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Article

An HPLC–DAD and LC–MS Study of Condensation Oscillations with *S*(+)-Ketoprofen Dissolved in Acetonitrile

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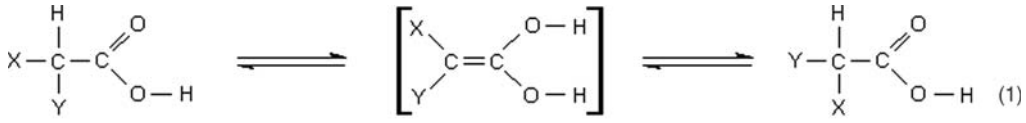
In our earlier studies, a spontaneous chiral conversion of the selected low-molecular-weight carboxylic acids (i.e., amino acids, hydroxy acids, and profen drugs) dissolved in aqueous ethanol medium, running *in vitro* was described. Then it became clear that this spontaneous chiral conversion is accompanied by the spontaneous condensation of the discussed compounds. With several acids, it was established that this condensation is also oscillatory in nature. The theoretical models were developed aiming to give a rough explanation of the observed non-linear processes. In this paper, the results of these studies on the dynamics of condensation with *S*(+)-ketoprofen, a very popular profen drug, when stored for certain amount of time dissolved in a non-aqueous medium (i.e., acetonitrile) is presented. These investigations were carried out with the aid of two independent high-performance liquid chromatographic systems with the diode array detection and of a third high-performance liquid chromatographic system equipped with mass spectrometric detection. In one cycle of chromatographic measurements, it was possible to monitor condensation of *S*(+)-ketoprofen in 25-min intervals for 30 h, thus obtaining kinetic information on the progress of this process. Mass spectrometric detection confirmed the presence of new species in the stored solution with molecular weights much higher than that of *S*(+)-ketoprofen, which can be attributed to the condensation products. The obtained data show that condensation of *S*(+)-ketoprofen dissolved in acetonitrile progresses in a rapid manner, and that the observed oscillatory concentration changes with *S*(+)-ketoprofen and with the main condensation product characterize with an irregularity and shallow amplitudes. A theoretical model was referenced that jointly describes the oscillatory chiral conversion and the oscillatory condensation with the low-molecular-weight chiral carboxylic acids.

Introduction

In our earliest studies, wide experimental evidence was provided on the spontaneous oscillatory chiral conversion running *in vitro* with the selected low-molecular-weight carboxylic acids belonging to the class of profens, amino acids, and hydroxy acids, when dissolved in the aqueous media. An overview of these investigations is given in the literature (1). Another work (2) signalized, for the first time, that the selected profen drugs (i.e., ibuprofen and naproxen) can undergo the spontaneous oscillatory chiral conversion in a non-aqueous medium also (i.e., in dichloromethane). Moreover, the oscillatory chiral conversion has been reported in the crystallization of the (+) and (-) enantiomers of 2-azabicyclo[2.2.1] hept-5-en-3-one (3).

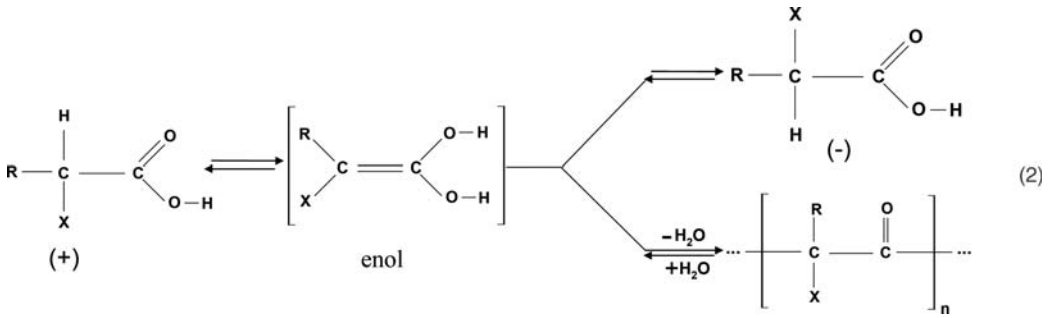
The general scheme of the spontaneous chiral conversions run with the low-molecular-weight carboxylic acids in anhydrous media or in presence of trace amounts of water can be given by Equation 1 (4):



(1)

where X: -NH_2, -OH, -Ar, etc., and Y: -R, etc.

Later, experimental evidence was collected pointing out the spontaneous peptidization of amino acids, and condensation of hydroxy acids and profen drugs (5) running in the aqueous media *in vitro*, in parallel with the process of the oscillatory chiral conversion. This evidence was collected by means of different analytical techniques, e.g., by means of thin-layer chromatography (TLC) and the ^{13}C NMR spectroscopy. A simple scheme of such process running with profens is given below in Equation 2.



where R = CH_3, and X = Ar

In another paper (6), it was experimentally demonstrated that the spontaneous oscillatory nature of peptidization of *R*-, *S*-, and *rac*-phenylglycine in the aqueous ethanol medium and theoretical models were proposed with an explanation of the observed phenomenon.

