New annotation tools for advanced 4D-Lipidomics workflows

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Introduction

The annotation of lipids can be demanding due to the large number of structural variations. The mass spectrometry-based identification typically relies on characteristic fragments from headgroups and side chains obtained from MS/MS experiments. Depending on the quality of the MS/MS data, the depth of the structure elucidation can cover different levels like molecular formula level, chain composition level, etc. While the matching of MS/MS spectral libraries[1] gives a broad and quick overview on the lipid content, the annotation level can be too detailed. The presented tools avoid this risk of over annotation and simplifies the automatic identification of lipid features by using selected fragmentation rules. The result visualization as Kendrick Mass Defect (KMD) Plots[2] allows for a simple validation.[3]

Methods

The total lipid extracts from Liver, Brain and *E. coli* (Avanti Polar Lipids) were investigated using mobility-enhanced LC-MS/MS data acquired on a timsTOF Pro (*Bruker Daltonics*). LC-MS/MS data of the lipid extracts[4] were acquired as triplicate injections (technical replicates) in positive and negative PASEF mode. Even from single runs, an almost comprehensive MS/MS coverage was achieved. The raw data were processed with a prerelease version of MetaboScape® 2021 (*Bruker Daltonics*) using four-dimensional feature extraction. All important qualifiers such as exact mass, isotopic pattern quality, retention times, MS/MS spectra and CCS values were extracted automatically for all specified adducts and neutral losses by the T-ReX® 4D algorithm. The retention time aligned features were listed in a bucket table. To increase the confidence for lipid ID, data of both polarities were merged. The MS/MS spectra were annotated using a new rule-based annotation tool implemented in MetaboScape®. The lipid annotation algorithm used $[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[M-H_2O+H]^+$, $[M-H]^-$, $[M+HCOO]^-$ and $[M+CH_3COO]^-$ ions as a basis for assigning 24 sub-classes out of four main categories (Glycerolipids, Glycerophospholipids, Sphingolipids and Sterol lipids). The confidence of annotations was rated using the visual annotation quality scoring applied in MetaboScape® for all qualifiers available. Finally, the annotations were checked for consistency and for false hits using a 4-dimensional and CCS-Aware Kendrick mass defect plot.

References

(1) http://fiehnlab.ucdavis.edu/projects/LipidBlast
(2) Kendrick, E., Analytical Chemistry 35.13 (1963): 2146-2154.
(3) Korf, A, *et al.* Rapid Commun Mass Spectrom. 2018; 32: 981- 991
(4) Matyash V., *et al.* J Lipid Research, 2008, 49(5):1137-46
(5) Vasilopoulou, C.G. *et al.*, Nat Commun 11, 331 (2020).

Results

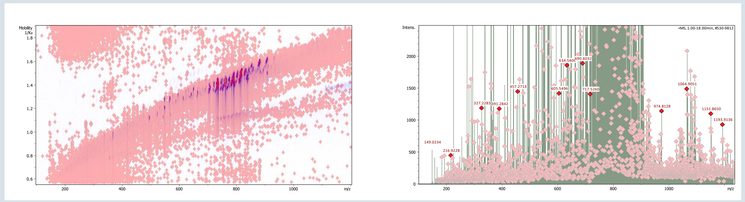


Figure 1 PASEF LC-MS/MS data generate comprehensive MS/MS coverage (red squares) from single injections – also from very low abundant precursors.

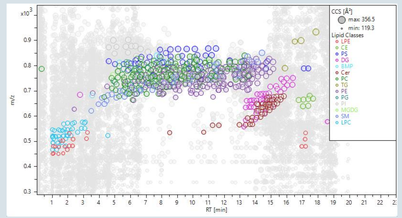


Figure 2 Overview on the annotated lipid classes in the total brain extract (pos mode). The CCS value is represented by the bubble sizes. Using this plot, apparent wrong annotations can be easily identified.

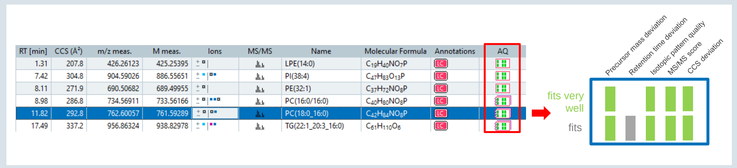
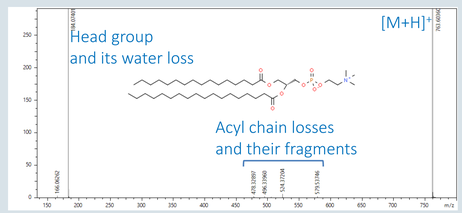


Figure 3 Screenshot of lipids with different levels of annotation. The AQ scoring (red box) represents the quality of exact mass, isotopic pattern and MS/MS coverage. The annotation of the selected lipid (blue bar) is explained in detail below



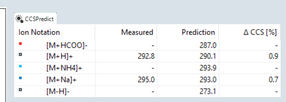


Figure 4 Rule-base annotation of PC(16:0_18:0) in positive mode. The head group qualifies the class, while the chain composition is based on acyl chain fragments

Figure 5 CCSPredict in MetaboScape can be used to increase the annotation confidence. The predicted values match very well.

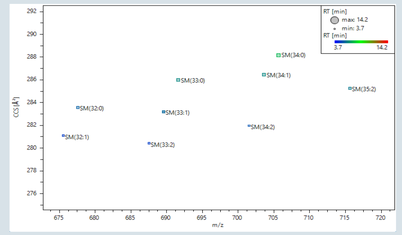


Figure 6 Plotting CCS as a function of m/z shows the benefit of the mobility dimension: lipids follow trend lines according to their saturation level (E. coli, ESI positive).

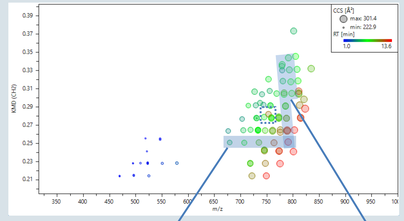
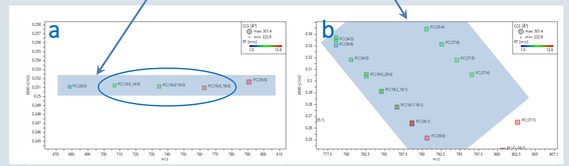


Figure 7 The KMD plot of an *E. coli* extract was filtered for PC lipids. It shows the RT using a color coding and the CCS values are represented by the bubble size. LPCs show consistently smaller CCS (bubble size) than PCs. An outlier in RT in the PC data is highlighted in the blue dotted box.

Figures 8 (a & b) The KMD plot can be used to analyze series or families with either similar KMDs (horizontal lines, a) or as diagonal lines (b). Some annotations show sufficient MS/MS spectra to annotate on a chain composition level (Figure 4 & blue circle in Figure 8a).



Conclusions

- The presented rule-based lipid class annotation allows for a **reliable and confident annotation** of lipids from 24 subclasses
- Mobility-enhanced PASEF LC-MS/MS data generates **comprehensive MS/MS coverage** from a single injection, enabling lipid annotation with higher confidence
- **CCS-Aware Kendrick Mass Defect** plots simplify the verification of lipid classes and the search for non-annotated candidates
- Acquired **CCS values** can be matched to predicted values or public repositories with high accuracy
- The presented **4D-Lipidomics™ workflow** enables deep profiling of lipid extracts from different sources

4D-Lipidomics™ on timsTOF Pro