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

CDR Peptides

The CDR-derived peptide isomers delivered small differences in their CCS, which were distinguishable in an automatic workflow. While the CDR-I exerted a CCS of 477.3+/-0.3 Å², the CDR-L had a CCS of 479.3+/-0.3 Å². Thus, given the standard deviation of the CCS determination of 0.06 %, the difference between the two forms with a 0.41 % CCS allowed for their unequivocal distinction according to their CCS in automated analysis (***Fig. 2,3***).

This approach may prove useful for *de novo* antibody sequencing as the unequivocal identification of isomeric peptides based on MS/MS alone remains difficult.

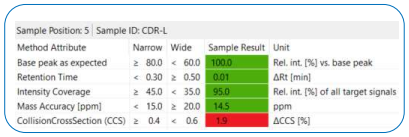

Fig. 2 Legend to the quality attributes displayed in ***Figs. 3, 4***. Sample **CDR-L** was tested with the Method to qualify CDR-I, which results in a perfect match of all attributes, except - perfectly correct - for ΔCCS [%], thus highlighting the specificity of the analysis.

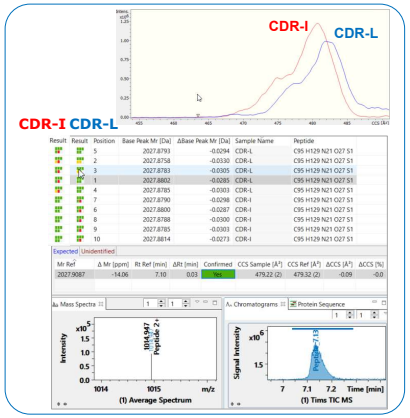

Fig. 3 Multi Attribute Analysis of 5 replicates of the peptides CDR-I and CDR-L by methods that are specific for the CCS values of either peptide. Top: mobilograms show a distinct shift between the peptides. Bottom: LC and MS signals for an analysis of CDR-L with a method specific for CDR-L. Left: same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

CDR-L: YYCLQYGSTPWTFGGGTK

CDR-I: YYCIQYGSTPWTFGGGTK

µ-conotoxin PIIIA: ZRL**CC**GFOKS**C**RSRQ**C**KOHR**CC**-NH_2

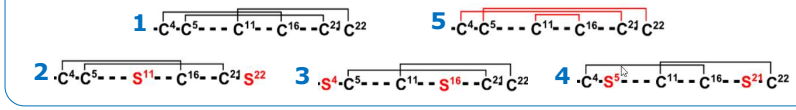

Fig. 1 Top Left: CDR-L and CDR-I are 2 CDR derived peptides that differ at position 4: Leu vs. Ile. Top Right: µ-conotoxin PIIIA. Bottom: Disulfide connectivity of the different disulfide-bonded variants: **1**: native PIIIA isomer, **5**: 3-disulfide bonded isomer, **2**-**4**: derivatives in which one disulfide bond of the native isomer was replaced by serine residues.

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. 1***). 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 $[M+5H]^{5+}$ ions.

Each dataset was tested for the presence of any of the isoforms (***Fig. 4***) and the 5th attribute (CCS) only matched (green) if the correct isoform was present.

The test for isomer **5** was also positive for the mixture of **1** and **5**, indicating the suitability of the method for isomer detection in mixtures [1].

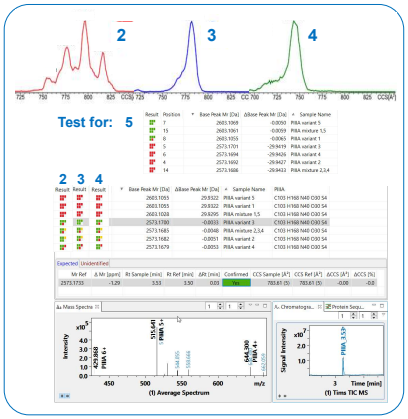

Fig. 4. Analysis of the disulfide-bonded conotoxin PIIIA variants. Top: Mobilograms are shown for isomers **2**,**3**,**4**. Centre: Results of the testing for the presence of variants **2**-**5**. Bottom: Full report of the test for variant **3** with matching Mr, Rt and CCS values (see legend in ***Fig.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

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-lab method transfer of peptide/substance identification.

References

1. LC-TIMS-TOF MS differentiation of 2- and 3-disulfide-bonded isomers of µ-conotoxin PIIIA (2020) T Schmitz, S Pengelley, E Belau, D Suckau, D Imhof, submitted.

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 0.06%. Isomers with CCS values differing by < 0.5% were safely and automatically distinguished.
- Leu/Ile differentiation in CDR derived peptides was achieved, which may prove useful in mAb *de novo* sequencing studies in the future.
- The presence or absence of the isomers were automatically determined using a traffic light reporting system, which allowed to detect isomers even in mixtures based on Mr, Rt and CCS values.

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