In the literature (7, 8), confirmatory reports are available on the spontaneous condensation similar to that observed in our studies, which are oscillatory in nature. Those results seem relevant to our own earlier results, as the oscillatory condensation documented by the authors was running in the aqueous organic media and involved chiral organosilanol substrates.

It is the aim of this study to investigate *S*(+)-ketoprofen with respect of its ability to undergo the spontaneous oscillatory condensation in acetonitrile as a non-aqueous medium and to trace the dynamics of such a process by means of high-performance liquid chromatography with diode array and mass spectrometric detection (HPLC–DAD and LC–MS, respectively). The earlier developed theoretical approach that captures the key aspects of the condensation and oscillatory chiral conversion processes with the low-molecular-weight chiral carboxylic acids seems valid for S(+)-ketoprofen also (6).

Experimental

Reagent

S(+)-Ketoprofen of analytical purity grade was purchased from Sigma–Aldrich (St Louis, MO, USA; cat. no 471909-1G). For the spontaneous condensation experiment, the solution of *S*-(+)-ketoprofen in acetonitrile (ACN) was used. Concentration of the optically pure enantiomer was 1 g/L (3.93×10^{-3} mol/L). The sample was stored in the tightly stoppered colorless glass vial, and its spontaneous ageing was carried out for several weeks at 22°C.

HPLC–DAD

HPLC analysis was carried out using two different liquid chromatographs equipped with the diode array detectors (HPLC–DAD) and the two different chromatographic columns, in order to obtain a double set of the experimental evidence.

The Gyncotek liquid chromatograph (Gyncotek, Macclesfield, UK) was equipped with a Gyncotek Gina 50 model autosampler, Gyncotek P 580A LPG model pump, Gyncotek DAD UVD 340U model diode array detector, and Chromeleon Dionex v. 6.4 software for data acquisition and processing. The analyses were carried out in the isocratic mode, using the Hypersil GOLD (5 μm particle size) column (250 mm × 4.6 mm i.d.; Thermo Scientific, Waltham, MA, USA; cat. no. 0694830N), and methanol–water (5:5, v/v) mobile phase at a flow rate of 0.6 mL/min. The chromatographic column was thermostated at 35°C with use of the Varian Pro Star 510 model column oven. The analyses with use of this system were carried out in the 25-min intervals for 30 h.

The Varian model 920 liquid chromatograph (Varian, Harbor City, CA) was equipped with Galaxie software for data acquisition and processing. The analyses were carried out in the isocratic mode, using the Pursuit 5 C18 (5 μm particle size) column (250 mm × 4.6 mm i.d.; Varian, Harbor City, CA; cat. no. A3000250C046), and methanol–water (5:5, v/v) mobile phase at a flow rate of 0.6 mL/min. The respective chromatogram presented in this study was recorded for the *S*-(+)-ketoprofen sample after 8 days of storage time.

LC–MS

LC–MS was carried out using an LC–MS System Varian (Varian, Palo Alto, CA) equipped with a Varian ProStar model pump, Varian 100-MS mass spectrometer, and Varian MS Workstation v. 6.9.1 software for data acquisition and processing.

The LC analyses were carried out in the isocratic mode, using a Pursuit X R_S 3-C18 column (50 mm × 2.0 mm i.d.; Merck KGaA, Darmstadt, Germany; cat. no. A6001050C020) and methanol–water (5:5, *v*/*v*) mobile phase at the flow rate of 0.20 mL/min.

MS detection was carried out in the ESI mode (full ESI-MS scan, positive ionization, spray chamber temperature 45°C, drying gas temperature 150°C, drying gas pressure 25 psi, capillary voltage 70 V, needle voltage 5 kV).

Results and Discussion

HPLC–DAD

In Figure 1, we show the chromatogram registered for the freshly prepared sample of *S*(+)-ketoprofen in ACN. Apart from the predominant peak of *S*(+)-ketoprofen, two more peaks of low intensity are also observed. Most probably, these two additional peaks are the condensation products, as the investigated compound does not decompose under the applied working conditions. The chromatogram is implemented with the three

