ASMS 2022, MP 316

Novel workflow providing improved sensitivity and annotation quality for eicosanoid analysis based on heated ESI source and PASEF data acquisition

Sven W. Meyer[1], Aiko D. Barsch[1], Nikolas Kessler[1], Ansgar Korf[1], Matthew Lewis[1]
[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany

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.

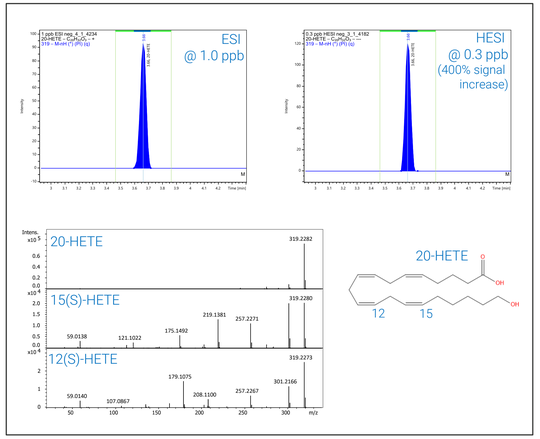

Fig. 1 Comparison of ESI to VIP-HESI data of 20-HETE: ESI (top, left) 1 ng/mL and HESI (top, right) 0.3 ng/mL. For standard electrospray ionization, 1 ng/mL is the lowest detectable concentration level. VIP-HESI data show a 3-4x lower detection limit.
Bottom: PASEF MS/MS spectra from 20-HETE, 15(S)-HETE and 12(S)-HETE (top to bottom)

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 5-minute RP chromato-graphy (*Bruker* Elute UHPLC, Intensity Solo HPLC column, C18, 2, 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 steps.)

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.

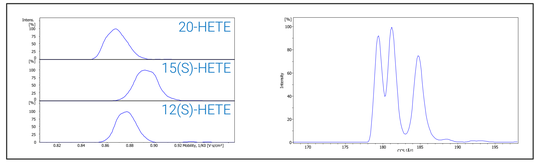

Fig. 2 Mobilogram traces of HETE compounds acquired with the standard PASEF method. Refining TIMS parameters and tailoring them for increased resolving power (~135) improves the separation achieved.

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 hydroxyeicosatetraenoic acids improved from 1 ng/mL down to 0.3 ng/mL. Even at these low concentration levels, CCS values deviate with < 2% [Å²] in TASQ. This enables the use of mobility-filtered and therefor cleaner chromatograms and returns clean MS/MS spectra (fig. 1). In the end, this improves the quality of targeted screening.

Likewise, CCS values can improve the quality of annotations in untargeted profiling studies. Either if the annotation databases contain such reference data, or if automatically predicted CCS values can be applied for matching.

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.

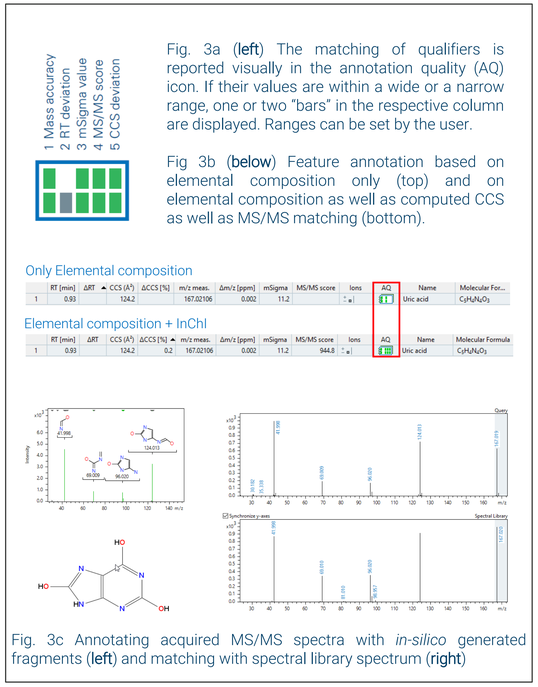

Fig. 3c Annotating acquired MS/MS spectra with *in-silico* generated fragments (left) and matching with spectral library spectrum (right)

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 users confidence in the achieved results.

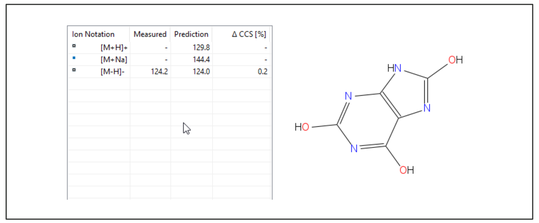

Fig. 4c Machine learning based prediction of CCS values is used to match CCS values automatically. The resulting deviation can be used to sort out false positives

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"

Mobility-enhanced analysis of eicosanoid compounds