Quality control tools to increase the confidence in lipid annotations

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Introduction

The field of lipidomics is attracting more and more interest in the research community, as the essential role of lipids in the emergence and progression of diseases is better understood. And with this, also the need for a standardized and high-quality reporting of lipid annotations is getting more important.

The usage of improved acquisition techniques increases the coverage and quality of the data from mass analyzers – and with this the total number of compounds that can be annotated. These increased numbers eventually need quality control, ideally automated and graphically clearly presented. Therefore, any tool that can be used to efficiently remove false annotations will simplify the researchers work.

Annotation of Lipids. In general, lipids can be annotated using different approaches. For instance, via MS/MS spectral library matching as usually applied in Metabolomics. If available, also CCS values will be utilized, like for the CCS-containing LipidBlast[1] library. To increase the confidence, library-free tools such as e. g. a rule-based annotation can be applied. This is more generic and will reduce false positives as specific fragmentation rules are applied to the acquired fragment spectra. MetaboScape offers all of the above-mentioned procedures to achieve high-quality annotations (**fig. 1a**). In addition, CCS values are automatically predicted for lipids assigned by the rule-based approach. This feature uses regression models, based on acquired CCS values. The additional CCS value serves as a further qualifier for annotation.
Importance of CCS values. If CCS is plotted against m/z, homologous series of lipids show trend lines (**fig. 1c**). CCS values are independent of Rt and can be used to intertwine for instance LC- and MALDI-data (SpatialOMx workflow) or different chromatographies.

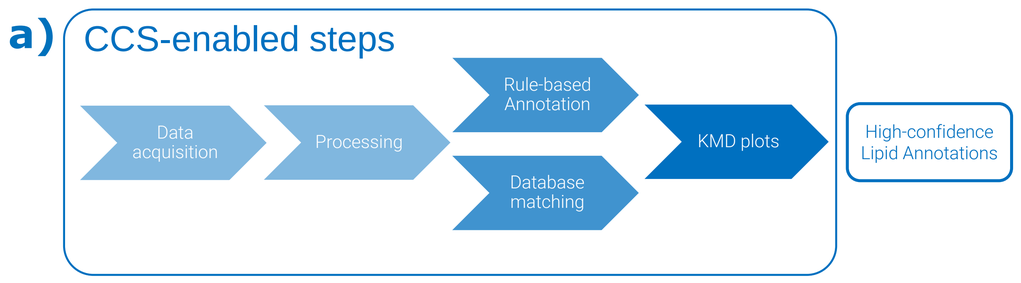
Sample preparation

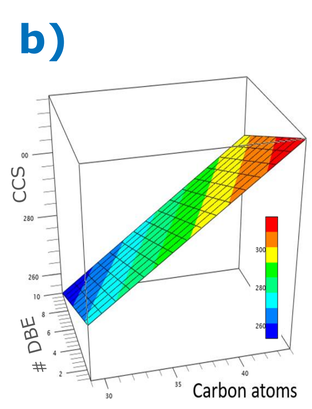
Lipids were extracted from anonymized skin fibroblast (corresponding to 1 mg of protein) following a procedure described in [2].

Methods

- timsTOF Pro 2 coupled with Elute UHPLC (*Bruker*),
- Bruker intensity C18 column (100 x 2.1 mm, 1.9 µm). 20 minute LC gradient
- Injection volume: 5 µL
- PASEF acquisition mode in ESI-(+) and ESI-(-) mode
- preliminary version of MetaboScape 2022a (*Bruker*) for 4D-processing, lipid annotation and quality control.

CCS values in the 4D annotation of lipids





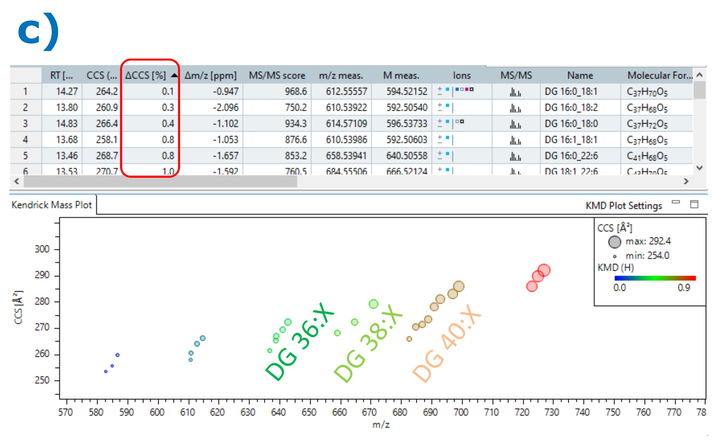


Fig. 1a: 4D-Lipid profiling in MetaboScape. The collisional cross section value is a physical property of an ion species in a gas phase. As such, CCS values can improve the annotation process as additional qualifiers. In MetaboScape 2022, several CCS-enabled annotation workflows can be used.