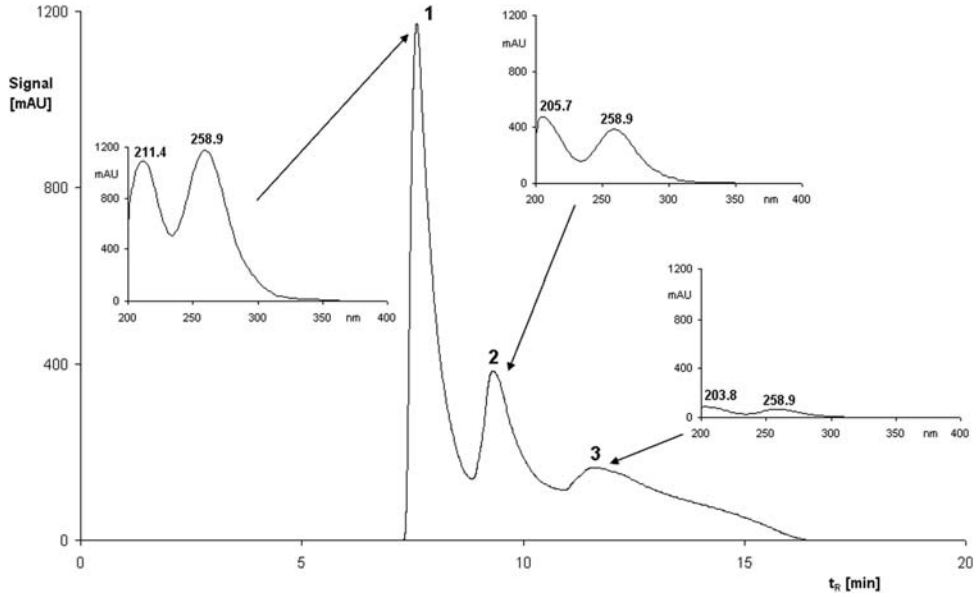


Figure 1. The chromatogram of the freshly prepared *S*(+)-ketoprofen solution in ACN registered with use of the Gyncotec liquid chromatograph at 259 nm. Retention times: peak 1, 7.66 min; peak 2, 9.68 min; peak 3, 11.80 min. Insets show UV spectra of the separated species recorded at the maxima of the respective peaks.

