Quality Control Tools to increase the confidence in Lipid annotations

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Introduction

The field of lipidomics is attracting an increasing interest in the research community, as the understanding of the role of lipids in the emergence and progression of diseases is essential.

The use of improved acquisition techniques can increase 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. Therefore, any tool that can be used to efficiently remove false annotations will simplify the researchers work.

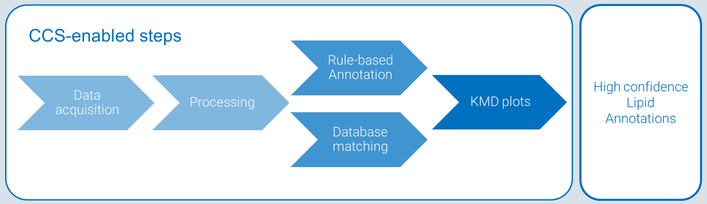
The collisional cross section (CCS) value is a physical property of an ion species in the gas phase. As such, CCS values can act as orthogonal qualifiers and improve the annotation quality. In MetaboScape 2022, different CCS-enabled annotation workflows can be used to achieve high-quality annotations (**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 (**fig. 1b**). The additional CCS value serves as a further qualifier for annotation. 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 different chromatographies or LC- and MALDI-data (in a so-called *SpatialOMx* workflow).

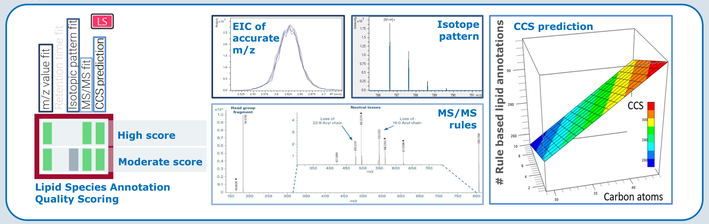
Sample preparation

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

1a



1b



High confident annotations

Fig. 1a: 4D-Lipid profiling in MetaboScape
In MetaboScape, lipid annotations can be achieved via a library-free rule-based annotation tool ("Lipid Species Annotation"). This tool uses a generic approach which can reduce the number of false positive annotations because specific fragmentation rules are applied to the acquired fragment spectra. Alternatively, MS/MS spectral libraries can be matched, like usually applied in Metabolomics projects. When using CCS-containing libraries, like e.g. the MS-Dial LipidBlast[1] library, CCS values can be utilized.

Fig. 1b: Lipid Species Annotation Annotation Quality Scoring
For lipids annotated via lipid species annotation, accurate m/z, isotope pattern, rule-based matching of lipid class specific MS/MS fragments, and automatic prediction of CCS values is applied. The latter uses CCS hyperplanes, where multiple linear regression models are calculated as a basis, using acquired CCS values as training data. The predicted CCS value serves as additional qualifier in the AQ Scoring.

Fig. 1c CCS deviations
The resulting CCS deviations can be used to filter the result table: all annotations with deviations > 2% should be manually reviewed. The CCS values can increase the annotation confidence considerably.

1c

	RT [...	CCS (...	ΔCCS [%]	Δm/z [ppm]	MS/MS score	m/z meas.	M meas.	Ions	MS/MS	Name	Molecular For...
1	14.27	264.2	0.1	-0.947	968.6	612.55557	594.52152			DG 16:0_18:1	$C_{37}H_{70}O_5$
2	13.80	260.9	0.3	-2.096	750.2	610.53922	592.50540			DG 16:0_18:2	$C_{37}H_{68}O_5$
3	14.83	266.4	0.4	-1.102	934.3	614.57109	596.53733			DG 16:0_18:0	$C_{37}H_{72}O_5$
4	13.68	258.1	0.8	-1.053	876.6	610.53986	592.50603			DG 16:1_18:1	$C_{37}H_{68}O_5$
5	13.46	268.7	0.8	-1.657	853.2	658.53941	640.50558			DG 16:0_22:6	$C_{41}H_{68}O_5$
6	13.53	270.7	1.0	-1.592	760.5	684.55506	666.52124			DG 18:1_22:6	$C_{43}H_{70}O_5$

Fig. 3

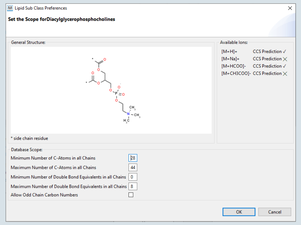
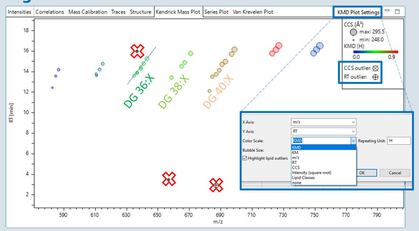


Fig. 2



Data evaluation

Fig. 2: Kendrick Mass Defect (KMD) analysis
KMD 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. Red crosses highlight the automatically detected and labelled outliers.

Fig. 3: GUI to setup Rule-based lipid annotation
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. The legend lists the ions where CCS predictions are available.

References

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

Kendrick Mass Defect analysis

Kendrick Masses (**KM**) and Kendrick Mass Defects (**KMD**) are mathematical transformations of the exact masses, using repeating units as a basis. They can 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 are observed, which is typical in RP chromatography. This enables the evaluation and detection of outliers using regression models with dynamic cross validation. In **fig. 2**, three retention time outliers are marked (red crosses), that were automatically labelled.

Methods

- timsTOF Pro 2 coupled with Elute UHPLC (*Bruker*),
- Bruker intensity C18 column (100 x 2.1 mm, 1.9 µm). 5-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.

Conclusions

timsTOF based 4D-Lipid profiling 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 Lipid Profiling