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Novel data processing workflow including in-silico derivatization for integrated CI & EI MS data acquired with the GC-ecTOF instrument

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Introduction

Combined suspect and non-target GC-MS analysis of volatile and semi-volatile compounds holds relevance for environmental monitoring & research, food, flavor & fragrance analysis as well as metabolomics studies. The combination of Chemical Ionization (CI) and Electron Ionization (EI) in the ecTOF instrument (Bruker Daltonics, Bremen, Germany) can improve the identification confidence of unknown compounds, especially in complex samples and when no standards are available. The simultaneous usage of two complementary ionization techniques brings additional advantages for quantification workflows. To leverage these benefits, a novel data processing approach has been developed, and applicability is shown in two study designs.

Methods

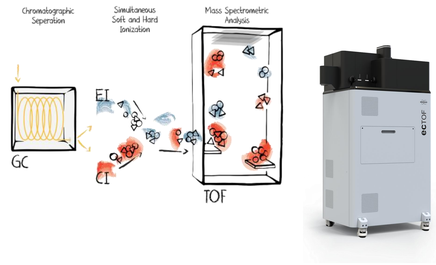
Derivatized samples from two studies were analyzed using the GC-ecTOF instrument. Data were converted to TSF format and processed with prototype versions of MetaboScape and TASQ. Untargeted feature detection was performed on the CI data stream, including de-isotoping, de-adducting for CI-specific ions $[M-H]^+$, $[M]^+$, $[M+H]^+$, $[M+NH_4]^+$, $[M-CH_3]^+$ and retention time alignment across all samples.

As applicational case study mice wound samples were derivatized using a two-step MeOX/MSTFA workflow, while fecal samples were prepared using MTBSTFA as derivatization reagent. An *in-silico* MTBSTFA model supported target list annotation based on accurate mass and isotopic pattern matching. Narrow and wide mass accuracy thresholds utilized for annotation were set to 2.0/5.0 mDa, with mSigma limits of 20/100.

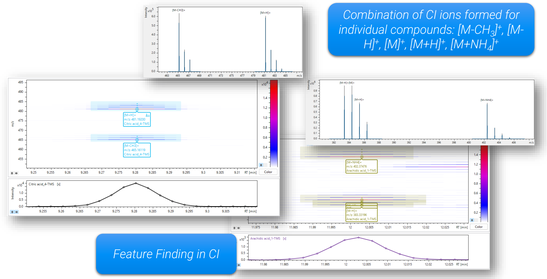
Literature
[1] NIST Reference Material 8048 Human Fecal Material - REFERENCE MATERIAL INFORMATION SHEET
https://tsapps.nist.gov/srmext/certificates/8048.pdf
[2] C Ruttkies, EL Schymanski, S Wolf, J Hollender, S Neumann (2016) MetFrag relaunched: incorporating strategies beyond in silico fragmentation Journal of Cheminformatics 8:3. DOI: 10.1186/s13321-016-0115-9

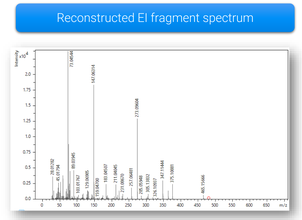
Results & Discussion

1) Untargeted feature finding compounds detected in CI data



Non-targeted feature finding is performed including de-isotoping and combination of typical adducts and CI fragments. Hereby, we obtain accurate mass and isotopic pattern information for all detected features in CI. Next, these features are registered across samples, and a consecutive retention time alignment is computed across all samples of the respective study.





Next, MetaboScape reconstructs and assign EI fragmentation spectra to features previously detected in CI. Here, a weighted average spectrum with reference to monoisotopic EIC of CI signal is computed from EI spectra coeluting with the CI feature.