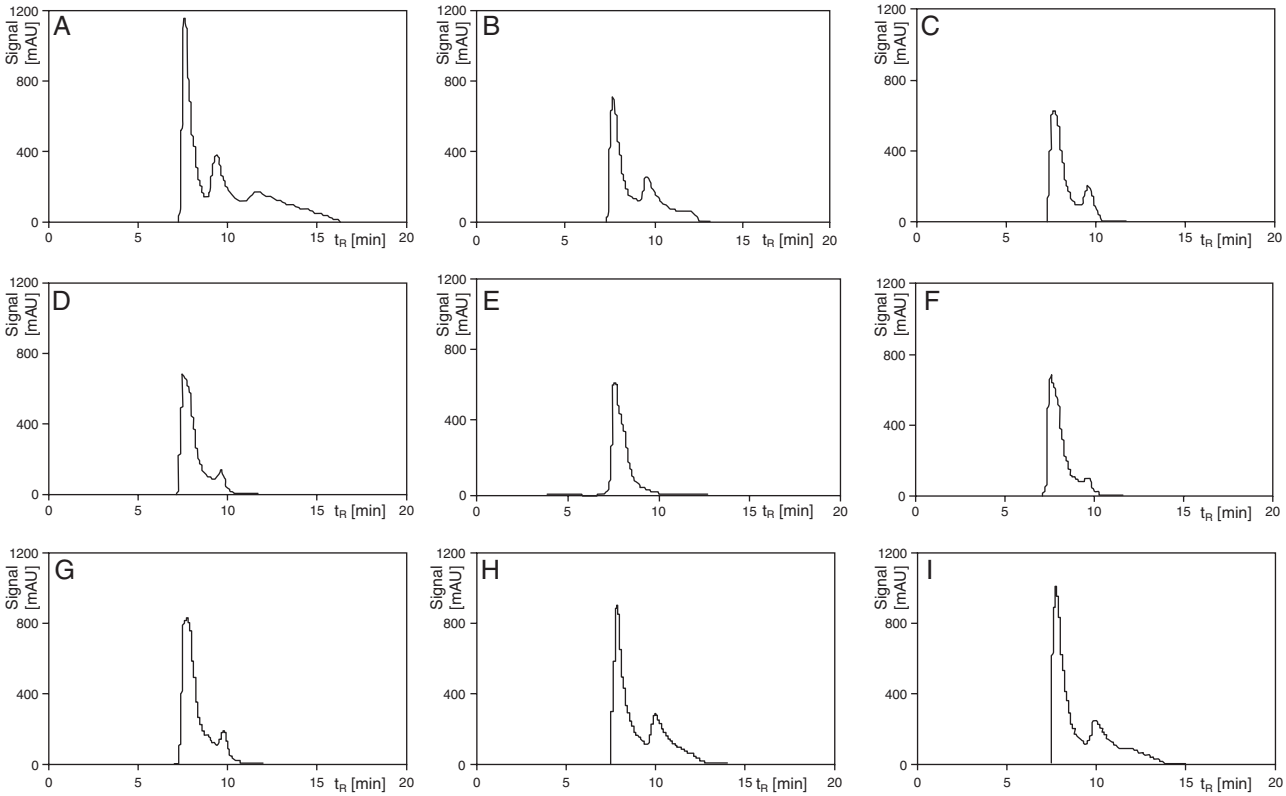


Figure 2. Sequence of the nine chromatographic concentration profiles registered with use of the Gyncotec liquid chromatograph at 259 nm for the *S*(+)-ketoprofen solution in ACN after (A) 0 h; (B) 5.5 h; (C) 9.5 h; (D) 18 h; (E) 19 h; (F) 20 h; (G) 24.5 h; (H) 28 h; and (I) 30 h storage time at 22°C.

inlets showing the UV spectra recorded from the respective peak maxima for the three separated species. The only difference among these three UV spectra consists in the intensity of absorbance, and the least intense spectrum is attributed to peak no. 3. One could simply conclude that the higher is the molecular weight of a condensate the lower is its yield.

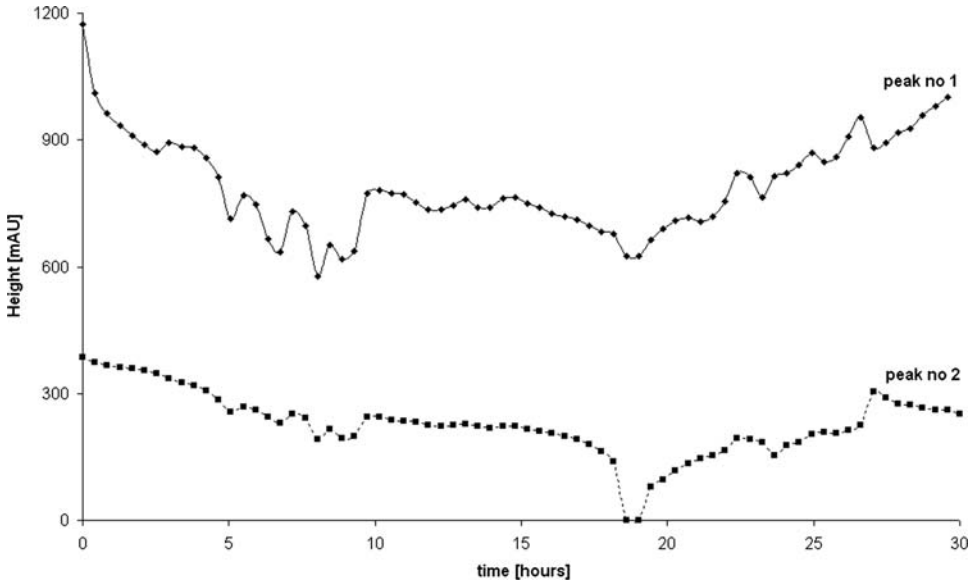


Figure 3. Time changes of the chromatographic peak heights for the *S*(+)-ketoprofen solution in ACN stored at 22°C for 30 h. Peak numbers, as given in Figure 1.

In the course of the 30-h sample storage period, the number of the peaks on the chromatograms recorded in the 25-min intervals periodically changed. This process is shown in Figure 2 in the form of nine chromatograms considered as specific snapshots. These snapshots are valid for the gradually ageing sample of *S*(+)-ketoprofen and the time evolution of the respective chromatograms is evident from these plots. In the initial period, the chromatograms clearly show three peaks (Figures 2A and 2B). After ca. 10 h storage period, only two peaks are left on the chromatogram (peaks numbers 1 and 2; Figures 2C and 2D) and disappearance of peak no. 3 is complete. After 18 h of running the experiment, only peak no. 1 can be seen (Figure 2E), but after 19 h peak no. 2 emerges again (Figures 2F–2H) and in the last hour of running the experiment, once more we can see the three peaks (Figure 2I). It is noteworthy that throughout the entire experiment, peak no. 1 (attributed to the starting sample, i.e., to *S*(+)-ketoprofen), is present in the chromatogram. The observed changes in the consecutive chromatograms are irregular yet oscillatory in nature. It is most probable that the discussed periodical changes of sample composition are due to formation and decomposition of ketoprofen condensates, represented by peaks numbers 2 and 3.

Changes in the peak heights for peaks numbers 1 and 2 (roughly equivalent to the respective concentrations of the two species) in the function of time are shown in Figure 3. We refrained from adding the analogous plot for peak number 3, as it appeared on the chromatograms in the first and the last (i.e., thirtieth) hour of the experiment only. Shapes of the two plots shown in Figure 3 are similar in this sense that they are both non-monotonic but oscillatory.

