Differentiation of Isobaric 3′-/5′-Oligonucleotide Clipping Products by Trapped Ion Mobility Spectrometry

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Introduction

Oligonucleotide analysis by mass spectrometry has gained significant interest recently with the increased use of RNA for pharmaceutical applications. Characterization of production related impurities or degradation products is of key importance to build an IND enabling package. LCMS is a well-established tool for the characterization of the side-products associated with the manufacturing of these synthetic molecules.

In our work, we use an additional technique, trapped ion mobility spectrometry (TIMS), to characterize oligonucleotide degradation products. By measuring the collisional cross sections (CCS) of the two isobaric oligonucleotide clipping products with an LC-TIMS-MS approach, we differentiate the molecules using an intrinsic biophysical property. Such measurements simplify method transfer as such parameters are independent from the analytical setup.

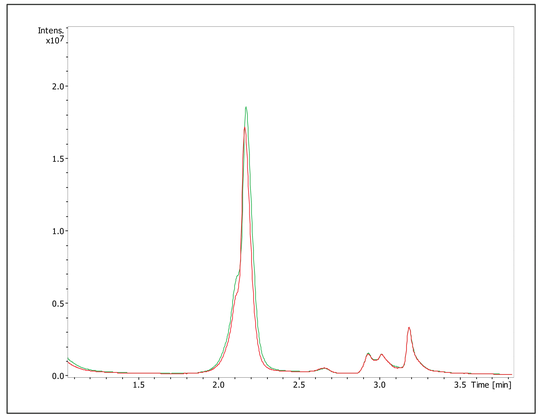

Fig. 1 Extracted Ion Chromatogram traces of isobaric 9mer oligonucleotides GCACGGCUC (red) and CACGGCUCG (red). No differentiation possible.

Methods

Two isobaric 9mer Oligonucleotides (Axolabs) were analyzed. These two forms are clipping products originating from a 10mer molecule with guanine as base at both termini. The two 9mer oligonucleotides are obtained by a loss of a guanosine nucleotide either at the 5′- or 3′-end, providing two isobaric molecules. The samples were measured with UPLC, utilizing a short gradient for desalting, connected to a MS instrument with TIMS functionality (timsTOF Pro2, Bruker) for differentiation based on the characteristics of the molecules' gas-phase behavior.

Extracted Ion Mobilogram (EIM) traces were created for the measured different charge states of each oligonucleotide. Collisional Cross Section values of detected charge states were then calculated to validate if a differentiation of these isobaric molecules is possible.

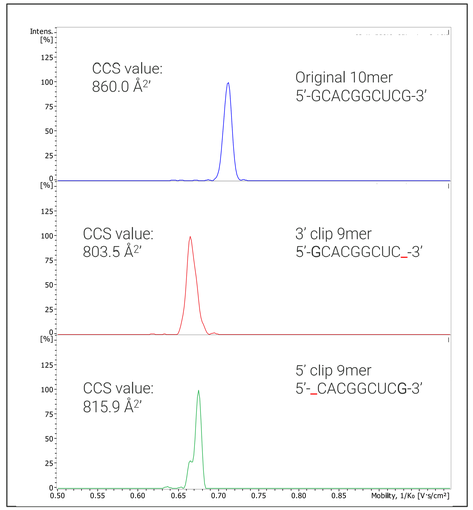

Fig. 2 Extracted Ion Mobilogram traces of charge state -6 of original 10mer Oligonucleotide and of isobaric 9mer clipping variants. Peak Maximum of clipping variants allows clear differentiation of the two molecules

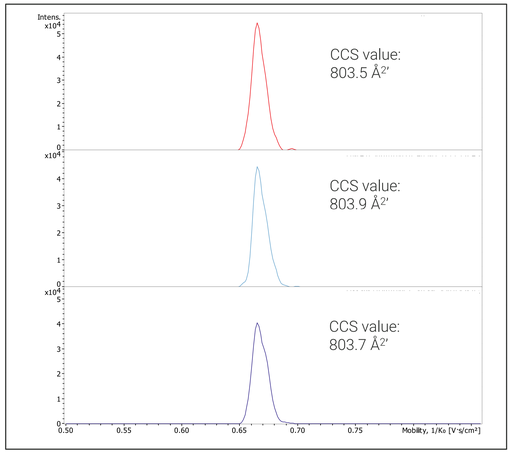

Fig. 3 Extracted Ion Mobilogram traces of three different injections of the 3′ clipping molecule. Shown is charge state -6. Highly consistent CCS values without need of data file recalibration.

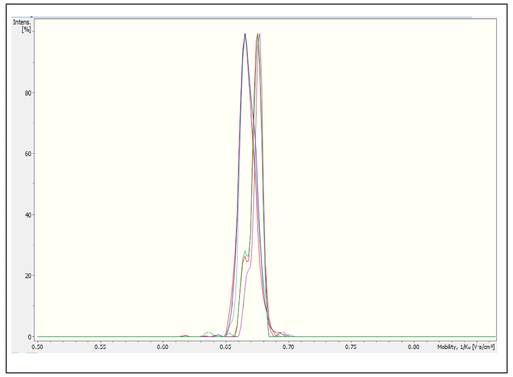

Fig. 4 Overlay of Extracted Ion Mobilogram traces of three injections each from the 3′ clipping and the 5′ clipping molecules. Clear differentiation by Trapped Ion Mobility Spectrometry (TIMS) and high reproducibility of obtained CCS values.

Results

- A short gradient was used to measure the 10mer product and two 9mer N-1 variants. No differentiation of the isobaric 9mer molecules was possible on the chromatographic time scale (Fig. 1).
- Use of the TIMS dimension and creation of extracted ion mobilogram traces revealed a clear differentiation of the two isobaric molecules (Fig. 2).
- Repeated measurements (n=3) provided highly reproducible CCS values for both oligonucleotides (Average CCS values: 3′ clip: 803.7 Å²; 5′ clip: 815.9 Å²) proving the accuracy of the TIMS technology for molecule characterization (Fig. 3 and 4).

Summary

An isobaric pair of Oligonucleotides, not distinguishable by the used chromatographic conditions, was measured with TIMS technology.

Differentiation of the isomeric molecules based on their behavior in the gas phase was achieved for selected higher charge states.

CCS values for both molecules could be determined with high reproducibility and this intrinsic molecule property can be used to identify which form is present in a sample.

Conclusion

- TIMS technology offers capabilities to differentiate isobaric oligonucleotide molecules based on the Collisional Cross Section of the molecules
- Differentiation of clipping variants from an oligonucleotide is providing an additional level of characterization capabilities
- The differentiation is based on selected charge states of the molecule. Higher charge states of oligonucleotides can be differentiated in the gas phase and offer characteristic CCS values

Technology