A workflow for simultaneous quantitation and discovery analysis using Revident LC-QTOF to follow CRISPRi knockdowns in *E. coli* metabolism

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Introduction

Quantitation und discovery in metabolomics.

With large cohort studies in metabolomics, researchers need to maximize the amount of information from metabolomics samples to reduce the need for reinjection. Often, the scope of metabolomics experiments is quantification or untargeted discovery separately. Unfortunately, the strategies for data collection and evaluation for these experiments are highly different. Herein, a workflow including data acquisition and chemometrics analysis is established that combines targeted and untargeted experiments in a single injection to increase efficiency and information returns per each sample.

HILIC metabolomics workflow implemented on the 1290 Infinity III BioLC with Revident LC-QTOF. From the seven available acquisition modes Auto MS/MS and All Ions were used for data acquisition.

Experimental

Quantitation in *E. coli* matrix.

Amino acid and TCA cycle organic acid standards were used to establish a matrix calibration in pooled *E. coli* matrix. For knockdown experiments aroC CRISPRi strains were isolated[1]. Using data-independent acquisition (DIA) amino and organic acids were quantified in control and knockdown *E. coli*. using Mass Hunter (MH) Quantitative Analysis software.

Samples were analyzed in negative ESI mode with the 23 min robust HILIC method providing reproducible retention times and peak shapes[2,3].

Stationary Phase:	Poroshell 120 HILIC-Z, 2.1 x 150 mm, 2.7 µm
Temperature:	15 °C
Mobile Phase A:	20mM ammonium acetate, pH 9.3 + 5µM medronic acid in H_2O
Mobile Phase B:	pure ACN
Autosampler Temp.:	5°C

Gradient:

Time [min]	% B	Flow rate [ml/min]
0.0	90	
1.0	90	
8.0	78	0.4
12.0	60	
15.0	10	
18.0	10	
19.0	90	0.5
23.0	90	0.4

Injection:	3µL
Needle Wash:	Multi Wash, S1: ACN, S2: IPA, S3: water

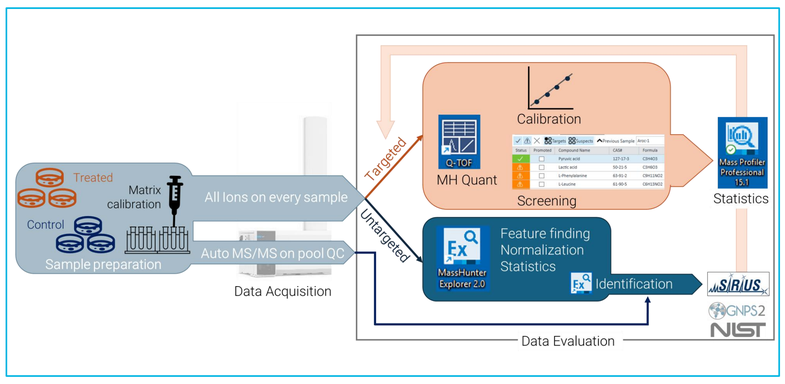
HILIC conditions for metabolite analysis with the Infinity III Bio LC.

Using the Screener tool for targeted data mining of DIA data metabolites were annotated based on RT and spectral information included within the Agilent HILIC library containing ~550 metabolites[2].

Experimental

An integrated software approach for untargeted data acquisition with different data mining strategies.

DIA data were collected for samples, calibrators, and pooled QCs. Two different data mining approaches were then established. 1st approach was targeted data mining for quantitation and discovery based on the HILIC library[2]. 2nd was untargeted data extraction including feature finding and ID. For enhanced IDs, auto MS/MS data and SIRIUS software can be included in the workflow.

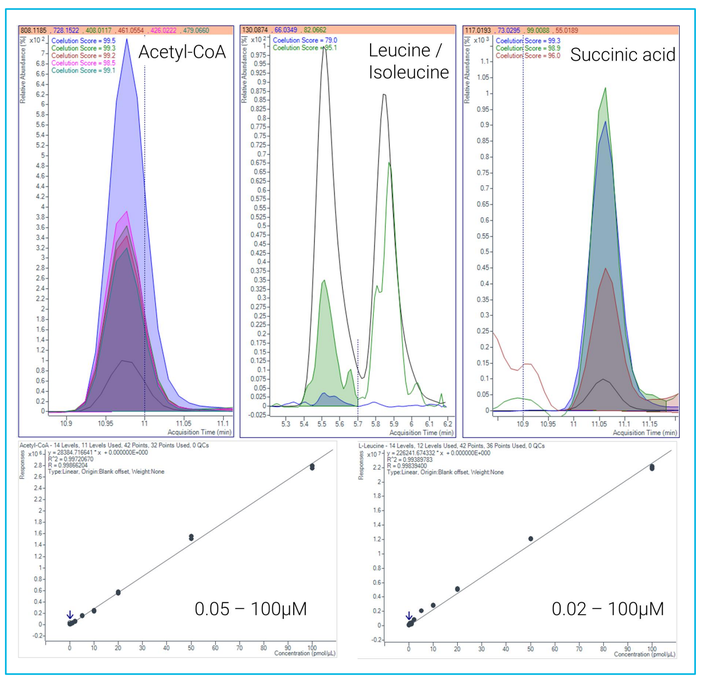


Workflow overview from samples to results.

Results and Discussion

Targeted data analysis using the Screener tool.

