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Mass spectrometric study of naproxen dimer anions generated from racemate and pure enantiomers

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ABSTRACT

The behaviour of naproxen dimer anions ([2Napr-H]$^-$) was studied by electrospray ionization mass spectrometry. Three different analyses were performed, namely direct inlet MS and HPLC/MS (performed for different cone voltages) as well as CID MS/MS (for different collision energies). The results obtained have shown that naproxen dimer anions generated from pure enantiomers are more stable than dimer generated from racemate. This finding indicates that in dimers the interactions between aromatic substituents take place. This observation can be used to differentiate between pure enantiomer and racemate.

Keywords*:* chirality, mass spectrometry, naproxen

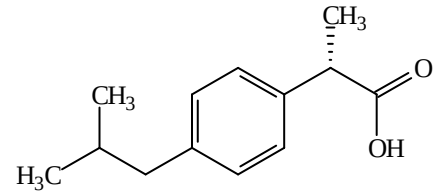
INTRODUCTION

Although mass spectrometry may be considered as a technique blind to chirality, there is a number of ways that allow the use of mass spectrometry for chiral analysis, as recently described in details in the excellent review papers [1,2]. One of the ways is to use liquid chromatography-mass spectrometry with a chiral stationary phase column [3] (nowadays such columns are commercially available), separation is necessary if we deal with a mixture of isomers. If we have only one isomer in the sample and we want to determine which one it is, the use of liquid chromatography may not be necessary. For example, diasteromers can be identified on the grounds of different mass spectrometric fragmentation patterns [4-7].

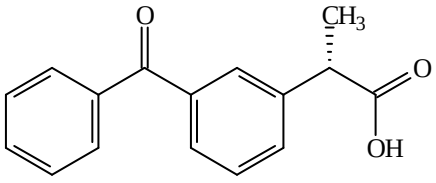
It is well known that in the electrospray ionization mass spectra the so-called cluster ions are often observed. The most common are dimer ions, namely $[2\mathbf{M}+H]^+$ and $[2\mathbf{M}+Na]^+$ in the positive ion mode and $[2\mathbf{M}-H]^-$ in the negative ion mode. The cluster ions can be either present already in solution and successfully transferred to the gas phase or they can be formed in the gas phase. If **M** is a chiral molecule, pure R or S enantiomer, the respective dimer ions formed can be

RR or SS, Such dimers are also enantiomers and must have identical mass spectrometric fragmentation patterns. If we deal with racemate, the dimer ion will be a mixture of RR, RS and SS. Statistically the ratio RR:RS:SS should be 1:2:1. Enantiomers RR and SS will be formed/decomposed with equal efficiency (provided that there is no interactions with other chiral compound). However RS is a diasteromer of RR and SS, thus the efficiency of formation/decomposition of RS may be slightly different than that of formation/decomposition of RR and SS (thus the ratio may be slightly different from 1:2:1). Anyway, the mass spectrometric behaviour of dimer ions obtained for pure enantiomer may be different from mass spectrometric behaviour of dimer ions obtained for racemate (in the first case the dimer ion is one diasteromer, in the second case the dimer ion is almost a 1:1 (RR+SS):RS mixture of diasteromers). The differences in mass spectrometric behaviour of the dimer ions may be useful if it is of interest to determine if we have pure enantiomer or racemate.

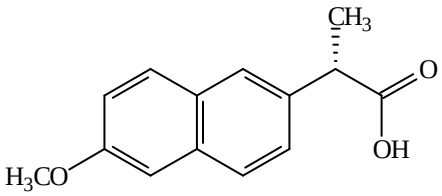
The exemplary important chiral compounds which as we found form respective dimer ions, are non-steroidal anti-inflammatory drugs (NSAIDs), namely naproxen, ketoprofen and ibuprofen. These three drugs are derivatives of propanoic acid containing at 2 position respective aromatic substituent, (ibuprofen - (*RS*)-2-(4-(2-methylpropyl)phenyl)propanoic acid, naproxen - (+)-(*S*)-2-(6-methoxynaphthalen-2-yl)propanoic acid and ketoprofen - (*RS*)-2-(3-benzoylphenyl)propanoic acid, Scheme 1).



