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ON PROBLEMS WITH LIQUID CHROMATOGRAPHIC QUANTIFICATION OF CHIRAL 2-ARYLPROPIONIC ACIDS BY USE OF UV-ABSORPTION-BASED DETECTION

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SUMMARY

In previous publications we reported abundant empirical evidence of the spontaneous oscillatory transenantiomerization of optically pure enantiomeric 2-arylpropionic acids (2-APAs), resulting in a racemic mixture of the pairs of enantiomers. Our experiments were performed with 2-phenylpropionic acid, ibuprofen, and naproxen. The last two compounds are non-steroidal anti-inflammatory drugs (NSAIDs) very popular throughout the world and sold in most countries over the counter. Before publication of our results pharmaceutical scientists were firmly convinced that the curative power of the *S*-(+) enantiomers of the 2-APAs was much greater than that of the *R*-(−) enantiomers.

In this paper we will show that – because of the spontaneous oscillatory transenantiomerization of the 2-APAs – not only is the conformational instability of these compounds when dissolved in very simple low-molecular mass solvents (e.g. water, ethanol, etc.) well proved, but also that they cannot be precisely quantified by use of liquid chromatographic detectors based on absorption of UV light. Quantification by means of HPLC–UV, HPLC–DAD, and TLC–densitometry is burdened by substantially more experimental error than for compounds that do not spontaneously racemize by keto–enol tautomerism.

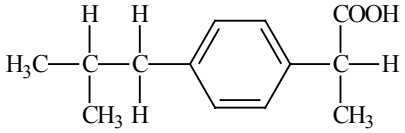
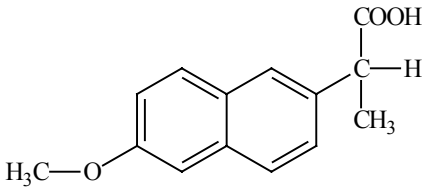
INTRODUCTION

In recent papers [1–3] we reported the striking phenomenon of repeated, i.e. oscillatory, transenantiomerization of selected 2-arylpropionic acids (2-APAs). This uncontrolled process eventually results in formation of a racemic mixture of the two enantiomers. Two out of the three 2-APAs investigated in our study were the popular non-steroidal anti-inflammatory

drugs (NSAIDs) *S*-(+)-ibuprofen and *S*-(+)-naproxen, the chemical structures of which are given in Table I.

Table I

Schematic representation of the chemical structures of the two NSAIDs discussed in this study

NSAID	Chemical structure
Ibuprofen	
Naproxen	

The oscillatory transenantiomerization of 2-APAs occurs in a similar manner to the first oscillatory reaction reported and explained by Belousov and Zhabotinskii [4,5]; it can be represented by the equation:

S-(+)-2-APA ⇔ keto-enol tautomer ⇔ *R*-(−)-2-APA

In Fig. 1. we show schematically the phenomenon of oscillatory transenantiomerization of *S*-(+)-2-APA to its *R*-(−) enantiomer as a change of concentration of the two species in a given solution as a function of time, *conc.* = *f*(*t*). If we start from a solution of the pure *S*-(+) species, it will turn, in an oscillatory manner, into a solution of the racemic mixture. The period and amplitude of these quasi-sinusoidal changes, and the duration of the racemization process, are highly dependent on many factors, e.g. the chemical structure of the given 2-APA, the solvent used, and the temperature of the solution.

Because keto–enol tautomerism involves a tautomer that is structurally different from both *S*-(+)-2-APA and its mirror image *R*-(−)-2-APA, we became curious of the effect of these continuous structural transitions on the UV spectra of 2-APA solutions. It is understandable that one expects

