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TIMS-enabled 4D-Metabolomics workflow for the automated analysis of derivatized analytes

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Introduction

Analysis of small hydrophilic molecule metabolites such as those in the TCA cycle are challenging for RP-LC-MS analysis, because of low LC retention and often low sensitivity. Chemical derivatization is an increasingly popular technique for improving LC retention and separation from matrix salts while also potentially improving sensitivity of detection. However, derivatization also substantially increases the complexity of the raw data and its interpretation, potentially confounding accurate metabolite annotation. Here we present an automated workflow that combines *in-silico* derivatization of library structures with CCS prediction and *in-silico* fragmentation to enable confident analysis and annotation of derivatized metabolites.

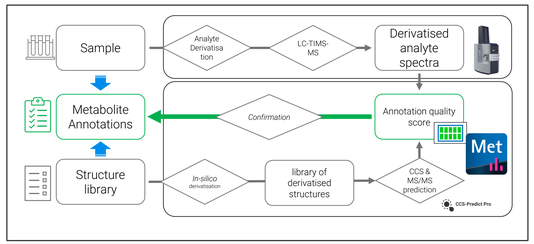

Fig. 1 Schematic of *in-silico* derivatization workflow supporting non-targeted metabolomics data evaluation.

Methods

Human plasma (SRM 1950 and plasma from inborn errors of metabolism) were extracted with 80% methanol and derivatized using 3-nitrophenylhydrazine (3-NPH) following [1]. Extracts were separated using RP chromatography. Data was acquired on a timsTOF HT in negative ionization mode using PASEF (DDA-TIMS-MS/MS) and processed using a preliminary version of the MetaboScape 2025 software (Bruker Daltonics GmbH & Co KG).

Results #1 – *In-silico* derivatization workflow exemplified on NIST SRM 1950 reference plasma