S-ibuprofen



S-ketoprofen



S-naproxen

Scheme 1. Enantiomers S of three common non-steroidal anti-inflammatory drugs.

In the negative ion mode, besides the abundant [**M**-H]$^-$ ions, there are [2**M**-H]$^-$ ions of notable abundances (the ions can be either present already in solution and/or can be formed in the gas phase) as shown in Figure 1.

The dimer anions [2**M**-H]$^-$ contain special class of hydrogen bond namely ionic hydrogen bond [8]. If besides this ionic hydrogen bond there are also interactions (attraction or repulsion) between aromatic substituents the mass spectrometric behaviour of dimer RS should be different from dimers RR and SS. Obviously, the dimers mainly exist due to the ionic hydrogen bond and

then the effect of interactions between aromatic substituents is low. Such interactions are expected to exist for naproxen dimer, since naproxen has large rigid aromatic substituent, enriched with electrons by methoxyl group. The repulsion between these substituents in dimer RS may be different from that repulsion in dimers RR and SS.

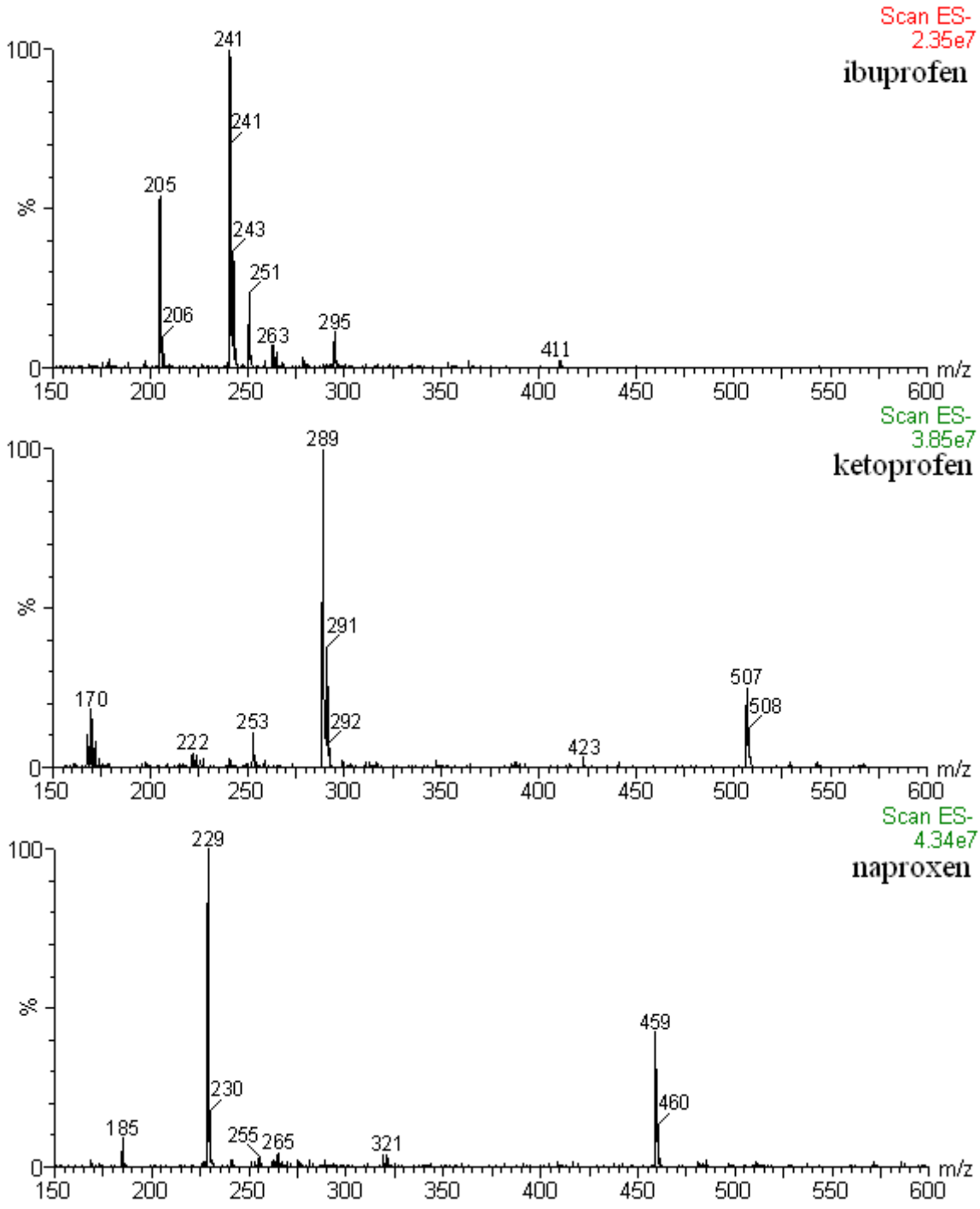


Figure 1. Full scan ESI mass spectra of three common non-steroidal anti-inflammatory drugs (NSAIDs) obtained for racemates by direct inlet at cone voltage 5 V.
[**Ibup**-H]$^-$ m/z 205, [**Ibup**+Cl]$^-$ m/z 241, [1**Ibup**-H]$^-$ m/z 411, [**Ket**-H]$^-$ m/z 253, [**Ket**+Cl]$^-$ m/z 289, [2**Ket**-H]$^-$ m/z 507, [**Napr**-H]$^-$ m/z 229, [**Napr+Cl**]$^-$ m/z 265, [2**Napr**-H]$^-$ m/z 459.

Therefore in this paper we wished to check whether the dimer ion [2**M**-H]$^-$ obtained from naproxen racemate (the ion is 1:2:1 mixture of RR:RS:SS isomers) can be differentiated from the

dimer ions obtained from pure enantiomers (the ions are RR or SS). Among three NSAIDs (naproxen, ketoprofen and ibuprofen), both R and S enantiomer of naproxen are easily commercially available (and of course racemate can be easily prepared from them). For ibuprofen and ketoprofen only enantiomers S and racemates are available. Therefore for naproxen it is possible to compare dimer anions obtained from racemate with dimer ions obtained from both pure enantiomers. For ibuprofen and ketoprofen it would be possible to compare dimer anions obtained from racemate with dimer ions obtained only for S enentiomer and it may be disputable if the results are accidental.

