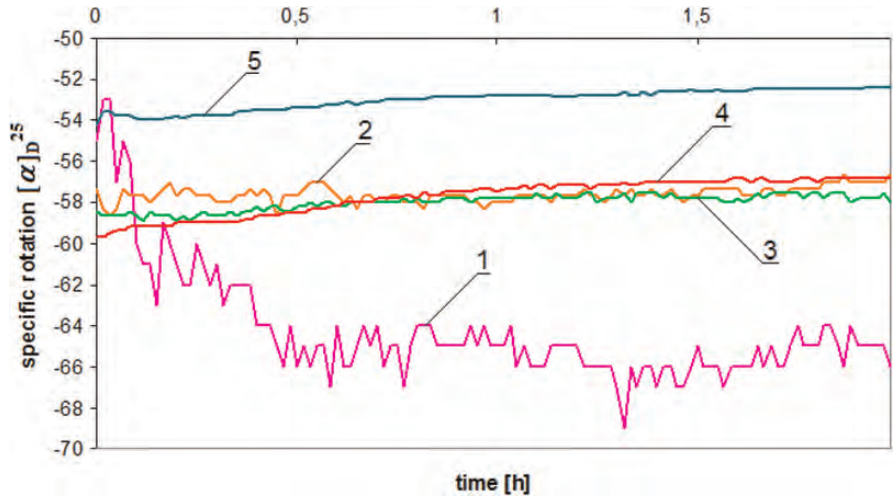

Figure 4. Changes of specific rotation ($[a]_D^{25}$) for the stagnant *R*(−)-naproxen solutions in 70% MeOH measured in the function of time at 25°C. Concentrations equal to 0.10 (plot 1), 0.30 (plot 2), 0.45 (plot 3), 0.60 (plot 4), and 0.80 g/100/mL solvent (plot 5).

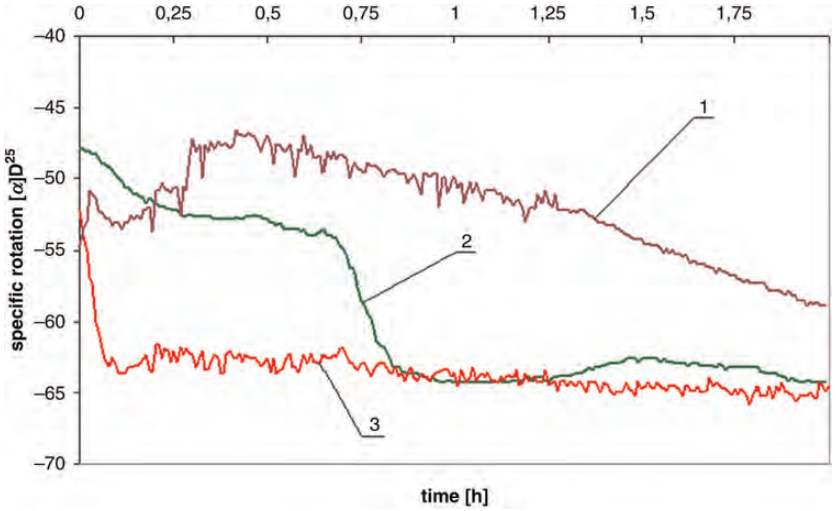

Figure 5. Changes of specific rotation ($[a]_D^{25}$) for the flowing *R*(−)-naproxen solutions in DCM measured in the function of time at 25°C. Concentration in all cases is equal to 0.68 g/100/mL solvent and the flow rates are equal to 0 mL/min (stagnant sample) (peak 1), 0.5 mL/min (peak 2), and 2.0 mL/min (peak 3).

supramolecular network becomes the most rigid, with the fewest degrees of freedom for a statistical *R*(−)-naproxen molecule to relocate in the supramolecular structure.

The influence of the forced flow exerted by the HPLC pump on the *R*(−)-naproxen solutions in DCM is illustrated in Figure 5. In this case, time dependences of $[a]_D^{25}$ were plotted for the single concentration of *R*(−)-naproxen under the forced flow rates of 0.5 and 2.0 mL/min, and the stagnant solution was also plotted for the sake of comparison. These measurements were conducted for 2 h, and it is clear that the specific rotation ($[a]_D^{25}$) for all investigated cases significantly changed with time. With the stagnant solution, the $\Delta[a]_D^{25}$ difference between the first and last measuring point equals approximately 10° (plot 1), and with those under the forced flow of 0.5 and 2.0 mL/min, the $\Delta[a]_D^{25}$ differences are approximately 16° (plot 2) and 14° (plot 3), respectively.

The analogous results shown in Figure 6 illustrate the case of *R*(−)-naproxen dissolved in 70% aqueous MeOH. Again, time dependences of the numerical $[a]_D^{25}$ values were plotted for a single concentration of *R*(−)naproxen under the forced flow rates of 0.5 mL/min (plot 2) and 5.0 mL/min (plot 3), and the stagnant solution was also plotted (plot 1) for the sake of comparison. Interestingly, all three trajectories are of a similar type. The difference of the specific rotation ($\Delta[a]_D^{25}$) calculated for the initial measuring points at the two flow rates was approximately 9°, and for the terminal measuring points, this difference only slightly diminished (and ultimately equaled approximately 6°). Although none of the measured specific rotation values can be considered to be a reference value, differences in the range from 6 to 9° indicate fluctuations ranging from approximately 10 to 15%, a result that demonstrates a profound impact of the forced flow on the specific rotation of *R*(−)-naproxen.