After eight days from preparation of the *S*(+)-ketoprofen solution in acetonitrile, the *S*(+)-ketoprofen sample was again analyzed by means of chromatography and the obtained results are shown in Figure 4. The chromatogram (Figure 4A) and the spectrochromatogram (Figure 4B) both witness to the presence of more than the three peaks in the aged sample (eight days old). Apart from the peaks numbered as (I) and (II), in the time range from 10 to 15 min one can perceive two additional bands. Peak (I) can again be ascribed to *S*(+)-ketoprofen, as an equivalent of peak no. 1 on the chromatograms shown in Figs 1 and 2. The anti-Langmuir shape of peak (II) (which characterizes with the desorption front much steeper than the tailing adsorption front) can signalize complex intermolecular interactions of the involved chemical species (maybe the presence in the analyzed sample of the effective lateral interactions through the hydrogen bonds). The UV spectrum shown in Figure 4C, identical for both peaks (I) and (II), can serve as an additional proof that peak (II) represents the condensed ketoprofen.

LC–MS

The chromatographic investigation on ageing of the *S*(+)-ketoprofen solution in acetonitrile (first performed with use of the HPLC–DAD system and described in the previous section) was now repeated with use of the LC–MS system. Due to the different working parameters of the liquid chromatographic systems (basically caused by the different column manufacturers, geometries and packings, and by the different column temperatures) and first of all, due to a different chromatogram-generating mode (MS detector in place of DAD), the obtained chromatograms are also different. A summary of the obtained results is shown in Figures 5 and 6.

Figure 5 shows a sequence of six chromatograms collected in the span of almost 50 h of the *S*(+)-ketoprofen ageing time. According to the changing pattern of the respective concentration profiles, all these chromatograms can be divided into the two groups and it is evident that one chromatogram type evolves to the other and *vice versa* in a cyclic manner. Type 1 chromatograms show several tiny peaks at $t_R \approx 1$ min and one predominant peak at $t_R \approx 10$ min (Figures 5A, 5B, and 5D). Type 2 chromatograms show the predominant sharp peak at $t_R \approx 1$ min and one broad band at $t_R \approx 5$ min (Figures 5C, 5E,