EXPERIMENTAL SECTION

Pure enantiomers of naproxen (as well as racemate and S enantiomers of ibuprofen and ketoprofen) have been obtained from Sigma-Aldrich (Poznań, Poland).
The direct inlet MS analysis was performed on a Waters/Micromass (Manchester, UK) ZQ2000 mass spectrometer (single quadrupole type instrument, Z-spray, software MassLynx V3.5). The sample solutions were prepared in methanol at the concentration of 10^{-4} mol/dm^3. The sample solutions were infused into the ESI source using a Harvard pump at a flow rate of 80 μl/min. The ESI source potentials were capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage (CV, most important parameter) 5-30 V (changed with the step 5 V). The source temperature was 120 ^{o}C and the desolvation temperature was 300^{o}C. Nitrogen was used as the nebulising and desolvating gas at the flow-rates of 100 and 300 l h^{-1}, respectively.

The HPLC/ MS analyses were performed by using the same (above) mass spectrometer (the same instrumental conditions) and by using a Waters model 2690 HPLC pump (Milford, MA, USA). Using an autosampler, the sample solutions were injected into the Atlantis C18 column (5 μm x 150 mm x 3.9 mm i.d., Waters). The gradient of methanol/water with flow rate 0.5 ml/min was applied, the gradient started from 0% CH_3OH reaching 100% CH_3OH after 15 min, maintained for 10 min.

The CID MS/MS analyses were performed on a Waters/Micromass (Manchester, UK) Q-tof Premier mass spectrometer (software MassLynx V4.1, Manchester, UK). The sample solutions were prepared in methanol at the concentration 10^{-4} mol/dm^3. They were infused into the ESI source by a syringe pump at the flow rate 5 μl/min. The electrospray voltage was 3.5 kV and cone voltage 30 V. The source temperature was 80^{o}C and the desolvation temperature was 250^{o}C. Nitrogen was used as the cone gas and desolvating gas at flow rate of 50 and 800 lh^{-1}, respectively. Argon was used as a collision gas at flow-rates 0.5 ml/min in the collision cell. The applied collision energies (most important parameter of CID MS/MS analysis) were 0-5 eV, changed with the step 0.5 eV.

