Novel workflow for improved sensitivity and annotation quality of eicosanoid LC-MS-based analysis

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Introduction

Eicosanoids are important signaling molecules and are correlated with pathological processes like inflammations. Commonly, they can be detected in urine or lipophilic extracts, for example.

The hydroxyeicosatetraenoic acids (HETE) are a subgroup of the eicosanoids and they are key precursors to many bioactive metabolites. Due to their chemical structures, hydroxy-eicosatetraenoic acids like 15(S)-HETE or 20-HETE are relatively labile and their LC-MS-based analyses needs optimized methods for a gentle desolvation and ionization.

We present enhanced sensitivity for eicosanoid analysis using a new heated ESI source. Additionally, the use of TIMS (trapped ion mobility spectrometry) separated QTOF data is discussed with respect to data quality and annotation confidence.

Methods

Eicosanoid standards (*Cayman Chemical*) were diluted in a range of 0.1-1000 ng/mL to acquire a dilution series (MeOH/ACN/H_2O/0.1% FA 47%/47%/6%). Also, the standards were spiked into diluted urine (1:100) at different concentration levels. Samples were separated by 6-minute RP chromatography.

LC parameters (*Bruker* Elute UHPLC, Intensity Solo HPLC column, C18, 2x100mm, 50°C column temp., 2 µL injection volume, solvent A: 0.1% FA in H_20, solvent B: 0.1% FA in ACN, 400 µL/min flow, linear gradient from 2% B (0.5 min) to 100% B (4 min) with 1 min wash and 1 minute reequilibration time.)

4D ESI-(-) MS data was acquired using a TIMS-MS setup (timsTOF Pro 2, *Bruker*) with either ESI or heated VIP-HESI sources. PASEF acquisition mode (DDA-TIMS-MS/MS) was applied. Source gas and ionization voltage settings were lowered to enable mild desolvation conditions.

Data processing was performed using preliminary versions of the TASQ 2023 and MetaboScape 2023 software (*Bruker*). Annotations of urine metabolites were performed with target lists containing compound name and molecular formula as basic input. Additionally, structural information (InChI) was used to automatically generate in-silico fragment spectra and predict CCS values.

Results

The standard PASEF MS/MS settings utilize a duty cycle of 0.3 s (1 MS + 2 MS/MS, 100 ms each). Thus, a 6 minute LC gradient will return about 7-8 MS data points across a 2.5 s LC peak. Using data acquired with a heated ESI source, the LOD for the hydroxy-eicosatetraenoic acids improved from 1 ng/mL down to 0.3 ng/mL.

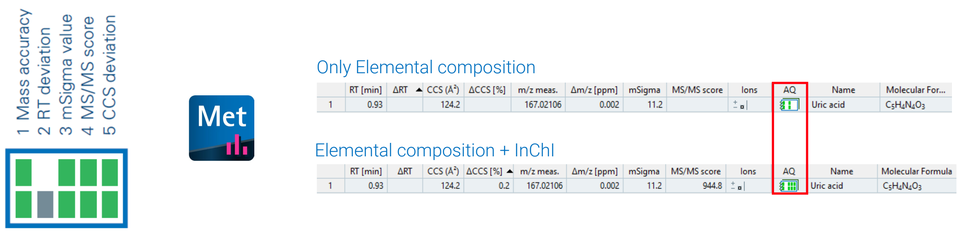


Fig. 2a
Visual reporting of annotation quality: The matching of certain "qualifiers" is represented in the annotation quality icon (AQ icon, **left**). If the data lies within a (user-defined) wide or narrow range, one or two "bars" in the respective column are displayed. Ranges can be set by the user. **Right side:** Feature annotation based on elemental composition only (top line) and on elemental composition as well as the matching with computed CCS values and MS/MS spectra (bottom line). The AQ symbol displays the increased confidence levels that can be achieved.

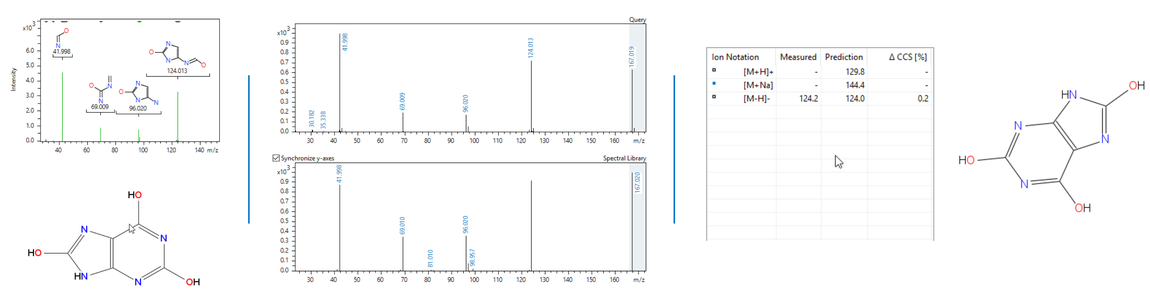


Fig. 2b
Left: Annotating acquired MS/MS spectra with in-silico generated fragments. **Middle:** Matching MS/MS with spectral library spectrum. **Right:** Matching predicted CCS values automatically. The resulting deviation can be used to sort out false positives.