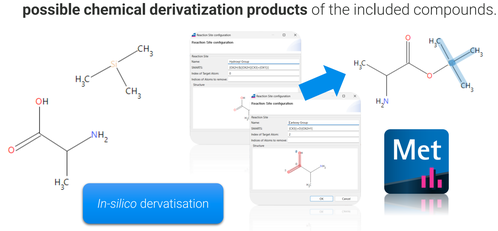
2) Annotation of compounds detected via CI profiling through MetaboScape's *in-silico* derivatization and *in-silico* fragmentation workflow

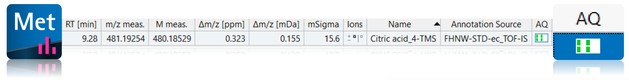
The starting point for the profiling workflow in MetaboScape is a list of target compounds with name, neutral molecular formula and structure (i.e. InCHI/SMILES).

For the feces samples, we utilized the compound information provided in the reference material sheet of the **NIST SRM 8048** [1].

Next, *in-silco* derivatization and *in-silico* fragmentation is applied on these structures in MetaboScape to create putative annotations of detected features, circumventing limitations of existing nominal mass EI spectral libraries.

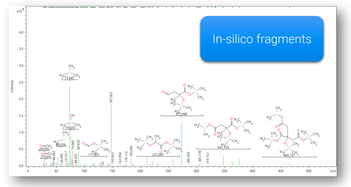
Target List **is expanded on the fly by *in-silico* derivatization** for **all possible chemical derivatization products** of the included compounds.



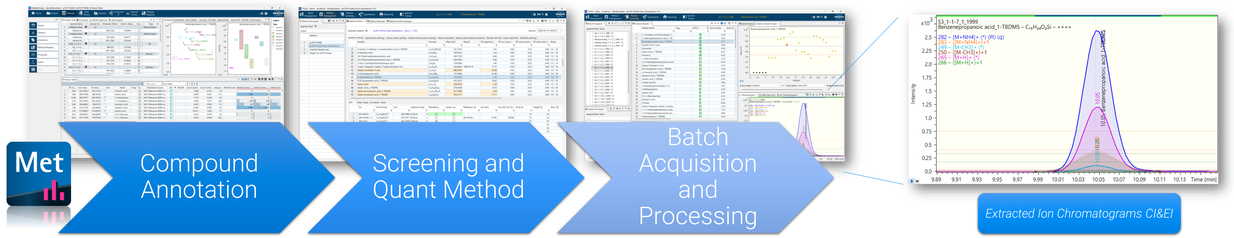


During automated annotation, matching of accurate mass and isotopic patterns as well as **automatic *in-silico* derivatization and fragmentation** is applied enabling multi-criteria evaluation and annotation of candidate structures [2].

Orthogonal information from both accurate mass CI ions as well as EI fragment ions contribute to annotation confidence in MetaboScape for ecTOF profiling experiments.



3) Seamless creation and application of Target Screening methods in TASQ software



Compound annotations in MetaboScape can now seamlessly be transfered to TASQ target screening and quantitation methods, a starting point for routine assays.

MetaboScape automatically creates a TASQ method containing:

- **Name, Molcular formula and Structure**
- **Retention time**
- **Accurate mass definitions of CI ions**
- **Accurate mass definitions of unique and abundant EI ions**

The TASQ software automates sample acquisition and data processing. The defined method can thereby be applied on complete batches of samples, reconstructing and assessing informative EIC traces from both EI and CI signals.

Confidence in identifications is obtained through accurate mass, isotopic fidelity and co-eluting characteristic ions in both CI and EI.

Note and Disclaimer:

- Authors Cory Lytle, Heiko Neuweger; Nikolas Kessler; Ilmari Krebs; Sonja Klee, Steffen Bräkling are employees of Bruker Corporation or one of its subsidiaries ("Bruker"). Bruker manufactures and sells analytical instruments including mass spectrometers and software. Bruker mass spectrometers and software were used in this study.

Conclusion

- Untargeted feature finding and streamlined annotation of ecTOF supported by *in-silico* derivatization and *in-silico* fragmentation extends GC-MS analysis for metabolomics and non-target screening.
- *In-silico* derivatization permits complementary annotation for compounds lacking reference standards.
- Simplified process from identified markers in profiling experiments to target screening and quantitation methods.

Metabolomics by GC-ecTOF

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