RESULTS AND DISCUSSION

Direct inlet analysis

At first, the ESI full scan mass spectra of naproxen (racemate and pure enantiomer) were obtained at different cone voltages by using direct inlet. Increase in this voltage leads to the so-called “in-source” fragmentation/dissociation, but a too low cone voltage may cause a drop in sensitivity. The pressure in this region is about 1.5 Pa, which is low enough to allow an ion a significant mean free path of travel before collision occurs, yet high enough for the probability of collision to be still significant [9]. It is possible that collisions may induce formation of some ions [10]. As shown in Figure 1 at a low cone voltage, for ibuprofen and ketoprofen there are

abundant chloride adducts, while they are less abundant for naproxen. Naproxen has a lower pKa than ibuprofen and naproxen [11] and the lower value of pKa the lower the abundance of chloride adducts [12]. Formation of chloride adducts may affect formation of dimer ions (both in the solution and in the gas phase). It is the next reason why for the purpose of this study naproxen is more suitable than ketoprofen and ibuprofen.

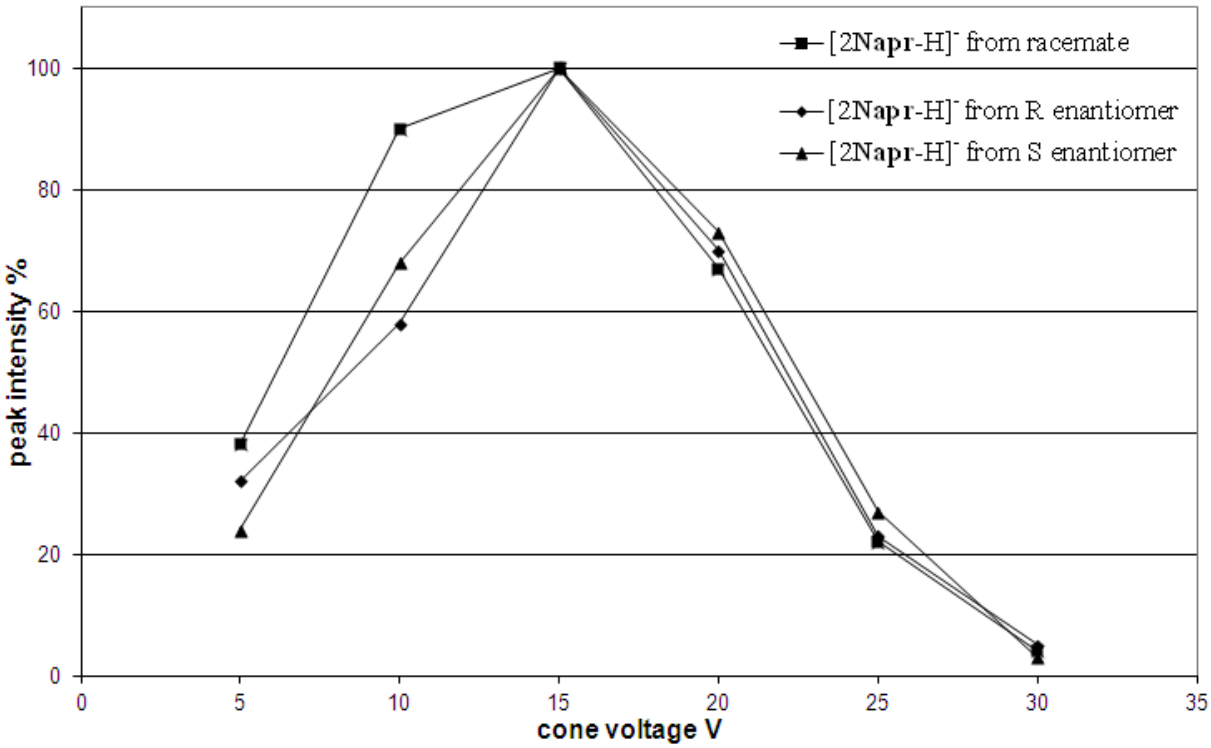


Figure 2. The peak intensities of the anionic dimer [2Napr-H]⁻ versus the cone voltage.

