

User Samples in the CSHS Workshop on Ion-Mobility Mass Spectrometry

On 14. and 15. February 2012, a CSHS workshop about ion-mobility mass spectrometry (IM-MS) took place in the Institute of Organic Chemistry and Biochemistry. With a total of 22 participants from all parts of Czech Republic, the resonance was overwhelming, and the organizers thank all participants for their interest and engagement.

In the interactive parts of the workshop, in total six samples from the participants were investigated. One of them was partially lost upon transport and from the rest left in the vial, we could only get a molecular ion signal. For a second sample, the signals of interest were too weak for a detailed analysis. For the remaining four samples, promising results were obtained which are briefly presented here (for the sake of confidentiality, no formula are displayed).

The first external sample was a rather complex extract from strawberry plants containing a wide variety of compounds, where the particular interest dealt with glycosylated polyphenols. Without any further purification, good IM-MS separations could be achieved for a variety of signals in the ESI mass spectrum of the sample. To this end, desired ions are selected from the source by means of a quadrupole mass filter, followed by a measurement of the ion-mobility traces of these specifically mass-selected ions (MS/MS type experiments with IM-MS). As an example, Figure 1 shows the IM-MS traces for mass selected ions at m/z 593 and 595, respectively. The ion at m/z 593 (red trace) has at least three components (at arrival times of ca. 4.9, 5.45, and 5.8 ms), while the signal at m/z 595 (blue trace) has two major components at 4.8 and 5.7 ms with raised baseline at about 5.2 ms and a shoulder above 6 ms indicating the presence of two additional minor components. This example demonstrates the significance of IM-MS for the analysis of complex mixtures. Combined with a HPLC in the front-end, multidimensional separation might be achieved.

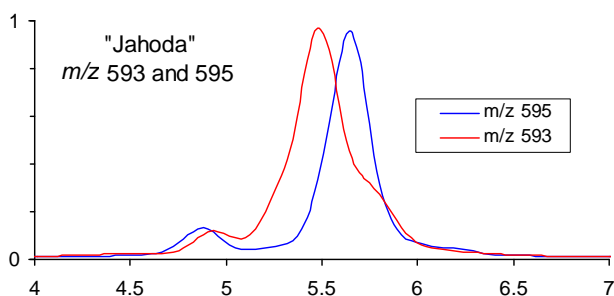


Figure 1. IM-MS traces of mass-selected m/z 593 and 595 in the negative-ion ESI mass spectrum of a strawberry extract.

The next sample dealt with a set of small molecules relevant within the detection of chemical warfare reagents. The task was to distinguish between different *N*-alkyl-substitutions. While similar patterns, e.g. *N,N*-diethyl versus *N*-methyl,*N*-propyl (both C_4 in total), could not be separated using ion mobility, the IM-MS profiles inter alia demonstrated an overlap of mono- and dicationic species in the ESI mass spectra which are tentatively assigned as $[M+H]^+$ and $[2M+2H]^{2+}$ in Figure 2; note that the dication denoted as $[2M+2H]^{2+}$ may also be an interference from one of the other samples rapidly measured in a row during the workshop. Irrespective of this uncertainty, Figure 2 shows the clean separation of the two isobaric components.

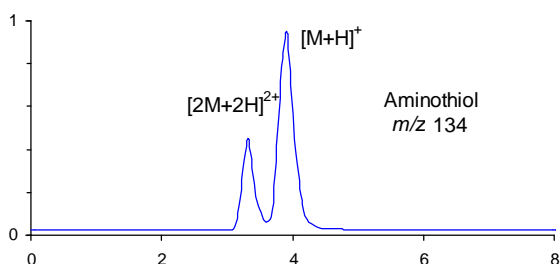


Figure 2. IM-MS traces of mass-selected m/z 134 in the positive-ion ESI mass spectrum of an aminothioliol. The protonated quasi-molecular ion $[M+H]^+$ shows a lower mobility than an interfering doubly charged ion (denoted as $[2M+2H]^{2+}$) co-generated upon ESI.

Another sample was a modified RNA fragment, for which previous HPLC analysis indicated a ca. 5:1 mixture of diastereoisomers. In this case, a reasonably clear separation of two components was achieved via IM-MS, although no baseline-separation could be realized in the short time of the workshop. Interestingly, the anions (m/z 363, blue trace in Figure 3) show the expected ratio of ca. 5:1, whereas the cations (red trace) are closer to 1:1. This indicates that in the positive ion mode the ionization efficiencies of the two diastereoisomers are different, thereby highlighting the importance of independent calibration in the quantitative analysis of IM-MS data.

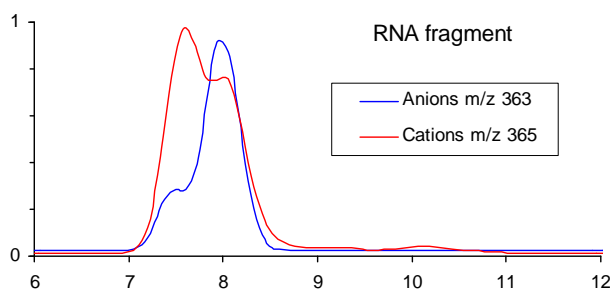


Figure 3. IM-MS traces of an RNA fragment for the mass-selected anion (m/z 363, blue) and cation (m/z 365, red).

For a sample containing three isomeric zeatines with m/z 380 for the deprotonated forms, we received a composite IM-MS profile, which could be modified by changing the source parameters. Because the differences between the isomeric ions were just double bond configuration and substitution pattern, respectively, this example demonstrates the power of IM-MS to easily differentiate isomeric ions with minimal experimental efforts.

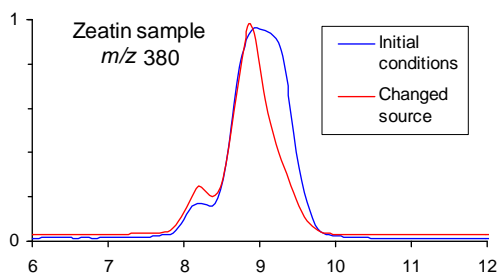


Figure 4. IM-MS traces of mass-selected m/z 380 in the positive-ion ESI mass spectrum of a zeatin sample. The protonated quasi-molecular ion $[M+H]^+$ shows a lower mobility than a doubly charged dimer $[2M+2H]^{2+}$ co-generated upon ESI.

In summary, the CSHS workshop on ion-mobility MS was not only successful in terms of the resonance from active researchers all over Czech Republic, but could also demonstrate the enormous potential of ion-mobility as a new dimension in mass spectrometry. If anyone else from the CSHS community would be interested in participating in a possible repetition of such a workshop in the future, please declare your interest in a formal email to schroeder@uochb.cas.cz.