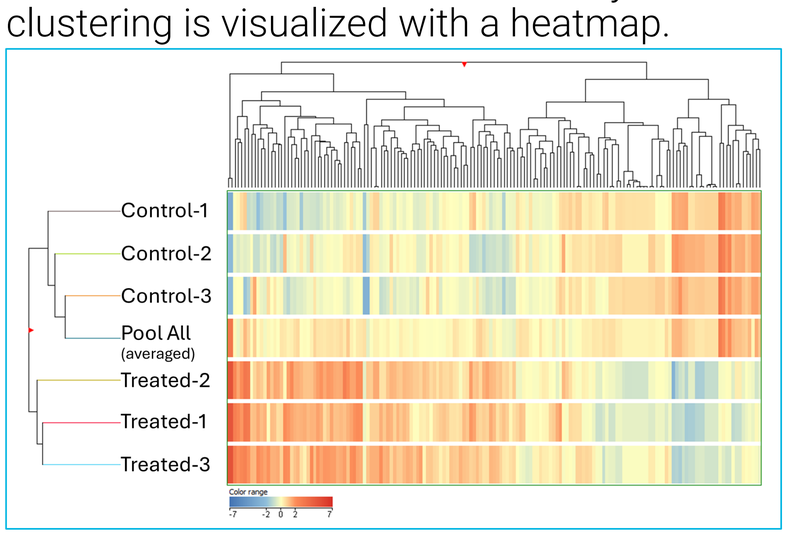
Using the HILIC library[2] for targeted data mining of ~550 metabolites quantification and discovery analysis are performed in parallel.



Top: standards spiked into pooled *E. coli* matrix for quantification. Colors indicate EICs of precursor and fragment ions in the coelution plot. Bottom: calibration curves for acetyl-CoA (left) and leucine (right) in matrix.

Multivariate statistical analysis for group differentiation.

For a global assessment of group differentiation between treated and control *E. coli* samples screener tool data are exported into MPP for statistical analysis and clustering is visualized with a heatmap.

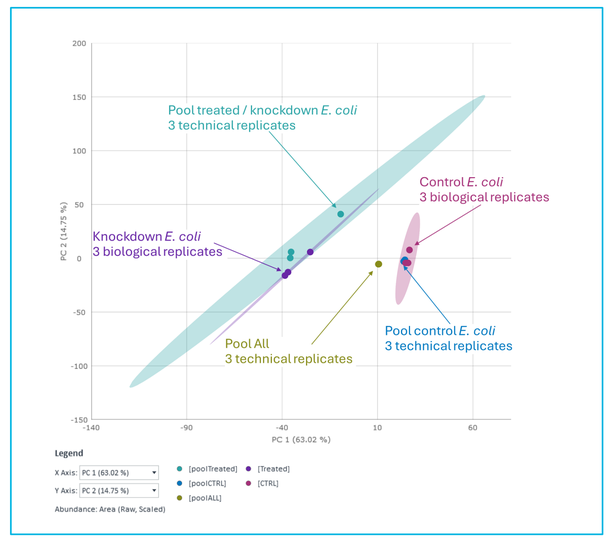


Heat map illustrating the clustering of compounds in the different sample groups.

Results and Discussion

Untargeted data analysis workflow.

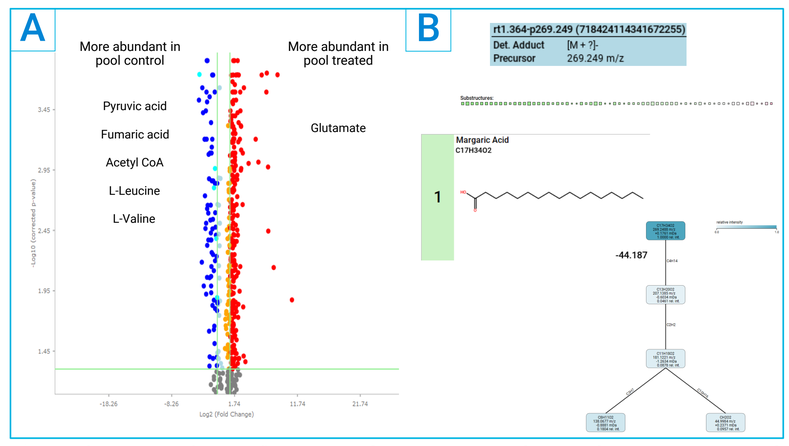
Enhanced untargeted analysis of DIA data is performed with MH Explorer 2.0 software following a classical approach including feature finding, external scalar normalization and statistics. The PCA plot confirms separation between treated and control *E. coli*.



PCA plot of knockdown (treated) *E. coli* compared to control, abundances were normalized by external scalar using optical density measurements in cell cultures.

Group differentiation analysis and identification allowing for customization of targeted data mining.

Significant compounds for group discrimination are visualized with a volcano plot and annotated with the Agilent HILIC and Metlin library. Remaining non-annotated compounds are then sent to SIRIUS for enhanced annotation.



A) Volcano plot of treated *vs* control pools. Annotated metabolites included in the panel for quantitation are highlighted. B) Annotation with SIRIUS.

Conclusions

A flexible workflow easily customizable for quantitation and discovery analysis.

- DIA data offer high flexibility for data evaluation while the standardized HILIC platform generates reproducible results and maintains confidence in quantitation
- ~550 metabolite containing HILIC library enables discovery analysis after targeted data mining
- Enhanced untargeted analysis, statistics and annotations with MH Explorer 2.0 and SIRIUS

Acknowledgements

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References

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3: Yannell, K et al. An End-to-End Targeted Metabolomics Workflow. Agilent Application Note 5994-5628EN. 2023.

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