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An efficient pseudo-targeted metabolomics workflow based on liquid chromatography coupled with mass spectrometry

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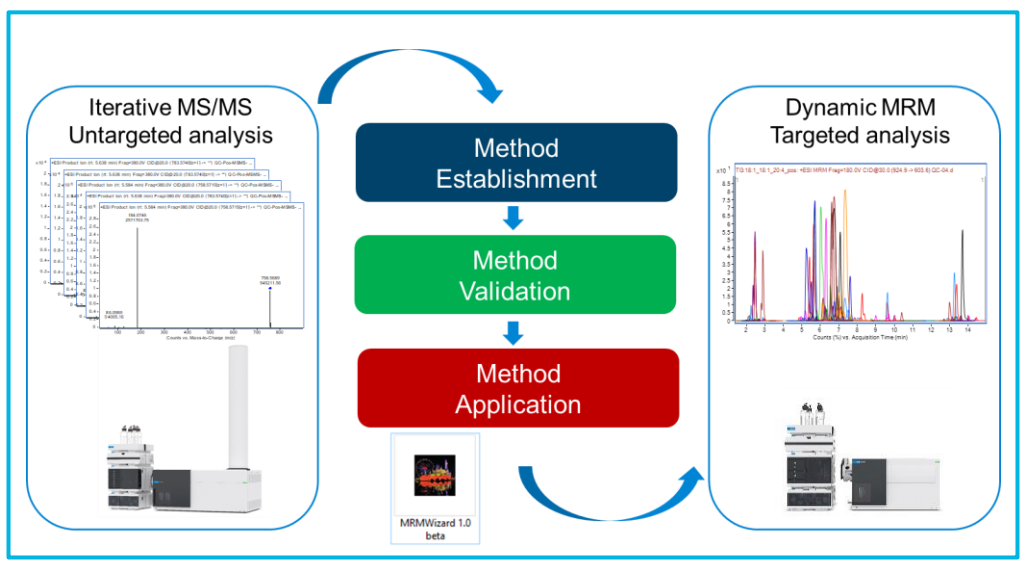
Introduction

An untargeted metabolomics method can provide the most comprehensive overview of the metabolome based on high-performance liquid chromatography coupled with high-resolution mass spectrometry, but it suffers from unsatisfactory repeatability, limited linear dynamic range and challenging requirements for complex data processing. A targeted method based on triple-quadrupole mass spectrometry is the gold standard for metabolite quantification, with high sensitivity, excellent stability, and a wide linear dynamic range. However, it is usually applicable to known compounds, limiting its applications in global metabolomics analysis. The pseudo-targeted metabolomics method was developed recently which combined the advantages of untargeted and targeted analysis and has a wide range of applications in metabolomics studies [1,2].

An efficient pseudo-targeted metabolomics workflow using Agilent high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC/Q-TOF), triple-quadrupole mass spectrometry (LC/TQ), MassHunter workstation and a novel program, MRMWizard is developed in this study. The entire workflow is efficient, high-throughput and high-coverage which enabled the simultaneously semiquantitative analysis of hundreds to thousands of metabolites.



Agilent pseudo-targeted metabolomics workflow

Experimental

Cell Samples

As a proof of this pseudo-targeted workflow, the developed method was applied to the discovery of differential metabolites in K562 cells with special gene knockout.

Instrumentation

LC conditions

1290 Infinity II UHPLC	
Column	Poroshell HILIC-Z, 2.1 x 100 mm, 2.7 µm, PEEK-lined
Mobile Phase	A: Water/ACN 9:1 with 15mM NH_4Ac, pH 9.0 B: Water/ACN 1:9 with 15mM NH_4Ac
Flow	0.3 mL/min
Gradient Elution	0 min 10% A 8 min 50% 10min 50%
Column Temperature	40 ℃
Injection Volume	1 µL