Figure 2 shows the breakdown plots of peak intensities (calculated from respective full scan mass spectra) of the dimer ions [2**Napr**-H]⁻ against the cone voltage (the peak intensities were normalized to the peak of the highest intensity).

All dimers, obtained from racemate as well as obtained from pure enantiomer, have the highest abundances at the cone voltage 15 V. Further cone voltage increase leads to decomposition of the dimers and practically there is no difference between decomposition of dimer obtained from racemate and decomposition of dimer obtained from pure enantiomers. On the other hand, there is a difference, namely a cone voltage increase from 10 V to 15 V leads to a substantial increase in the peaks of pure enantiomer dimers, whereas the corresponding increase is much lower for the peak of racemate dimer (Figure 2). In other words, for pure enantiomer dimers in the cone voltage range 10-15 V we deal with a significant increase in sensitivity and/or significant increase in dimer formation. For racemate dimers the increase in sensitivity and/or in dimer formation is much lower or the increase in sensitivity is affected by dimer decomposition. Thus, the conclusion can be drawn that pure enantiomer dimers are more stable then racemate dimer.

HPLC/MS analysis

In order to confirm the above conclusion the racemate and pure enantiomers were analysed by HPLC/MS technique. We use simply a C18 column, not a chiral column, thus the enantiomers were not separated, but their separation was not the purpose. The HPLC/MS conditions are slightly different from direct inlet conditions: naproxen is separated from impurities and the flow rate of mobile phase is much higher than that of the solution in direct inlet (the higher flow rate

leads to an increase in pressure in the electrospray source). Therefore, the efficiency of formation/decomposition of the ions of interest may be different for direct inlet MS analysis and HPLC/MS. Figure 2 was prepared by using intensities (heights) of mass spectrometric peaks of ions [2**Napr**-H]$^-$ (obtained from direct inlet) against the cone voltage. Figure 3 was prepared by using areas of chromatographic peaks (in arbitrary units) of ions [2**Napr**-H]$^-$ (obtained from HPLC/MS) against the cone voltage. The HPLC/MS analysis were performed in negative modes for 6 values of cone voltage, simultaneously (5, 10, 15, 20, 25 and 30 V - during the HPLC/ MS analyses the mass spectrometer was switched in the fast mode between these values).