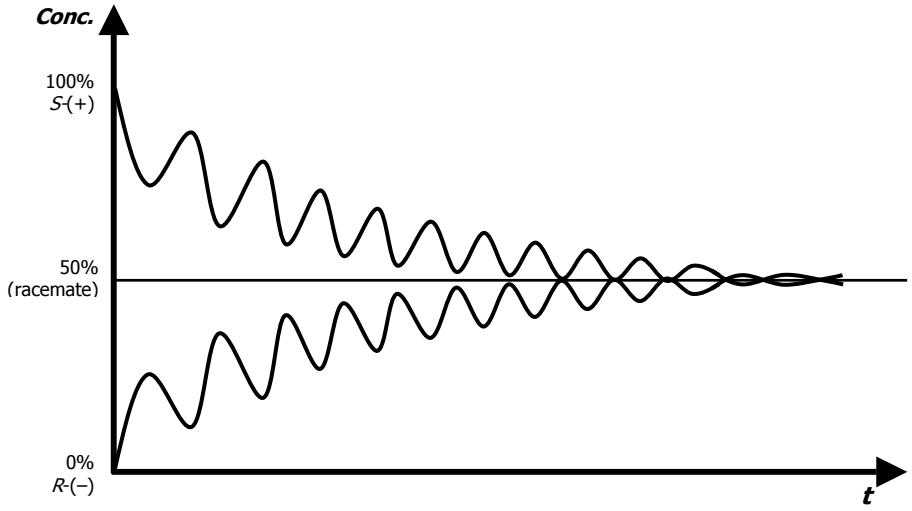


Fig. 1

Schematic representation of the oscillatory transenantiomerization of *S*-(+)-2-APA into *R*-(−)-2-APA. The oscillatory plot mirrors the fluctuating, decreasing concentration of the *S*-(+) species with the corresponding increase in concentration of the *R*-(−) species

the UV spectra of the *S*-(+) and *R*-(−) species to be identical (or almost) both in terms of the positions of the absorption maxima on the wavelength scale and of the respective molar extinction coefficients. The UV spectrum of the tautomer (which is also present in solution) should, however, differ from those of the *S*-(+) and *R*-(−) species because of the different arrangement of the double bonds in its molecule. Thus recording of the UV spectra of 2-APA solutions spontaneously and continuously undergoing transenantiomerization should result in superimposition of the identical spectra of the two 2-APA enantiomers and that of the tautomer. Because oscillatory transenantiomerization of the 2-APAs is a dynamic process, one should expect steadily changing quantitative proportions of the *S*-(+) and *R*-(−) species and tautomer. How is this reflected in the UV absorption spectra and how does it affect the quality of chromatographic quantification of these compounds based on UV absorption? The objective of the work discussed in this paper was to answer these questions.

EXPERIMENTAL

Our investigations were performed by using three independent measurement techniques, all based on UV absorption. The working conditions for each technique are given below.

S-(+)-ibuprofen and *S*-(+)-naproxen (Sigma–Aldrich, St Louis, MO, USA; #I-106 and #28,478-5, respectively) were dissolved in 95% ethanol, 70% ethanol, or pure tetrahydrofuran (THF) of HPLC grade. The respective concentrations in these solvents were 4.85×10^{-4} and 4.34×10^{-4} mol L^{-1}.

ACN, MeOH, and H_2O, used for mobile phases, were of HPLC grade and glacial acetic acid was of analytical grade.

UV–Visible Spectrophotometry

UV–visible spectrophotometry was performed with a Thermo Spectronic (Waltham, MA, USA) Genesys 6 spectrophotometer. UV spectra of the solutions were acquired at 10-min intervals for 4 h in the wavelength range 190 to 500 nm.

HPLC–DAD

HPLC was performed with a Gynkotek (Germering, Germany) Gina 50 autosampler, P 580A LPG pump, and UVD340V diode-array detector (DAD), with Dionex Chromleon v. 4.32 software.

Compounds were separated on a 250 mm × 4 mm i.d. LichroCART cartridge containing 5-µm particles of LiChrospher 100 RP-18 as stationary phase (Merck, Darmstadt, Germany; #1.50983.0001). The mobile phase was ACN–H_2O, 6:4 (*v*/*v*), at a flow rate of 0.60 mL min^{-1}.

Samples (10 µL) were injected by means of the autosampler at 30-min intervals, for up to 5 h, and spectra were acquired continuously in the wavelength range 200 to 500 nm by means of the DAD.

TLC–Densitometry

TLC was performed on 20 cm × 20 cm precoated silica gel 60 F_{254} TLC plates (Merck; #1.05715) Before use the plates were carefully washed by predevelopment with methanol–water, 9:1 (*v*/*v*), and dried at ambient temperature for 3 h. 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, and then dried. The concentration of the impregnating solution was calculated as depositing 0.5 g L^{-1} arginine per 50 g dry silica

gel. The washed, impregnated, and dried adsorbent layers were ready for chromatography.