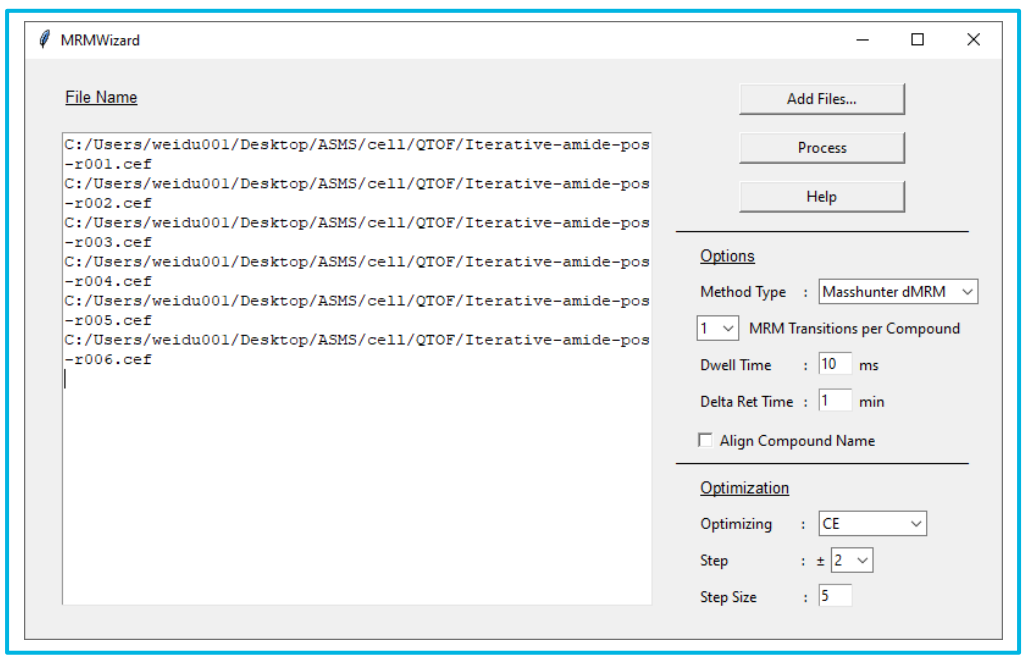
MS conditions

MS Source	
Ion source mode	ESI
Drying gas temperature	280 ℃
Drying gas flow	8 L/min
Sheath gas temperature	325 ℃
Sheath gas flow	11 L/min
Nebulizer pressure	35 psi
Capillary voltage	3500V (pos) / 3000V (neg)
Nozzle voltage	1000 V (pos) / 1500V (neg)
6546 LC/Q-TOF	
Scan type	Iterative autoMS/MS
6470 LC/TQ	
Scan type	Dynamic MRM