Summing up, the experimental results presented in this study emphasize the evident impact of the sample flow rate combined with its storage period on the specific rotation ($[a]_D^{25}$) values of

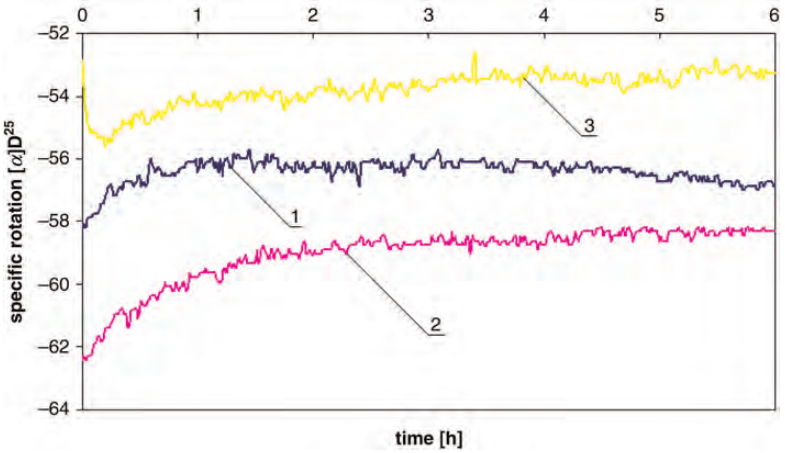

Figure 6. Changes of specific rotation ($[a]_D^{25}$) for the flowing *R*(−)-naproxen solutions in 70% aqueous MeOH measured in the function of time at 25°C. Concentration in all cases is equal to 0.68 g/100/mL solvent and the flow rates are equal to 0 mL/min-1 (stagnant sample) (peak 1), 0.5 mL/min (peak 2), and 5.0 mL/min (peak 3).

R(−)-naproxen, both in non-aqueous and aqueous solutions. The two selected solvents can rightfully be regarded as model mobile phases employed in HPLC, and so can the tested flow rates. Finally, the employed concentrations of *R*(−)-naproxen in DCM range from 0.42 to 3.00 g/100/mL, and those in 70% MeOH range from 0.10 to 0.80 g/100/mL. These concentrations are typical of analytical liquid chromatography systems (13). The column was purposely exempt from this experiment, to focus exclusively on the polarimetric detector's response to the sample (without attributing instability of the analyte's specific rotation to the weird action of the column).

Because this study was limited to a demonstration of the sample concentration, its storage time and the flow rate impact on the specific rotation ($[a]_D^{25}$) of *R*(−)-naproxen, the authors refrained from speculating about the detailed molecular-level mechanisms responsible for the observed instabilities. All valid $[a]_D^T$ values reported in the literature and valid for *R*(−)-naproxen (registered in a close temperature range for the chloroform solutions) (17–20) fall within the scope from −65.4 to −67.2°, thus showing a difference of several percent. This discrepancy can be due to the differences in the concentrations, which were 1.096 g/100/mL of chloroform in the study by Svoboda *et al.* (18) and 1.0 g/100/mL of chloroform in all remaining cases (17, 19, 20) (and hence, the discrepancy can be attributed to the differentiated concentration-dependent gelating and/or chiral conversion effects). These reference data furnish additional support for the observations reported in this study, witnessing fuzziness of the specific rotation values for *R*(−)-naproxen (which is an apparent example of the deterministic chaos).

Conclusions

The results of the experiments discussed in this study clearly show that despite the use of the same solvent and a constant measuring temperature, the specific rotation of *R*(−)-naproxen measured at static conditions is not constant, hence it does not characterize this compound in a unique manner.

The flow rate of *R*(−)-naproxen solution strongly affects its specific rotation, which appears to be very sensitive to the magnitude of the applied flow rate.

Instability of the specific rotation ($[a]_D^{25}$) value of *R*(−)-naproxen, both in the static and dynamic conditions, clearly suggests that quantification of this compound cannot be reliable with the use of an HPLC system equipped with a polarimetric detector.

The numerical values presented in Figures 2–6 understandably cannot undergo any statistic evaluation, because the molecular level organization inside *R*(−)-naproxen solutions (selected as the test samples) represents so-called deterministic chaos, due to the profen's gelation property and to its ability to undergo spontaneous oscillatory chiral conversion. Thus, the only observable repeatability is (oscillatory to a large extent) instability of the test compound's specific rotation values.

Conclusions drawn for *R*(−)-naproxen can certainly be extended to the numerous other chiral molecules able to physically gelate the organic solvents, act as molecular rotors and/or characterize with reversible or irreversible interconversion of the enantiomers.

The presented discussion justifies a limited use of HPLC with polarimetric detection for the analysis of chiral compounds, which is motivated not only by economical reasons, but by physicochemical reasons.