Samples (10 μL) were applied to the plates by means of a Desaga (Heidelberg, Germany) AS 30 autosampler. Ten (ibuprofen) or five (naproxen) samples were applied to the plates 2 cm above the bottom of the plate and 2 cm from each other. ACN–MeOH–H_2O, 5:1:1 (*v*/*v*) containing several drops of glacial acetic acid was used as mobile phase for *S*-(+)-ibuprofen and ACN–MeOH–H_2O, 5:1:1.5 (*v*/*v*) containing several drops of glacial acetic acid was used for *S*-(+)-naproxen. The developed lanes were scanned densitometrically at 200, 205, and 210 nm for *S*-(+)-ibuprofen and 202, 215, and 225 nm for *S*-(+)-naproxen, by means of a Desaga CD 60 densitometer with Windows-compatible ProQuant software (Desaga). These particular wavelengths were carefully chosen after study of fluctuations of the UV absorption spectra of the two 2-APAs showed that the UV spectra of the compounds fluctuated more vigorously in the lower regions of the wavelength scale than in the higher regions.

RESULTS AND DISCUSSION

UV–Visible Spectrophotometry

In this section we show a small selection only of the UV spectra acquired for samples of *S*-(+)-ibuprofen and *S*-(+)-naproxen dissolved in ethanol–water and in THF and then stored for up to 4 h at 22 ± 2°C (Figs 2–4). Changes of peak positions and intensities are clearly visible in the

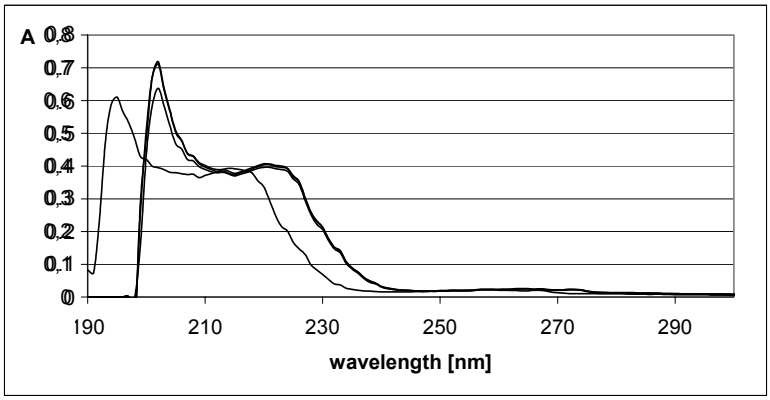


Fig. 2

Changing UV spectra of *S*-(+)-ibuprofen dissolved in 95% ethanol and stored at 22 ± 2°C. Spectra were recorded repeatedly for several hours by means of a UV–visible spectrometer

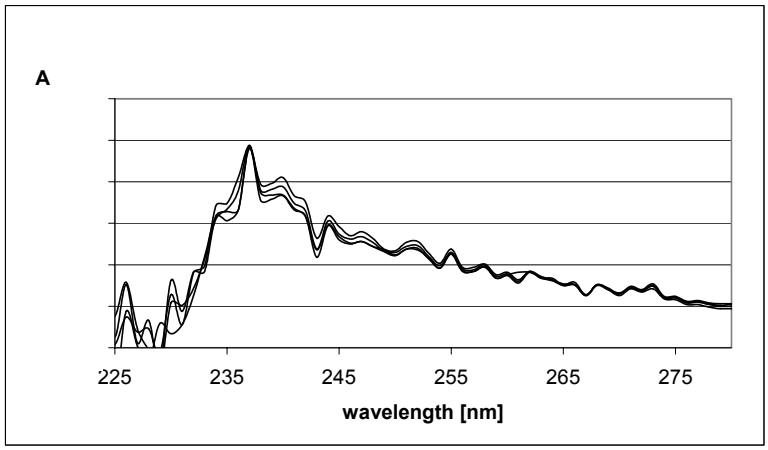


Fig. 3

Changing UV spectra of *S*-(+)-ibuprofen dissolved in THF and stored at 22 ± 2°C. Spectra were recorded repeatedly for several hours by means of a UV–visible spectrometer