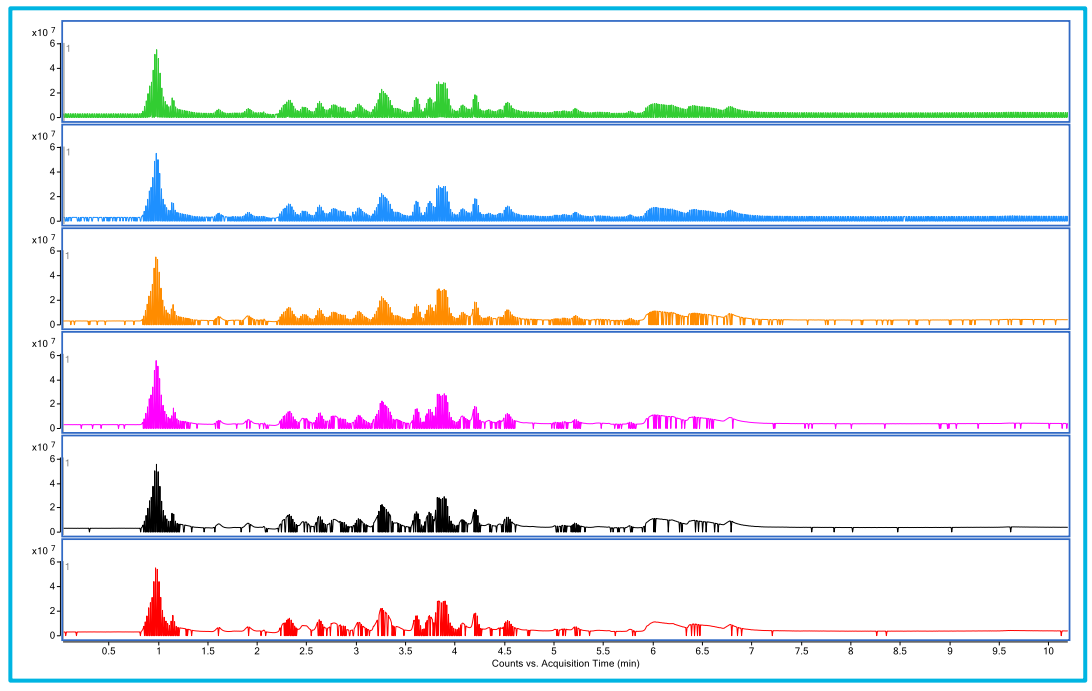
MRMWizard

The setup of the pseudo-targeted method was time consuming and laborious, especially the process of choosing ion pairs for thousands of metabolites. To define ion pairs automatically and systematically, a novel Python program named MRMWizard[3] was developed which can generate MRM transitions from MS/MS spectra of LC/Q-TOF data and evaluate optimization of MRM transitions efficiently and easily. It is able to:

- Generate the LC/TQ MRM list from Q-TOF MS/MS data.
- Support Masshunter MRM, dMRM, and Skyline format output
- Support MRM CE / Fragmentor optimization, automatically evaluate optimal MRM conditions through the quantmethod.xml file from MassHunter Quantitative Analysis software.



MRMWizard



TICs of iterative MS/MS runs of the QC samples

Method transformation from HRMS to TQMS

MRMWizard can automatically choose the most responsive product ion from MS/MS spectra of each compound in a CEF file and generate an MRM list with the format suitable for LC/TQ acquisition. 1337 MRM transitions were created in positive mode and 982 MRM transitions in negative mode.