Fig. 1b: Automatic prediction of CCS values based on CCS hyperplanes is implemented in MetaboScape 2022. As a basis, multiple linear regression models are calculated using acquired CCS values as training data.

Fig. 1c Resulting CCS deviations can be used as rating factor in the result table: all annotations with deviations > 2% should be manually reviewed. Homologous series of lipids show trend lines in the mobility dimension.

Advanced 4D-Kendrick Mass Defect Analysis

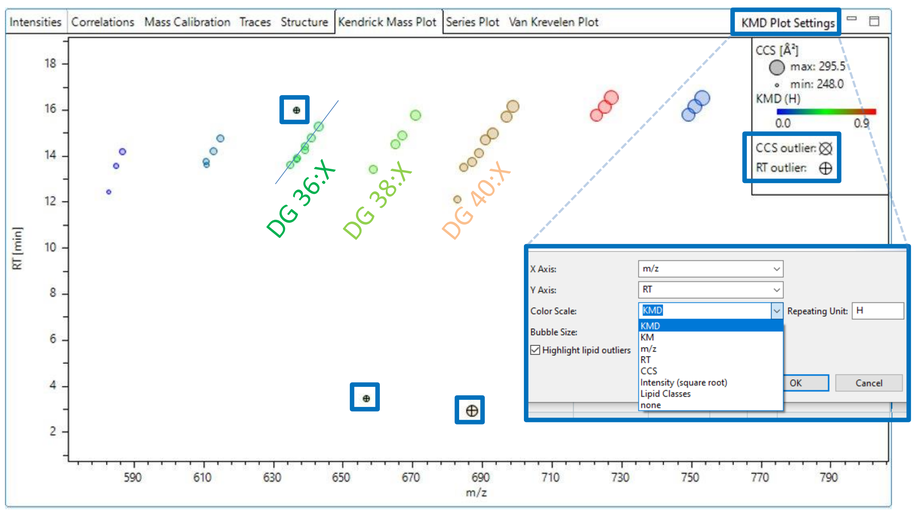


Fig. 2: Kendrick Mass Defect Plot filtered for DG lipids. The selected dimensions (x: *m/z*, y: Rt, color: KMD(H), bubble size: CCS) simplify the investigation of homologous series. The KMD(H) was plotted as color gradient to separate series of DGs with identical numbers of carbon atoms and different number of double bonds (X) in the side chains.

Kendrick Mass Defects

Kendrick Masses (**KM**) and Kendrick Mass Defects (**KMD**) are methematical transforamtions with the aim to simplify the analysis of homologous compounds differing only by one or more repeating units, e.g CH_2. In MetaboScape, KMD plots can be adjusted to address different scientific questions and to serve as a data validation tool. In **fig. 2**, KMDs were calculated for the repeating unit **H** to group lipids of the same class and with the same number of carbon atoms in all side chains together - indicated by the same color. By plotting the retention time on the y-axis, almost linear trends, typical for lipids in RP chromatography, are observed. This enables a quick evaluation for possible outliers.

Outlier Detection

Retention time and CCS outliers are automatically identified and labelled using regression models with dynamic cross validation. In **fig. 2**, three retention time outliers were automatically marked (blue rectangles).

Rule-based lipid annotation setup

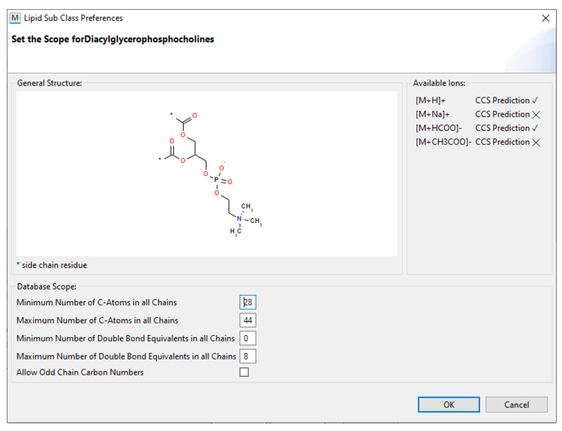


Fig. 3: Personalized lipid database The parameters for the lipid annotation can be personalized to match the scientific question. Easily, VLCFAs (Very Long Chain Fatty Acids) can be enabled, or odd side chains allowed. Ions where CCS predictions are available are mentioned.

References

1) Fiehn et al., Tsugawa et al. https://www.nature.com/articles/s41587-020-0531-2
2) Molenaars *et al., Dis Model Mech* **2021,** 14 (**4**)

Conclusions

timsTOF-based 4D-Lipidomics workflows take full advantage of the mobility separation:

- In MetaboScape, CCS values are predicted automatically for assigned lipids. The deviations can be used to filter possible false positives
- Retention times as well as CCS-values are used for outlier detection based on advanced regression models
- 4D-Kendrick Mass Defect plots simplify deep analysis of results

4D Lipidomics

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