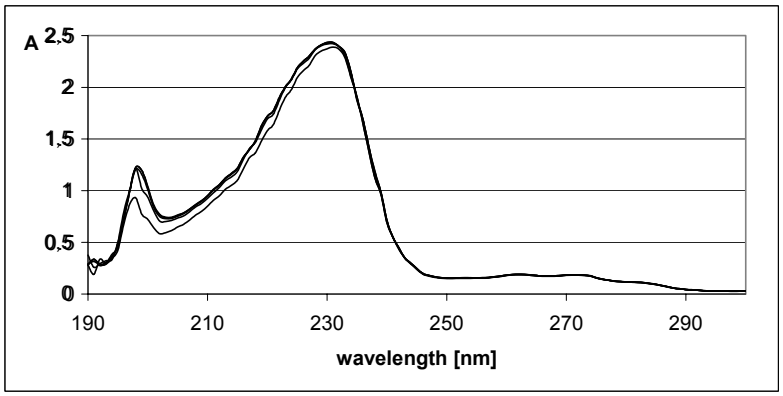


Fig. 4

Changing UV spectra of *S*-(+)-naproxen dissolved in 70% ethanol and stored at 22 ± 2°C. Spectra were recorded repeatedly for several hours by means of a UV–visible spectrometer

figures (and in those which are not reproduced here). It is apparent that all these changes and shifts – far surpassing the experimental error of the method – are because of oscillatory transenantiomerization of the 2-APAs in the solvents. For compounds that are not chiral and not undergoing repeated structural transformation, UV spectra recorded repeatedly with our equipment remained almost unchanged.

The largest changes always occurred during the first hour after dissolution of a given 2-APA and then the situation partially (although not completely) stabilized. The only sensible explanation seems to be that within the first hour – despite the oscillatory transenantiomerization occurring in each solution for many hours or even days – the concentration of the intermediate tautomer attains a quasi-stationary level. The rather large fluctuations of the UV spectra of the *S*-(+)-ibuprofen and *S*-(+)-naproxen solutions in the initial period of storage evidently result from fluctuating quantitative proportions of the chiral forms of the species and of the non-chiral keto–enol tautomer.

HPLC–DAD

In this section we again show only a small selection of the UV spectra acquired for samples of *S*-(+)-ibuprofen and *S*-(+)-naproxen dissolved in 70% ethanol and then stored for up to five hours at 22 ± 2°C. In this work, however, acquisition of the UV spectra was performed differently. The stock solutions of each 2-APA were injected at regular time intervals by means of an autosampler and UV spectra of the analytes were recorded by means of the diode-array detector. It is apparent from Figs 5 and 6 that fluctuations of peak positions and intensities are again observed, as was shown in Figs 2–4. Obviously, such unstable UV spectra cannot enable precise quantification of the 2-APAs by HPLC.

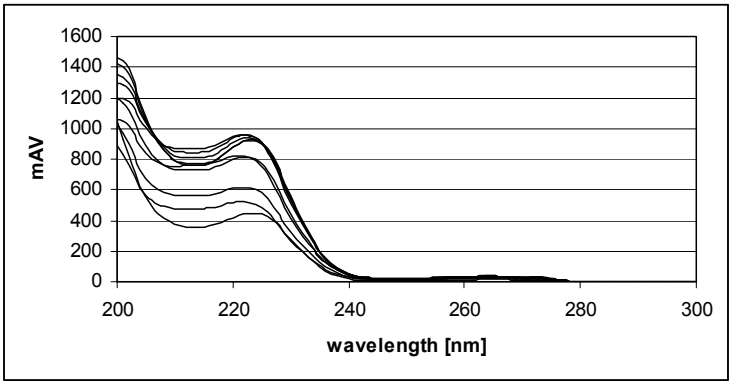


Fig. 5

Changing UV spectra obtained from ten ibuprofen samples dissolved in 70% ethanol and automatically injected, from the same stock solution, into the chromatograph in equal quantities and at regular time intervals. RP-HPLC was performed on a C_{18}-type stationary phase with ACN–H_2O, 6:4 (*v*/*v*), as mobile phase. Measurements were performed at ambient temperature (22 ± 2°C)

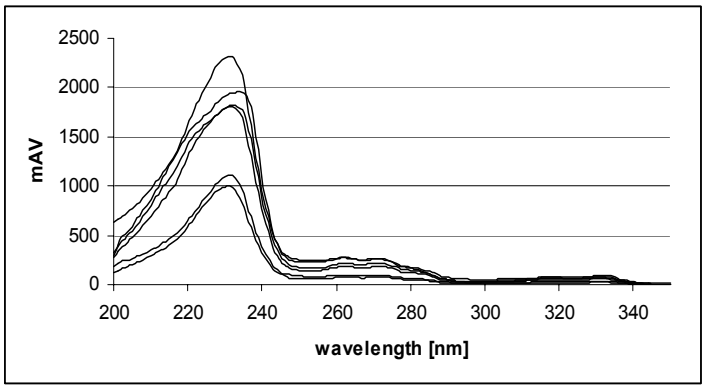


Fig. 6

Changing UV spectra obtained from six naproxen samples dissolved in 70% ethanol and automatically injected, from the same stock solution, into the chromatograph in equal quantities and at regular time intervals. RP-HPLC was performed on a C_{18}-type stationary phase with ACN–H_2O, 6:4 (*v*/*v*), as mobile phase. Measurements were performed at ambient temperature (22 ± 2°C)