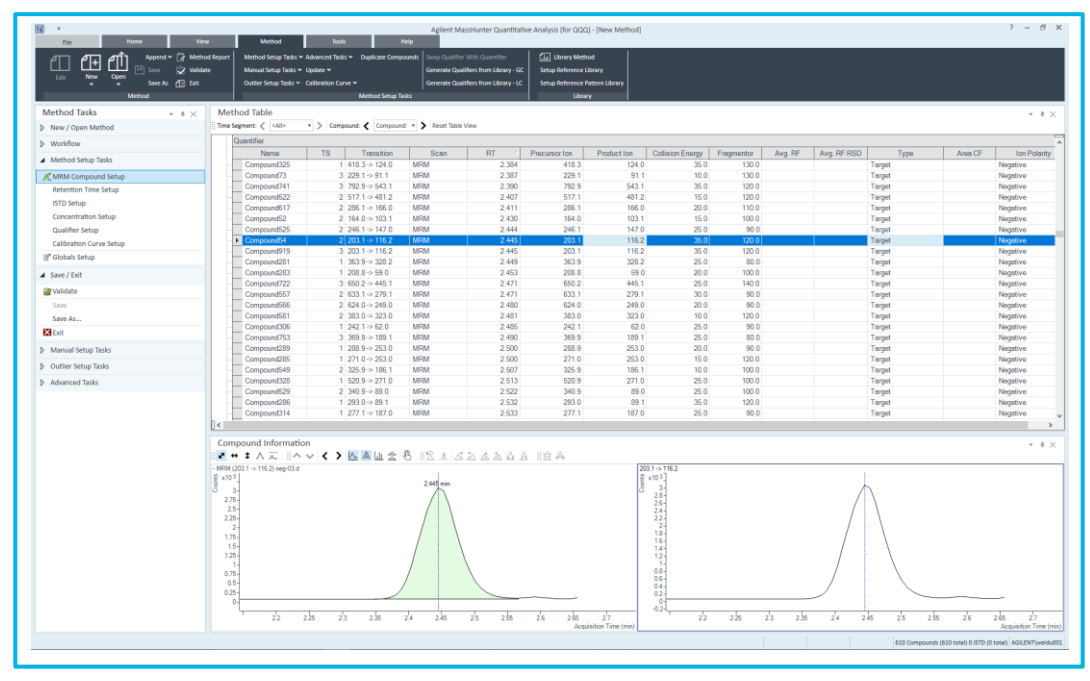
Optimization of MRM CE and Fragmentor

MRMWizard can generate an MRM list with different CE or fragmentor values. A dynamic MRM acquisition method was developed on an LC/TQ, then it imported the list and acquired QC sample data. A quantitative method was created from the QC data using MassHunter Quantitative Analysis 10.2 software. After opening the quantitative method in MRMWizard the optimal collision energy or fragmentor of each MRM was obtained to generate a final MRM list. After optimization of the CE and fragmentor values, the MRM transitions with low abundance and poor repeatability were removed.

Pseudo-targeted workflow

Acquisition of MS/MS spectra of metabolites

Data-dependent MS/MS acquisition of a mixed QC sample was performed on an LC/Q-TOF at different collision energies in both positive and negative mode. Automated Iterative functionality should be used to enhance the coverage of the cell metabolites. Subsequently, molecular feature extraction was performed of all the iterative data files, then identification with METLIN metabolite database and library, and results were exported to CEF files using MassHunter Qualitative Analysis workstation 10.0.