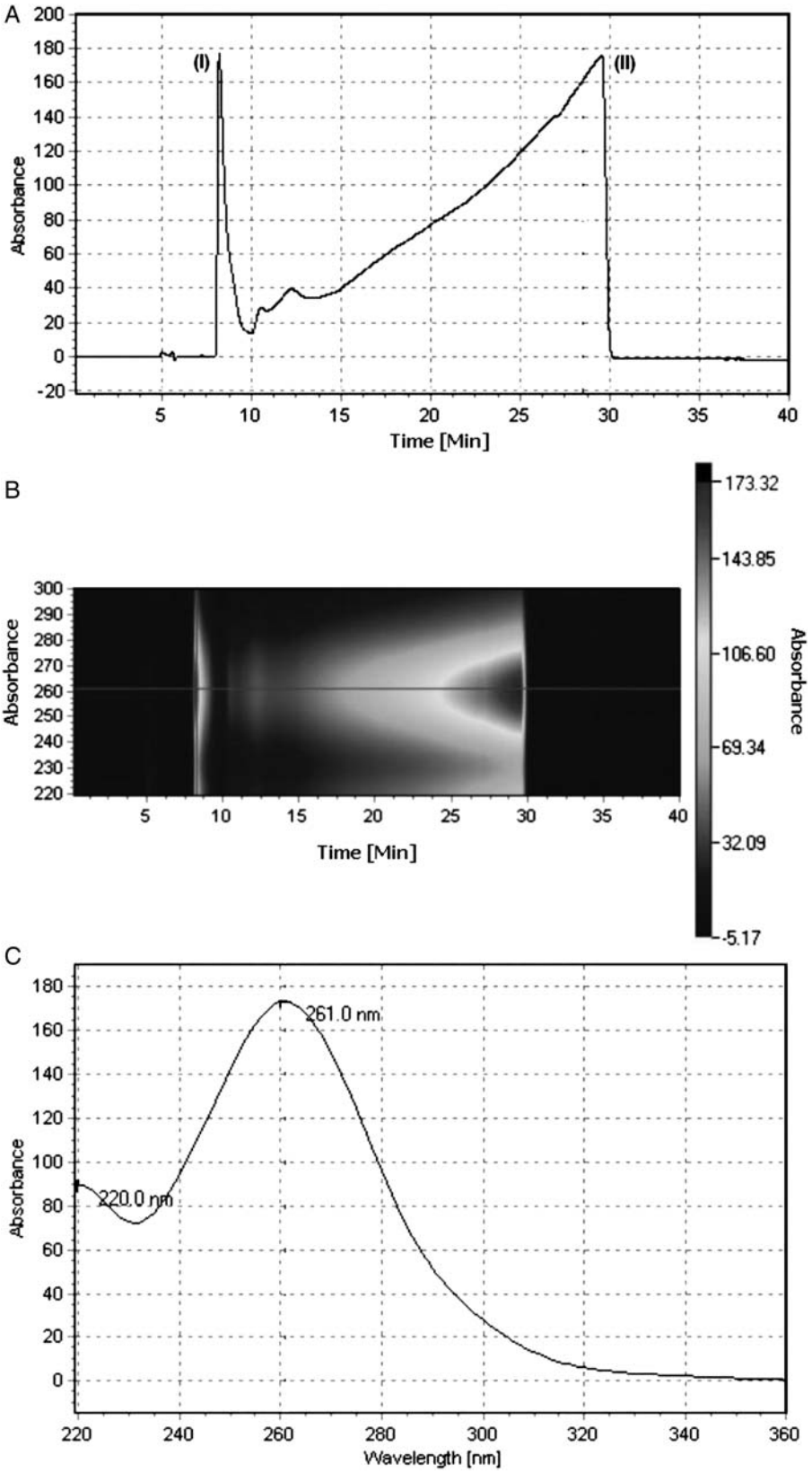


Figure 4. (A) The chromatogram of the *S*(+)-ketoprofen solution in ACN after eight days storage period registered with use of the Varian liquid chromatograph at 257 nm. Retention times: peak (I), 8.28 min, peak (II), 28.45 min. (B) The 2D spectrochromatogram registered for the same sample. (C) The UV spectrum of the peaks at 8.28 min (*S*(+)-ketoprofen) and at 28.45 min (the ketoprofen condensate) retention time.