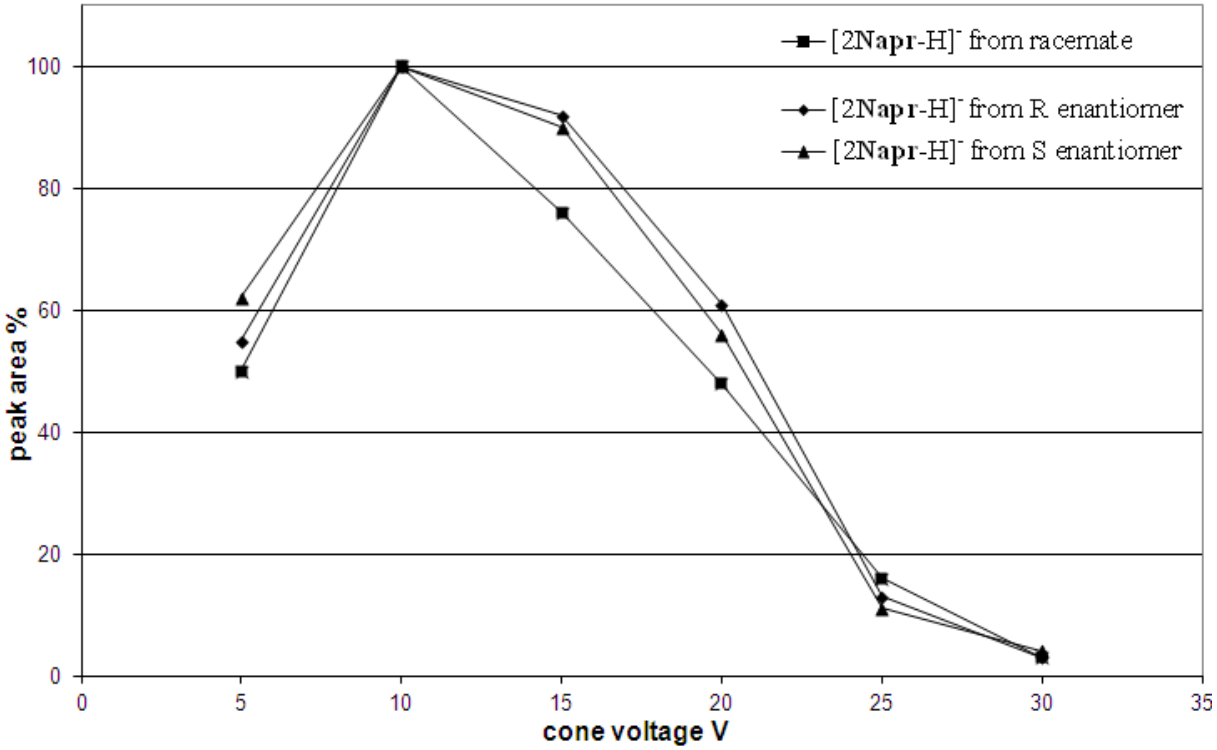


Figure 3. The intensities (areas) of the chromatographic peaks of the anionic dimer [2Napr-H]$^-$ versus the cone voltage.

The behaviour of naproxen dimer ions generated in HPLC/MS analysis is not the same as that of naproxen dimer ions generated in direct inlet MS analysis (for example the highest abundance is at cone voltage 10 V). On the other hand, the conclusion concerning the stabilities of ions [2**Napr**-H]$^-$ generated from racemate and generated from pure enantiomers is valid. A cone voltage increase from 10 V to 15 V leads to a substantial decrease in the peak of racemate dimer, whereas the peaks of pure enantiomer dimers are much less decreased. In other words, under HPLC/MS conditions, a cone voltage increase from 10 V to 15 V leads to substantial decomposition of racemate dimer, whereas decomposition of the pure enantiomer dimers occurs with lower efficiency. Thus, pure enantiomer dimers are more stable then racemate dimer.

MS/MS analysis

Next, the dimers studied were subjected to the CID MS/MS experiments. In these experiments we deal only with decomposition (no with formation) of a selected ion (provided that collision energy is high enough). CID MS/MS is a better technique to study the fragmentation of the ions than CID "in-source". As expected decomposition of [2**Napr**-H]$^-$ ions leads to the formation of monomer ion [**Napr**-H]$^-$ and at higher collision energy the ion [**Napr**-H]$^-$ loses a CO_2 molecule producing fragment ion [**Napr**-HCOO]$^-$ (Figure 4) [13, 14].