Method setup in MassHunter Quantitative Analysis

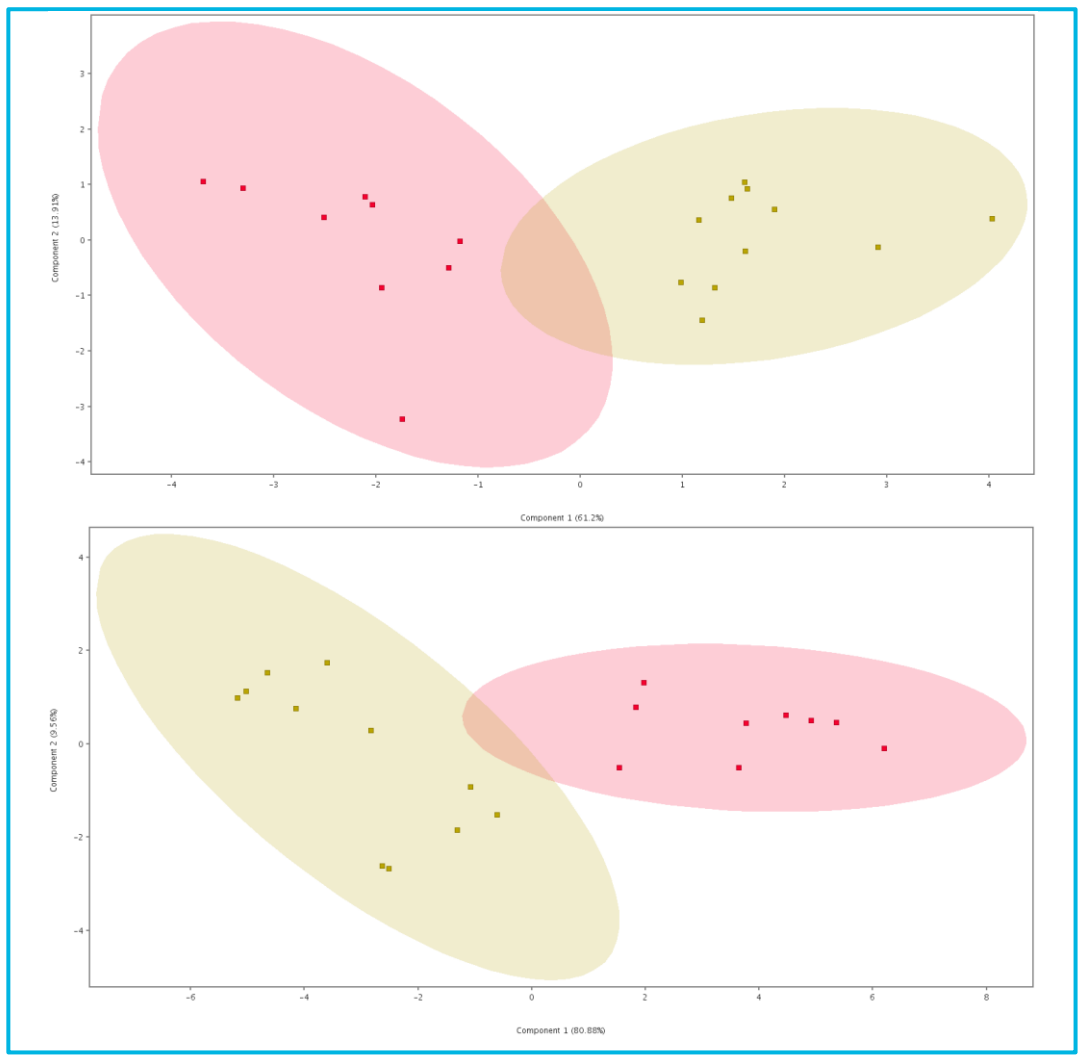
Results and Discussion

Metabolomics data acquisition

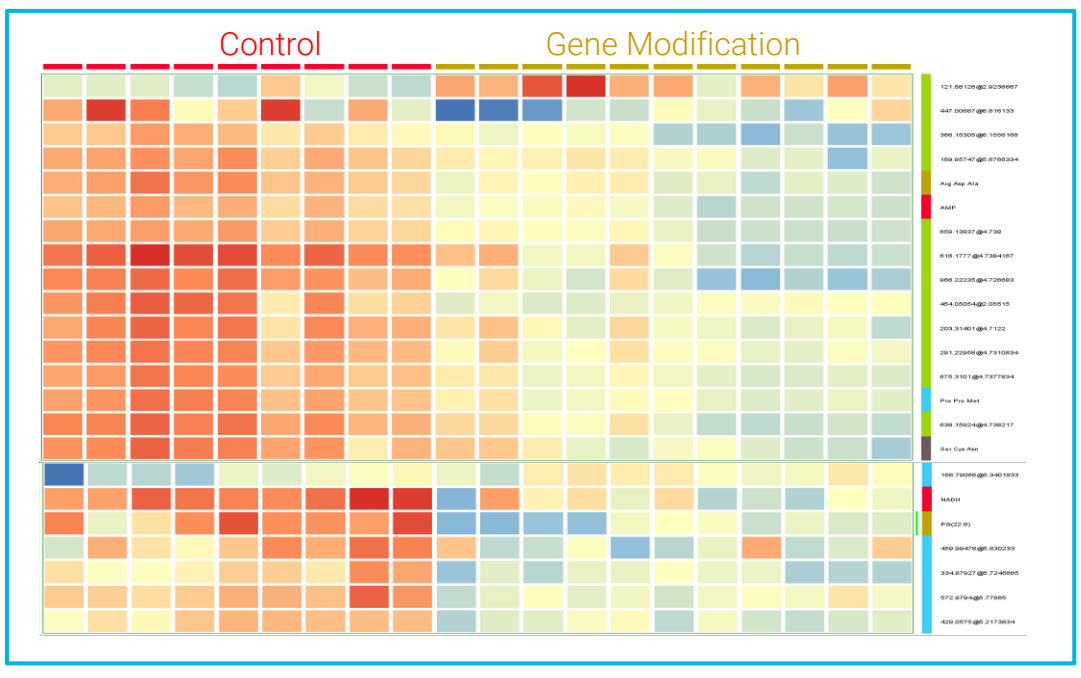
After optimization and validation, acquisition methods of LC/TQ with 706 MRMs in positive mode and 408 MRMs in negative mode were performed on the cell extract.