References

1. Lloyd, D.K., Goodall, D.M.; Polarimetric detection in high-performance liquid chromatography; *Chirality*; (1989); 1: 251–264.
2. Bobbitt, D.R., Linder, S.W.; Recent advances in chiral detection for high performance liquid chromatography; *Trends in Analytical Chemistry*; (2001); 20: 111–123.
3. Wolf, C.; Dynamic stereochemistry of chiral compounds – Principles and applications. The Royal Chemical Society, Cambridge, UK, (2008), p. 171.
4. Aranayi, A., Ilisz, I., Pataj, Z., Szatmári, I., Fülöp, F., Armstrong, D.W., *et al.*; High-performance liquid chromatographic enantioseparation of Betti base analogs on a newly developed isopropyl carbamate-

cyclofructan6-based chiral stationary phase; *Chirality*, (2011); 23: 549–556.
5. Gergely, A., Horváth, P., Noszál, B.; Deconvolution of composite chromatographic peaks by simultaneous dual detections; *Journal of Chromatographic Science*, (2000); 38: 425–429.
6. Gergely, A.; A review of the application of chiroptical methods to analytical chemistry; *Journal of Pharmaceutical and Biomedical Analysis*, (1989); 7: 523–541.
7. Schoonbeek, F.; Making it all stick together – The gelation of organic liquids by small organic molecules. PhD Thesis, University of Groningen, Groningen, (2001).
8. Sajewicz, M., Pietka, R., Kuś, P., Kowalska, T.; On the gelation of profens as a property causing their oscillatory transenantiomerization; *Acta Chromatographica*, (2006); 16: 181–191.
9. Marczak, W., Sajewicz, M., Bucek, M., Piotrowski, D., Szewczyk, K., Kowalska, T.; The aggregation of naproxen in acetonitrile and tetrahydrofuran studied by the ultrasonic, volumetric and viscometric methods; *Journal of Molecular Liquids*, (2008); 141: 8–16.
10. Sajewicz, M., Pietka, R., Pieniak, A., Kowalska, T.; Application of thin-layer chromatography (TLC) to investigating oscillatory instability of the selected profen enantiomers; *Acta Chromatographica*, (2005); 15: 131–149.
11. Sajewicz, M., Pietka, R., Pieniak, A., Kowalska, T.; Application of thin-layer chromatography (TLC) to investigate oscillatory instability of the selected profen enantiomers in dichloromethane; *Journal of Chromatographic Science*, (2005); 43: 542–548.
12. Sajewicz, M., Pietka, R., Pieniak, A., Kowalska, T.: Application of thin-layer chromatography to the investigation of oscillatory instability of selected profen enantiomers in physiological salt; *Journal of Liquid Chromatography and Related Technologies*, (2006); 29: 2059–2069.
13. Sajewicz, M., Gontarska, M., Wojtal, Ł., Kronenbach, D., Leda, M., Epstein, I.R., *et al.*; Experimental and model investigation of the oscillatory transenantiomerization of L-α-phenylalanine; *Journal of Liquid Chromatography and Related Technologies*, (2008); 31: 1986–2005.
14. Sajewicz, M., John, E., Kronenbach, D., Gontarska, M., Kowalska, T.; TLC study of the separation of the enantiomers of lactic acid; *Acta Chromatographica*, (2008); 20: 367–382.
15. Polanski, J., Sajewicz, M., Knas, M., Gontarska, M., Kowalska, T.; Lateral relocation in thin-layer chromatography; *Journal of Planar Chromatography – Modern TLC*, (2012); 25: 208–213.
16. Snyder, L.R., Kirkland, J.J., Dolan, J.W.; Introduction to modern liquid chromatography, 3rd edition. Wiley, Hoboken, NJ, (2010), pp. 119–121.
17. Tsuchihashi, G., Mitamura, S., Kitajima, K., Kobayashi, K.; Synthesis of optically active 2-arylalkanoic acids by the use of 1,2-rearrangement of the aryl group; *Tetrahedron Letters*, (1982); 23: 5427–5430.
18. Svoboda, J., Capek, K., Palecek, J.; Esters of arylpropionic acids with 1,2:5,6-di-O-isopropylidene- and 1,2-O-isopropylidene-α-D-glucofuranose; *Collection of Czechoslovak Chemical Communications*, (1987); 52: 766–774.
19. Fisher, L.E., Caroon, J.M., Stabler, S.R., Lundberg, S., Zaidi, S., Sorensen, C.M., *et al.*; Mild hydrolysis or alcoholysis of amides. Ti(IV) catalyzed conversion of primary carboxamides to carboxylic acids or esters; *Canadian Journal of Chemistry*, (1994); 72: 142–145.
20. Fujiwara, T., Sasaki, M., Omata, K., Kabuto, C., Kabuto, K., Takeuchi, Y.; An efficient procedure for the resolution of α-cyano-α-fluoro-p-tolylacetic acid (CFTA) via the diastereomeric N-carbobenzyloxy-cis-1-amino-2-indanol esters; *Tetrahedron Asymmetry*, (2004), 15: 555–564.