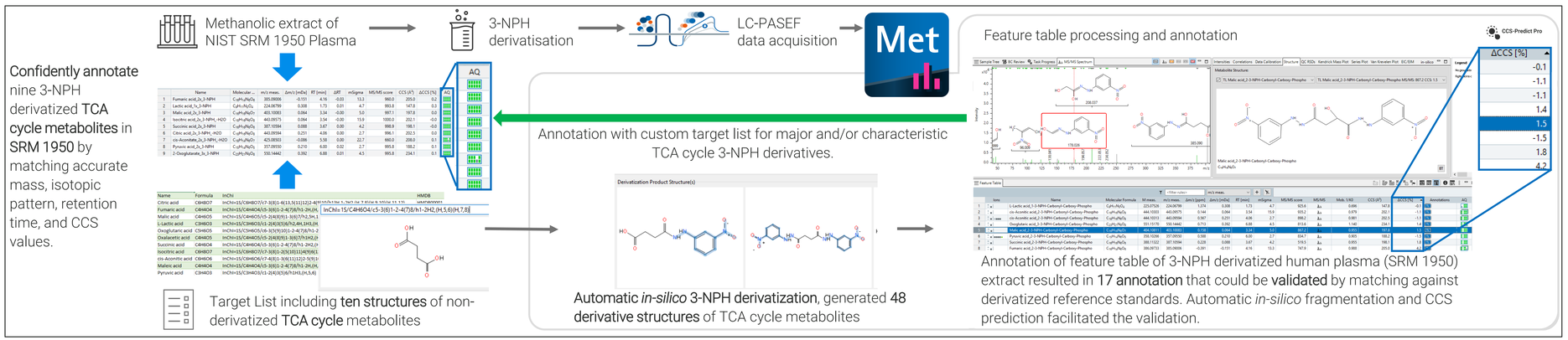

Fig. 2 3-NPH was used as an exemplar reagent due to its growing use in metabolomics studies in recent years [1; 2; 3]. Using ten TCA cycle- related metabolites, the software automatically performed *in-silico* 3-NPH derivatization of carboxy- and carbonyl groups, generating 48 derivative structures of TCA cycle metabolites. This list was used to annotate a feature table of a 3-NPH derivatized human plasma (SRM 1950) extract. Out of 28 annotated features, 17 could be validated by matching against derivatized reference standards. Several targets displayed multiple derivatives, including cis/trans isoforms of carbonyl derivatives. Matching to authentic standards allowed for the creation of a custom target list for major and/or characteristic derivatives. By applying this custom target list and matching accurate mass, isotopic pattern, retention time, and CCS values, we were able to confidently annotate 9 TCA cycle metabolites in SRM 1950.

Results #2 – Non-targeted metabolomics on derivatized plasma samples

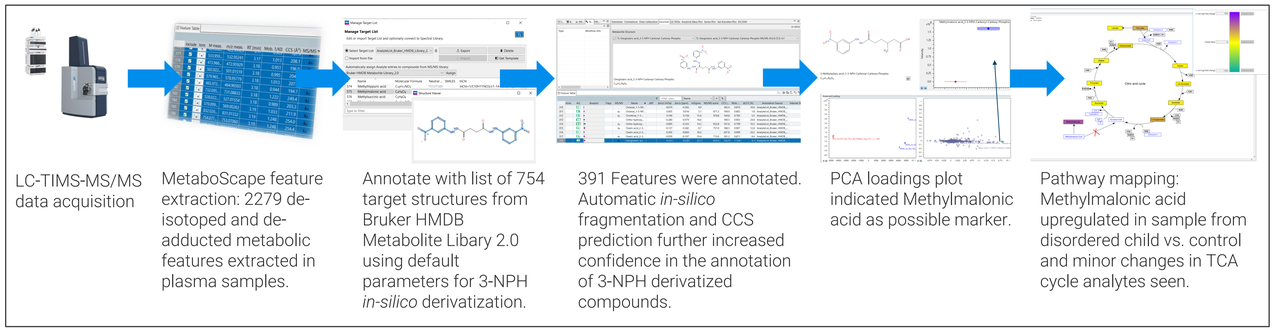

Fig. 3: Aim of this proof of concept was to understand if one can perform non-targeted metabolomics on derivatized samples, just as one would do with non-derivatized samples. For this purpose, plasma metabolic profiles of a healthy infant and child with a congenital metabolic disorder were compared. Automatic *in-silico* derivatization enabled to readily annotate 3-NPH derivatized compounds. Statistical analysis pointed to possible markers. Pathway mapping of derivatized TCA cycle metabolites revelated minor changes in these analytes between individuals.

Note and Disclaimer:

- Clinical samples were provided in accordance to local ethics: Samples and data were used for method development and QA purposes only in this study.
- Authors Aiko Barsch, Nikolas Kessler, Sofie Weinkouff, Heiko Neuweger, Matthew R. Lewis, Ryo Nakabayashi 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.
- Authors Jesper Havelund and Nils J. Færgeman declare no competing financial interest.

Literature

[1] https://doi.org/10.1038/s41467-018-07019-x
[2] https://doi.org/10.1016/j.jchromb.2023.123719
[3] https://doi.org/10.1021/acs.analchem.0c04686

Conclusion

- Derivatization allows to explore alterations in metabolic pathways associated with polar compounds, including the TCA cycle, which are often overlooked when analyzing non-derivatized samples.
- The novel MetaboScapes in-silico derivatization workflow enables to perform non-targeted metabolomics on derivatized samples, just as one would do with non-derivatized samples.
- Beginning with a list of target compounds, the structures undergo on-the-fly in-silico derivatization according to the chosen mechanism. The result is an automatic annotation with all possible chemical derivatization products of the included compounds.
- For providing increased confidence in compound annotation MetaboScapes in-silico derivatization workflow integrates automatic library structure derivatization with CCS prediction and *in-silico* fragmentation.

Metabolomics by LC-TIMS-MS/MS