Peptide structure confirmation based on molecular weight and collision cross section obtained with trapped ion mobility separation

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Overview

• Quality control for synthetic peptides typically involves intact mass determination.
• Chromatographic variables (molecular weight and retention time) alone fail short if isomeric peptides need to be safely distinguished.

We used trapped ion mobility (TIMS) QTOF-MS data to determine collision cross sections (CCS) routinely alongside molecular weights as quality control (QC) parameters for synthetic peptides.

Data were automatically interpreted for QC purposes using multiple quality attributes within the BioPharma Compass 2021 software.

Several synthetic isomeric peptides, including Leu/Ile peptide isomers and µ-conotoxin PIIIA variants with different disulfide connectivities were used to establish CCS values as reference points for future identification or synthesis quality control.

Methods

Peptides: Two 18mer isomeric peptides from an antibody CDR (complementarity determining region) with an isobaric L vs. I exchange and five cyclic µ-conotoxin PIIIA variants were used (Fig. 1).

LC-TIMS-MS: Peptides were purified using short reverse HPLC gradients with 15 min cycle times and analysed by TIMS-MS on a QqQTOF Pro (Bruker).

Data analysis: Datasets were processed in BioPharma Compass 2021 (Bruker) and matched against previously obtained reference CCS values to distinguish isomeric peptides.

Conotoxin PIIIA Isomers

Native PIIIA (1), its isomer 5 and 3 disulfide-deficient µ-conotoxin PIIIA variants with variable disulfide connectivities (2, 3, 4) were also analysed (Fig. 2). They are indistinguishable by molar mass, MS/MS and retention time alone. TIMS established distinct mobilograms for each and enabled the identification based on the CCS values of the [M+H]+ ions.

Each dataset was tested for the presence of any of the isomers (Fig. 4) and the 5th attribute (CCS) only matched (green) if the correct isoform was present. The test for isoform 5 was also positive for the mixture of 1 and 5, indicating the suitability of the method for isomer detection in mixtures.

BioPharma Compass 2021 software (Bruker) and matched data analysis (Bruker).

Fig. 2 Legend to the quality attributes displayed in Figs. 3, 4. Sample CDR-L was tested with the Method to qualify CDR-L, which results in a perfect match of all attributes, except - perfectly correct - for CCS [%], thus highlighting the specificity of the analysis.

Fig. 3 Multi Attribute Analysis of 5 replicates of the peptides CDR-L and CDR-I, by methods that are specific for the CCS values of either peptide. Top: mobilograms show a distinct shift between the peptides. Bottom: LC and MS signals for an analysis of CDR-L with a method specific for CDR-L. Left: same analysis with a method specific for CDR-I.

Fig. 4 Analysis of the disulfide-bonded conotoxin PIIIA variants. Top: Mobilograms are shown for isomers 2, 3, 4. Centre: Results of the testing for the presence of variants 2-5. Bottom: FDR report of the test for variant 3 with matching Mr, Rt and CCS values (see legend in Fig. 2).

References

1. LC-TIMS-QTOF analysis permitted the reproducible determination of peptide CCS values with standard deviations smaller than 0.05% suitable to distinguish subtle isomer differences. Molecular weight, retention time and CCS were developed as acceptance criteria in the quality control of synthetic peptides which will facilitate cross-tab method transfer of peptide/substrance identification.

Conclusions

• Trapped ion mobility-LC-MS permitted to distinguish peptide isomers by mass and CCS in a format suitable for automatic isomer assessment.

• The observed rel. standard deviation of CCS value was 0.96%. Isomers with CCS values differing by < 0.5% were safely and automatically distinguished.


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