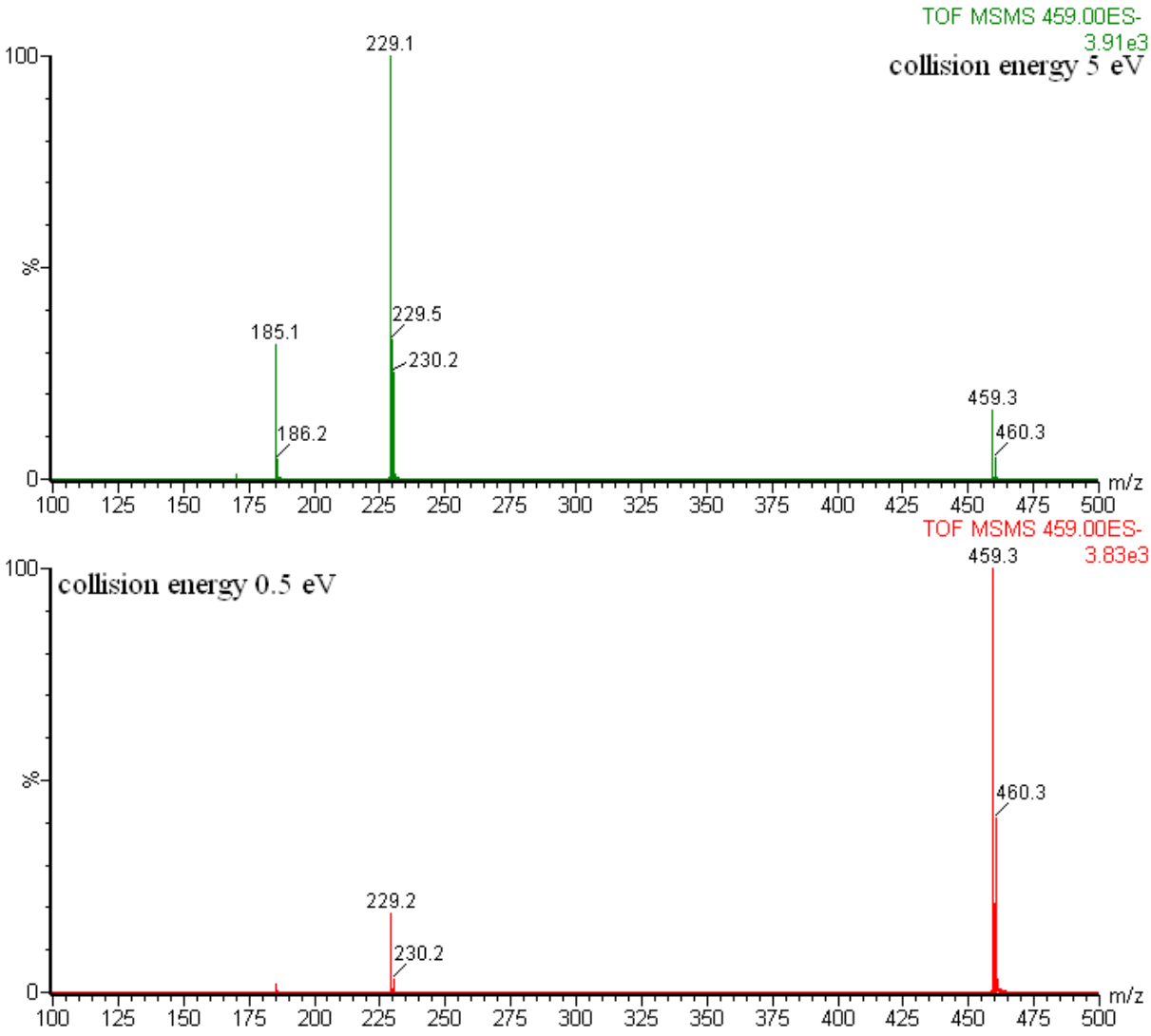


Figure 4. Exemplary CID MS/MS spectra of naproxen dimer anion obtained for R enantiomer.
[2**Napr**-H]$^-$ m/z 459, [**Napr**-H]$^-$ m/z 229, [**Napr**-HCOO]$^-$ m/z 185.

It is of interest if the differences between decompositions of the racemate dimer and pure enantiomer dimers could be detected. The following equation was used to calculate the relative intensity (RI) of the surviving naproxen dimer anions:

$$RI = \frac{[2Napr\text{-}H]^-}{[2Napr\text{-}H]^- + [Napr\text{-}H]^- + [Napr\text{-}HCOO]^-}$$

The breakdown plots of relative intensities (RI, calculated from the respective MS/MS spectra) of the dimer ions [2**Napr**-H]$^-$ against the collision energy did not show the differences between the behaviour of racamate dimer and pure enantiomers dimers. On the other hand, analysis of the ratio of relative intensities $RI_{racemate}/RI_{pure\_enetiomer}$, reveals a tendency towards higher stabilities of pure enentiomer dimers than that of racemate dimer (Figure 5). For $RI_{racemate}/RI_{R\_enetiomer}$ the points are arranged better than in the case of $RI_{racemate}/RI_{S\_enetiomer}$, but the tendency was identical. Decrease in the ratio $RI_{racemate}/RI_{pure\_enetiomer}$, with increasing collision energy indicates that

dimers of pure enantiomer are more stable (are decomposed with lower efficiency) than racemate dimer.

