Targeted and untargeted metabolomics – a powerful hybrid workflow enabled by a biocrates kit on timsMetabo HRMS

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Introduction

We investigated a hybrid workflow that integrates the quantitative rigor of a standardized targeted LC-MS approach with the structural depth of nontargeted metabolomics enabled by TIMS separation. Quantification is achieved using the biocrates kit methodology, ensuring reproducible retention times, validated calibration curves, and absolute quantification across key metabolic pathways. In parallel, fullscan TIMS-QTOF acquisition using the timsMetabo™ platform with PASEF generates rich ion mobility resolved MS/MS data that support untargeted feature discovery and structural annotation. By combining targeted quantification with untargeted TIMS-enabled 4D-Metabolomics analyses in a single integrated workflow, we bridge the gap between standardized absolute quantification and broad metabolome coverage, enabling comprehensive interpretation of metabolic responses.

Methods

The biocrates kit includes two patented 96-well filter plates, system suitability test mixtures, calibration standards, internal standards, and quality controls (QCs). Experimental samples consisted of 21 individual human plasma samples (11 male (incl. one lipemic) / 10 female) and NIST SRM 1950. Samples were registered in WebIDQ and arranged on a 96-well plate layout.

A total of 30 µL of sample was distributed across the two plates (10 µL and 20 µL), followed by PITC or 3-NPH derivatization, respectively, and extraction. Plates were analyzed using optimized LC-HRMS timsMetabo (Bruker) MoRE PASEF methods. All samples were measured in triplicate. Data were processed in TASQ 2026b / WebIDQ for targeted absolute quantitation and MetaboScape 2026b for untargeted discovery and feature annotation. Quantified results were exported to Microsoft Excel for visualization and evaluation of analytical performance.

Spectral Libraries used for annotation were “NIST 2023”; “Maurer, Meyer, Helfer, Weber: LC-HR-MS/MS Library of Drugs, Poisons” and “Bruker HMDB Metabolite Library 2.0”. Structures in Target Lists used for *in-silico* derivatization were derived from the latter two spectral libraries.

Summary

This unified workflow guarantees smooth transition between targeted and untargeted data, enabling the application of standardized and scalable biocrates kit technology on the timsMetabo HRMS platform.

Note and Disclaimer:
All authors of this Poster are employees of Bruker Corporation or one of its subsidiaries (“Bruker”). Bruker manufactures and sells analytical instruments including mass spectrometers, mass spectrometry consumables and software. Bruker mass spectrometers, consumables and software were used in this study.

Results

1) Hybrid workflow scheme

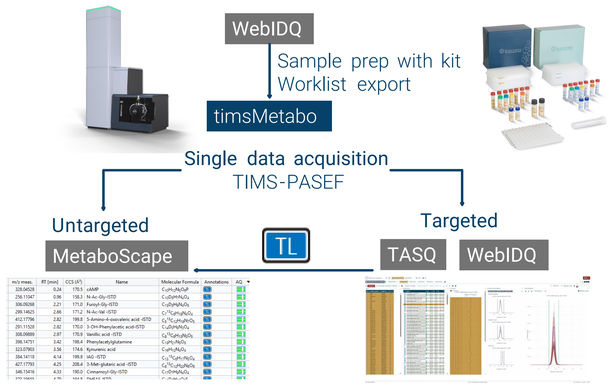


Fig. 1 Targeted and untargeted metabolomics enabled by a biocrates kit on timsMetabo HRMS

2) Standardized and quantitative targeted workflow – matching TQ performance

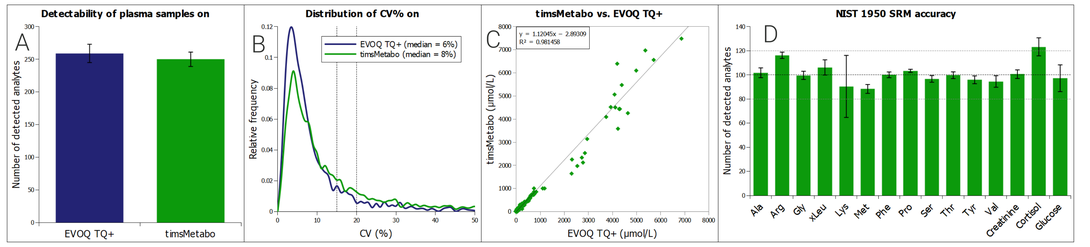
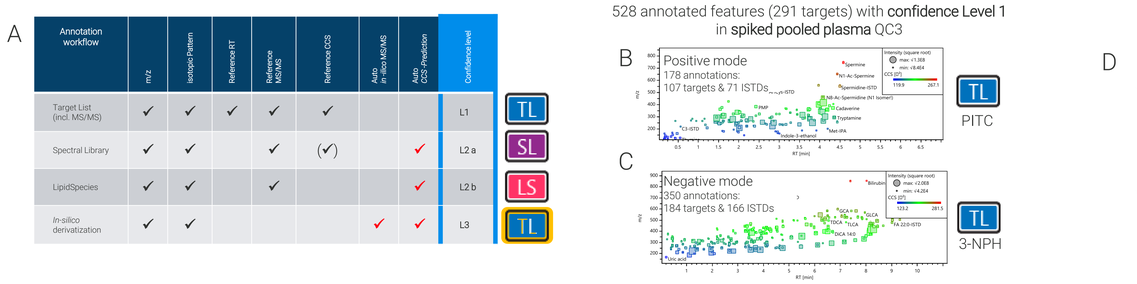