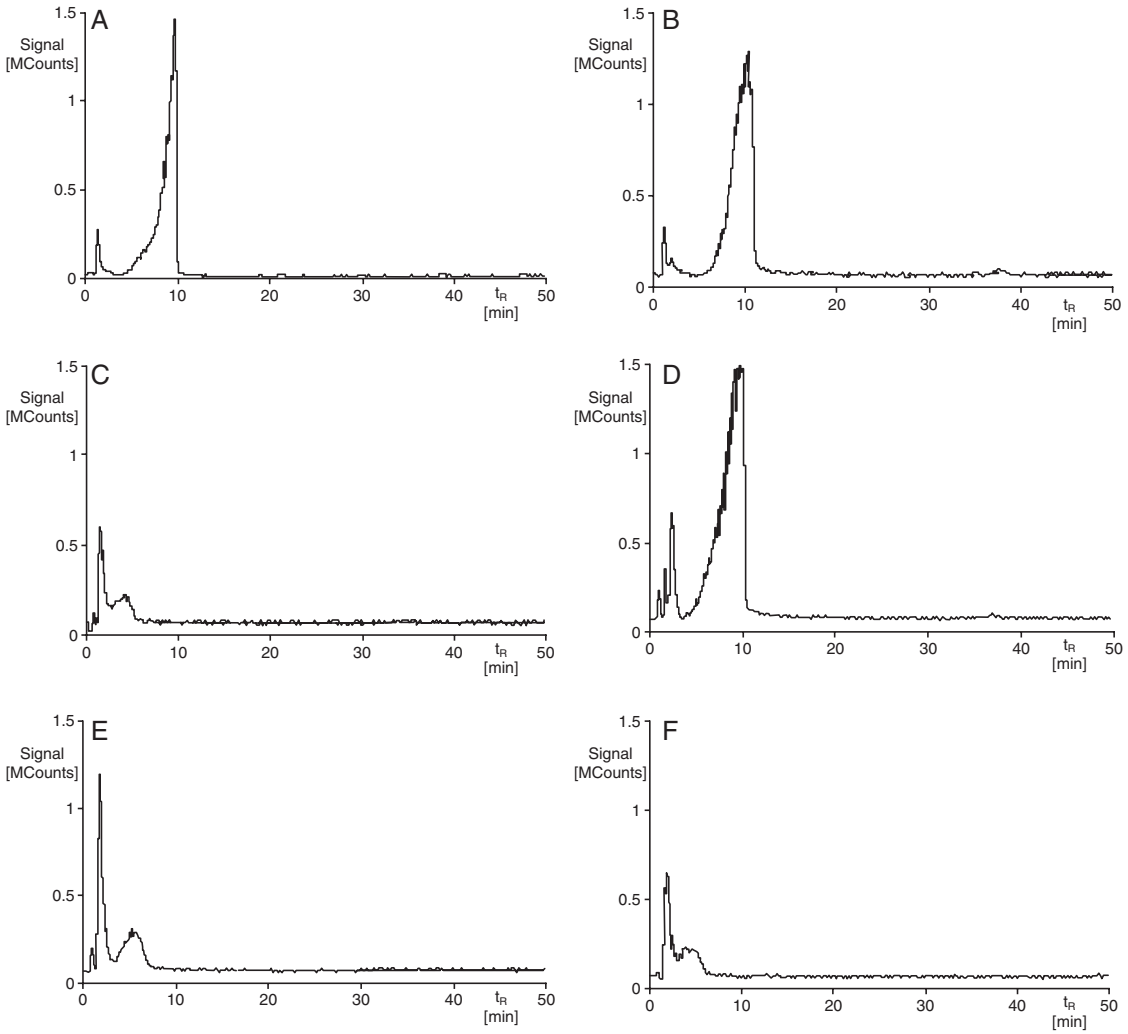


Figure 5. Sequence of the six chromatographic concentration profiles registered with use of the Varian LC-MS system for the *S*(+)-ketoprofen solution in ACN after (A) 0 h; (B) 24 h; (C) 43.5 h; (D) 44.5 h; (E) 45.5 h; and (F) 47.5 h storage time at 22°C.

and 5F). Peak at the retention time $t_R \approx 1$ min observed in the both types of the chromatograms most probably originates from ketoprofen. Cyclic changes of peak positions and areas for the peaks appearing at t_R equal to ca. 5 and 10 min can be due to the oscillatory formation and decay of ketoprofen condensates in the ageing solution. Growth of the condensate concentration is most probably represented by the chromatograms type 1 (Figures 5A, 5B, and 5D). This supposal can be confirmed by at least two observations. Firstly, the predominant peak at $t_R \approx 10$ min has an anti-Langmuir shape which suggests a multi-layer adsorption of the condensates on stationary phase, with the respective condensate molecules kept together by the covalent chemical bonds and the H-bonds. Secondly, high intensity of the predominant peak at $t_R \approx 10$ min can be due to cumulating a considerable number of phenyl groups per one condensate molecule. With chromatograms type 2 (Figs 5C, 5E, and 5F), the peak at $t_R \approx 1$ min tends to be more intense than that at $t_R \approx 5$ min. Moreover, position of the second peak at $t_R \approx 5$ min suggests that the molecular weight of the respective condensates is lower than of those appearing at $t_R \approx 10$ min.

Figure 6 shows two chromatograms with the insets of mass spectra for each separated peak. The chromatogram shown in Figure 6A represents type 1 (analogous to those shown in Figs 5A, 5B, and 5D), while the chromatogram shown in Figure 6B represents type 2 (like those shown in Figures 5C, 5E, and 5F). In the mass spectra of the peaks with the higher retention times, the signals with $m/z > 500$ are abundant and they most probably correspond with ketoprofen oligomers derived by coupling of several monomer units. Upon the numerical m/z values it is not very difficult to speculate about an anticipated composition of the respective molecules or molecular fragments, which does not automatically mean though that our guess is always correct. To this effect, we would like to limit our comments on the mass spectra shown in Figure 6 to the following statement: The m/z values equal to 710.8 and 714.7 are indicative of the presence of the molecular structures derived from