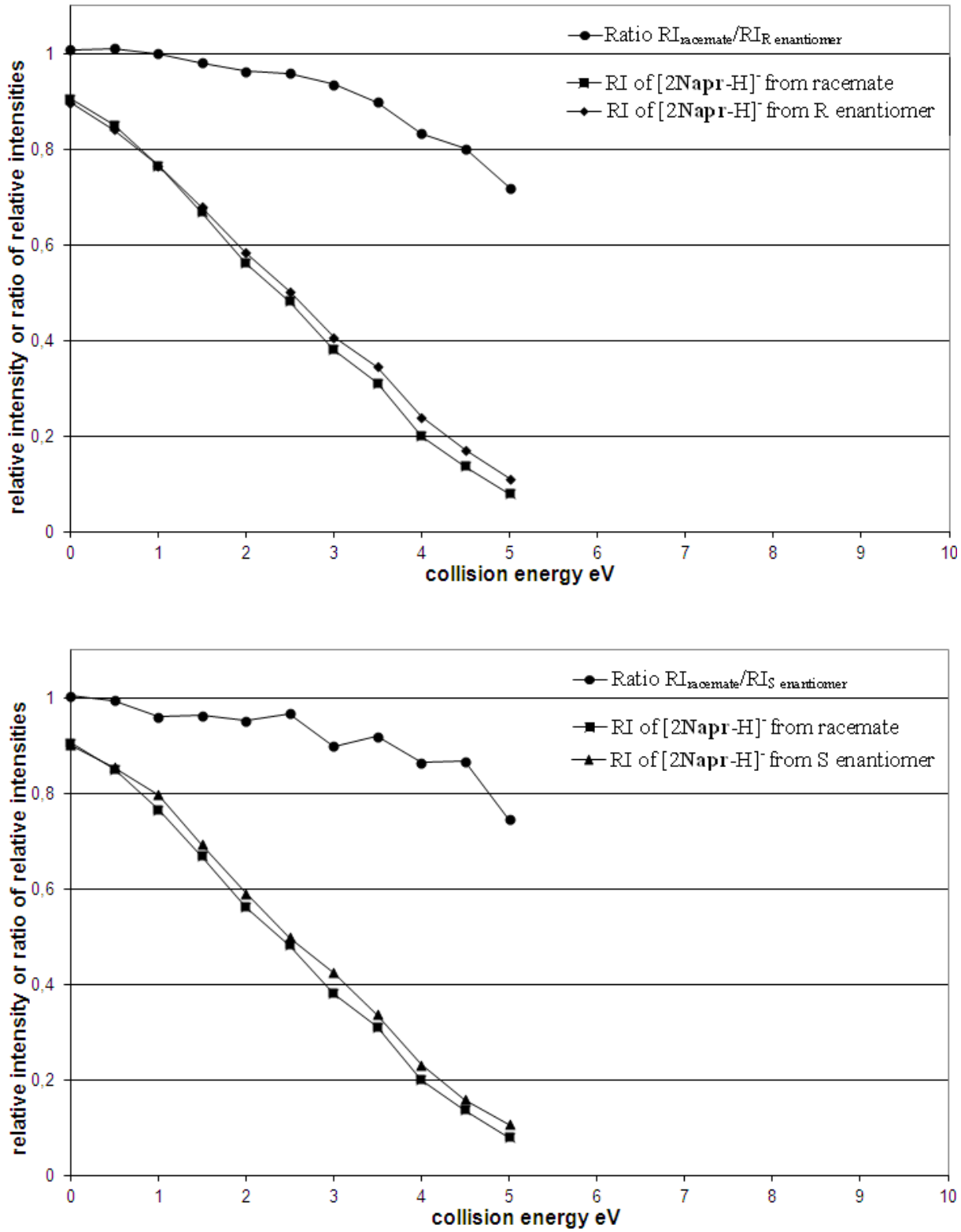


Figure 5. The relative intensities and the ratio of relative intensities of the anionic dimer [2Napr-H]$^-$ versus the collision energy.

CONCLUSION

The results of three analyses (direct inlet MS, HPLC/MS and CID MS/MS) have shown that naproxen dimer anions ([2**Napr**-H]$^-$) generated from pure enantiomers are more stable, under the conditions of analysis, than naproxen dimer anions generated from racemate. This finding indicates that, in dimmers, aromatic substituents interact. Most probably, we deal with repulsion and the repulsion is stronger in SR dimer than in SS and RR dimers. Our results do not allow answering the fundamental question on the orientation of the aromatic substituents in space (theoretical calculations at a high level could give the answer). Our finding does nor allow identification of the enantiomer, but may be useful if the problem is to differentiate between pure enantiomer or racemate. For this purpose the results of an unknown sample should be compared with the results obtained for racemate and pure enantiomers. The methodology presented here for naproxen may be used for other chiral compounds provided that respective interactions exist in relevant dimers.

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