TLC–Densitometry

TLC was performed with densitometric detection (although diode-array detection is now being slowly introduced to TLC also). In densitometric detection – fully analogous with HPLC with UV or diode-array detection – quantification of an analyte depends on the peak height and/or area of the concentration profile of the analyte, recorded at a given wavelength.

We present results from quantification of *S*-(+)-ibuprofen and *S*-(+)-naproxen dissolved in 70% ethanol then spotted automatically on the adsorbent layer. The results were obtained from use of different plates but

Table II

Average peak heights and respective standard deviations (*SD*) for *S*-(+)-ibuprofen, as measured densitometrically at three different wavelengths (200, 205, and 210 nm). Measurements were performed on two plates and ten equal volumes were applied to each plate

Plate no.	Average peak height (±*SD*) [mAV] at the wavelength λ [nm]:		
	200	205	210
1	465.58 (±28.04)	465.60 (±27.63)	465.69 (±26.46)
2	561.56 (±75.01)	562.08 (±75.22)	552.20 (±74.75)

with equal amounts of sample applied to the same plate. The average peak heights and their standard deviations (*SD*) are presented in Tables II and III.

Table III

Average peak heights and respective standard deviations (*SD*) for *S*-(+)-naproxen, as measured densitometrically at three different wavelengths (202, 215, and 225 nm). Measurements were performed on four plates and five equal sample volumes were applied to each plate

Plate no.	Average peak height (±*SD*) [mAV] at the wavelength λ [nm]:		
	202	215	225
1	177.53 (±10.78)	337.74 (±17.43)	285.18 (±13.03)
2	422.17 (±26.18)	653.99 (±24.75)	555.49 (±20.10)
3	234.97 (±21.10)	427.59 (±22.32)	354.50 (±20.12)
4	416.77 (±13.66)	661.47 (±18.29)	569.61 (±16.37)

It is readily apparent from the data in these tables that large standard deviations of the peak heights were obtained for each plate and for each UV wavelength. Here it must be stated that for compounds which do not undergo structural transformation during storage as their solutions (i.e. the vast majority of compounds analyzed) the *SD* of peak height is usually ±2%. For *S*-(+)-ibuprofen, numerical values of *SD* at 200, 205, and 210 nm were – because of the relatively narrow range of wavelengths compared – very similar. For plate 1 *SD* values were ca. ±6% of the mean values and for plate 2 they were even higher, ca. ±13% of the mean values.

For *S*-(+)-naproxen, the highest *SD* (up to ca. ±9% of the experimental mean value) were observed at 202 nm; *SD* were lower (up to ca. ±6% of the recorded mean values) for densitograms recorded at the higher wavelengths of 215 and 225 nm. These observations are in good agreement with the fluctuations of the UV spectra of *S*-(+)-ibuprofen and *S*-(+)-naproxen shown in Figs 2 and 4, respectively. From the plots shown in these figures it is clearly apparent that at lower wavelengths these fluctuations are more pronounced than at higher wavelengths.

CONCLUSIONS

Oscillatory transenantiomerization of 2-APA solutions in ethanol–water and in some non-aqueous solvents is responsible for fluctuations of

the UV absorption spectra of these compounds during storage. The changes observed are apparent both from peak intensities and from their positions on the wavelength scale. These changes occur because of steady fluctuation of the molar proportions of the three species present in the solutions, i.e. the *S*-(+) and *R*-(−) enantiomers, and the intermediate tautomer. The UV spectra recorded are, in fact, superimpositions of two identical spectra originating from the *S*-(+) and *R*-(−) enantiomers of a 2-APA and of a different spectrum originating from the intermediate keto–enol tautomer.

Because quantification of liquid chromatograms in both HPLC and TLC is most often performed by use of UV-absorption-based detectors, any uncontrolled and spontaneous change of the UV spectra of the analytes can negatively affect calibration plots and, more generally, the accuracy of quantification of the compounds.

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