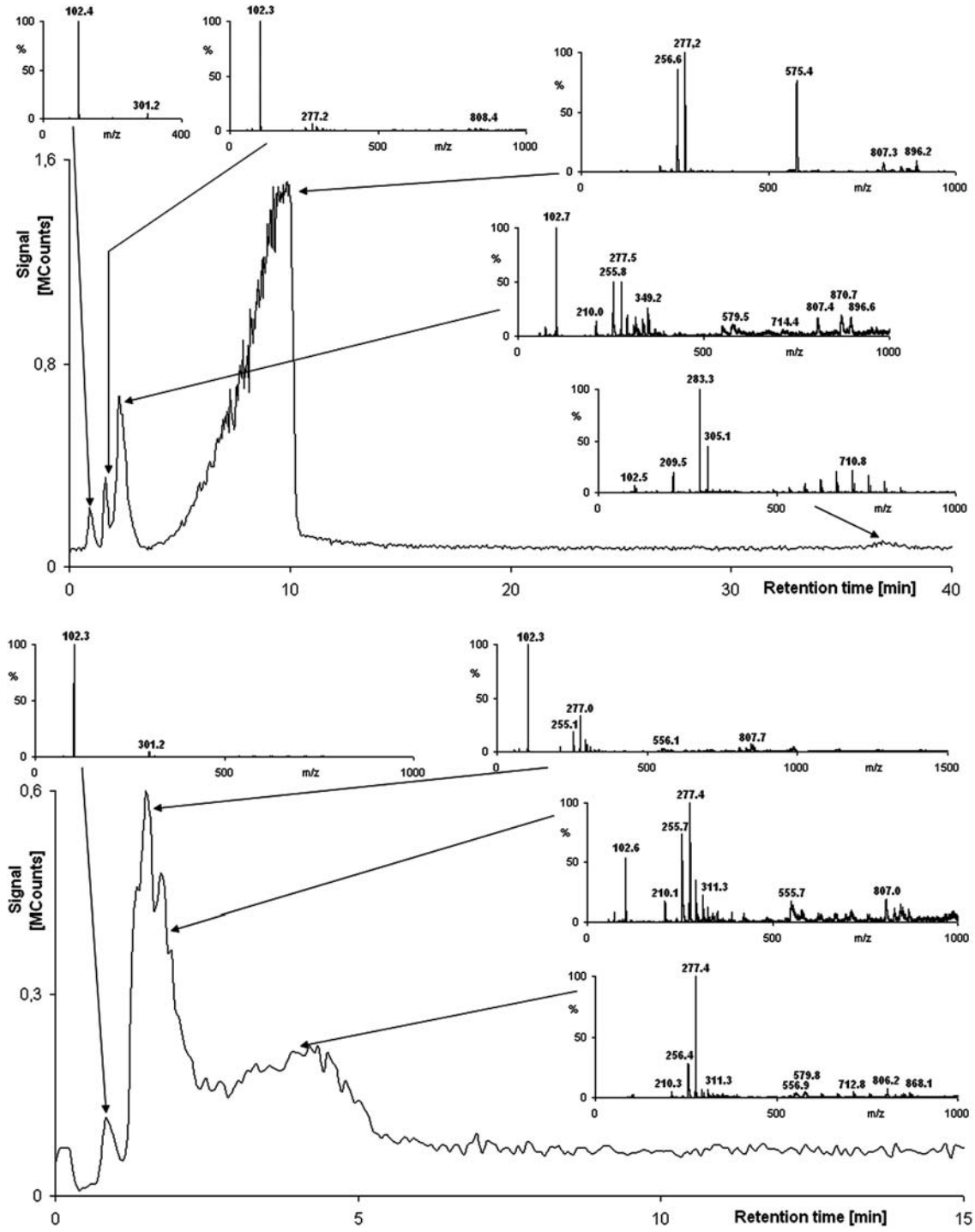


Figure 6. The chromatogram of the *S*(+)-ketoprofen solution in ACN registered with use of the Varian LC–MS system after (A) 44.5 h and (B) 43.5 h storage time. Insets show mass spectra of the separated species recorded at the maxima of the respective peaks.

ketoprofen trimer in the aged *S*(+)-ketoprofen solution, and those equal to 807.3, 870.7, and 896.6 are indicative of the presence of the structures related to ketoprofen tetramer. From the discussed mass spectra it is also evident that certain amounts of ketoprofen pentamer (and even slightly higher oligomers) in the aged sample seem also possible.

Two peaks which are particularly well visible in almost each mass spectrum appear at m/z equal to 255.8 and 277.3. The most probable structures of the respective peaks are given in Figure 7.

In another paper (6), possible mechanisms of chemical processes were discussed, such as the spontaneous oscillatory

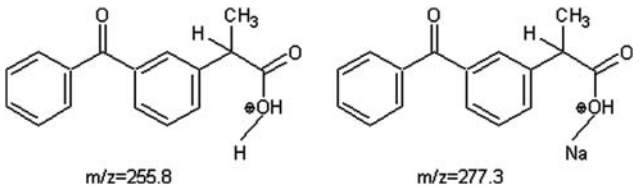


Figure 7. The most probable structures of the respective peaks which are particularly well visible in almost each mass spectrum appear at m/z equal to 255.8 and 277.3.

chiral conversion and the spontaneous oscillatory condensation of the low-molecular-weight carboxylic acids belonging to the groups of profen drugs, amino acids, and hydroxy acids running in parallel. These mechanisms are presented in the form of the three different theoretical models, all of them anticipating aggregation of the starting monomers through the H-bonds and oligomerization thereof through the covalent bonds. In each model, the oscillatory changes of the polycondensation products are due to attributing different importance (and hence, the different ranges of the reaction rate constants) to the different elementary reaction steps. The proposed mechanisms seem valid for the case of *S*(+)-ketoprofen also.

Conclusions

A variety of the chromatographic techniques have been employed to demonstrate that *S*(+)-ketoprofen generates oligomers in the course of storage in the nonaqueous acetonitrile medium. This oligomerization process takes place in an irregular, oscillatory fashion. Although present study on the popular pain-killing drug (i.e., *S*(+)-ketoprofen) was performed *in vitro* in the non-physiological acetonitrile solution, one can expect that the analogous oligomerization process can develop in the aqueous media, and not only *in vitro*, but *in vivo* also. An ability of the *S*(+)-ketoprofen molecules to easily polymerize can exert a negative impact on the curative process with use of this particular drug, which in the worst case might result even in clogging of the patient's capillary blood vessels.

Acknowledgment

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