Metabolomics data processing

Statistical analysis was performed using Mass Profiler Professional 15.1 (MPP). Principal Component Analysis (PCA) showed that the genetic modification and wild-type samples sorted into separated groups, which suggests differences between the two groups.

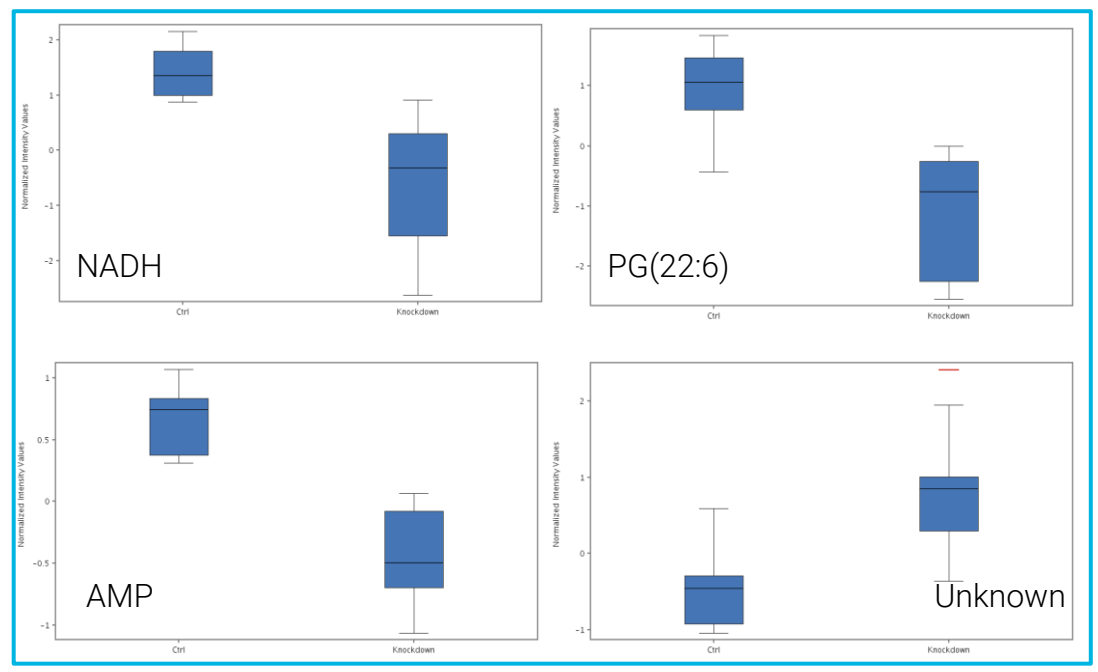


PCA score plot



Heatmap of differential metabolites

A differential list of 23 metabolites were obtained by significant test (Moderated t-test, p<0.01, fold change>2, VIP>1) including nucleotide, lipid, peptide and some unidentified metabolites.



BoxWhisker plots of differential metabolites

Conclusions

Based on the Agilent LC/Q-TOF, LC/TQ, and a novel program MRMWizard, the pseudo-targeted workflow in this study exhibited high efficiency, excellent repeatability and precision by improving the coverage and detection limit of low-abundance metabolites. It provides a reliable basis for achieving efficient metabolomics analysis for the discovery of potential biomarkers on Agilent's mass spectrometry platform.

References

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[2] Jing Xu, Jiangshuo Li, Ruiping Zhang, Jiuming He, Yanhua Chen, Nan Bi, Yongmei Song, Luhua Wang, Qimin Zhan, Zeper Abliz, Development of a metabolic pathway-based pseudo-targeted metabolomics method using liquid chromatography coupled with mass spectrometry, Talanta, 192, 160-168 (2019).

[3] https://drive.google.com/drive/folders/185bb2bP5v7k-8G_8ag-fjG1SLffCyg_D?usp=sharing

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