Even at these low concentration levels, CCS values deviate with < 2% [Å²]. This enables the use of mobility-filtered and therefor cleaner chromatogram traces and returns cleaner MS/MS spectra (fig. 1). In the end, this improves data quality for targeted screening.

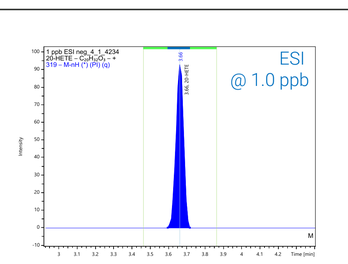
Likewise, CCS values can improve the quality of annotations in untargeted profiling studies. Either if the annotation databases contain such reference information, or if CCS values can be predicted on the fly.

MetaboScape was used to process the 4D-data for an untargeted profiling of the endogenous compounds in urine. It enables the generation of computed cross section values as well as *in-silico* generated fragment spectra if structural information is available (e.g. InChI code). This can enhance the annotation confidence, especially when using "low quality" databases. Also, it simplifies the process of reviewing results and removing false positives. To evaluate the influence of the additional computed qualifiers, an HMDB-based target list containing > 4700 compounds was used for the annotation of spiked urine data.

With elemental compositions as the only input for identification (i.e. use of m/z and isotopic pattern quality), >300 annotations returned. These rather large numbers have to be handled with caution and need to be checked thoroughly.

Alternatively, the same database containing in addition structural information (InChI code) returned about 130 unique annotations. These results still need checking. But due to the use of computed CCS values and fragment spectra, they are more solid. In addition, the process of reviewing annotation results and of removing false positives is simplified.

Ultimately, using automatic prediction tools increases the user's confidence in the achieved results.



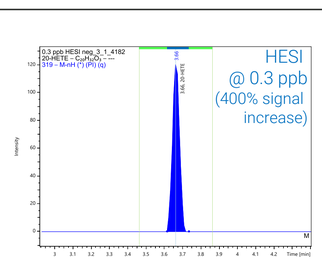
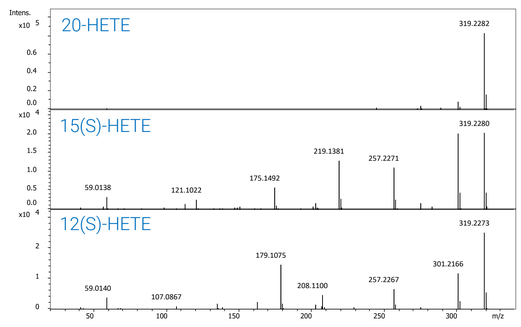


Fig. 1a
Comparison of 20-HETE data: ESI 1 ng/mL (left) and HESI 0.3 ng/mL (right).
For standard electrospray ionization, 1 ng/mL is the lowest detectable concentration level. VIP-HESI data show a 3-4x lower detection limit. (increase in peak signal 400%)



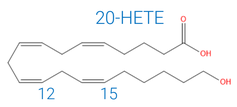


Fig. 1b
PASEF MS/MS spectra from 20-HETE, 15(S)-HETE and 12(S)-HETE (top to bottom)

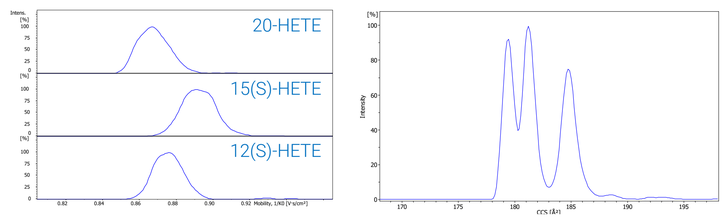


Fig. 1c
Mobilogram traces of HETE compounds acquired with the standard PASEF method. Refining TIMS parameters and tailoring them for increased resolving power (~135) improves the separation achieved.

Conclusion

Increased sensitivity and annotation quality for eicosanoid analysis using rapid chromatographic separation

- Increased sensitivity for eicosanoid standards based on heated ESI
- Improved screening and annotation quality due to additional CCS
- Use of structural information to improve untargeted profiling using "low quality target lists"

Technology