Fig. 2 The performance of the timsMetabo on the biocrates’ kit panel of 326 targeted analytes is very comparable to Triple Quad MS based measurements (EVOQ TQ+). The quantitation is based on MS1 accurate mass. A) While EVOQ TQ+ can detect 259 ± 14 target analytes in plasma samples, timsMetabo can detect 250 ± 11 (6 as sum-signals of coeluting isomers, B) timsMetabo shows comparable reproducibility of replicates with a median CV of 8%, compared to 6% from EVOQ TQ+. C) Measured concentrations of quantified analytes are comparable between instruments, and D) Accuracies of certified analytes in NIST 1950 SRM measured on timsMetabo are within 80-120% range.

3) 4D untargeted metabolomics expands annotation depth beyond targeted workflows



*L1: TL; L2a: SL with MS/MS score >800; L2b: LS with MS/MS coverage >60%; L3: TL *in-silico* derivatization with explained MS/MS coverage >60%

Fig. 3 A) MetaboScape enables complementary annotation workflows with varying confidence levels. B&C) L1 TargetList-based annotation of derivatized metabolites included in the biocrates kit yielded 528 annotated features (291 analytes and 237 internal standards) in spiked pooled plasma QC3 samples. RT vs. m/z heatmap plotted for: B) PITC-derivatized compounds (positive mode), C) 3-NPH-derivatized compounds (negative mode). D) Overview of annotated features in real world human plasma extracts (further investigation of positive mode data (PITC) – see Fig. 4).

4) Biological insight from 4D metabolomics: human plasma metabolomics

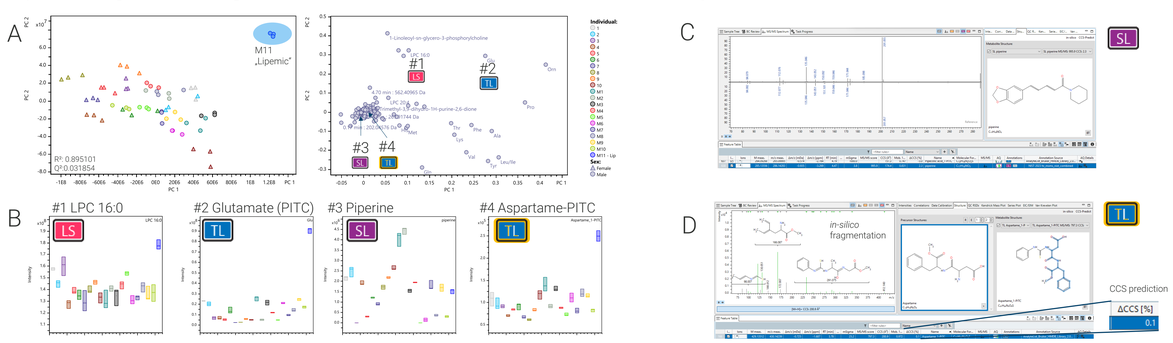


Fig. 4 A) PCA scores and loadings of 21 PITC-derivatized human plasma samples (triplicates). Sample M11 (lipemic) separates clearly from all others. Partial sex-based separation is observed in PC1 vs. PC2 (stronger in PC1 vs. PC3, not shown). Loadings highlight features annotated by complementary workflows (see Fig. 3). B) Box plots of four representative features, illustrating differences across samples and the benefit of applying complementary annotation approaches. C) Example of spectral library-based annotation: Piperine (the main alkaloid in black pepper), showing inter-individual variation (see B, #3). D) Example of *in-silico* derivatization-based annotation: Aspartame (PITC), a common artificial sweetener, elevated in sample M11 (lipemic).

Conclusion

- Reference plasma and cross-platform checks confirm strong analytical performance.
- Hybrid workflow unites robust quantification with broad untargeted TIMS-resolved metabolite coverage: three-fold increase of annotations.
- Untargeted TIMS-PASEF data reveals biology beyond the targeted panels, including LPC markers for lipemic sample and exposome molecules.

Metabolomics by LC-TIMS-MS/MS