A Systematic Approach to Transform Untargeted Profiling to Pseudo-targeted Analysis for Metabolomics Study Based on Liquid Chromatography Mass Spectrometry

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ABSTRACT

Purpose: Develop a high coverage workflow to transform wide range of metabolites collected from LC-HRMS data to LC-QQQ for metabolomics profiling for natural products or biological matrix.

Methods: We defined a workflow to transform the untargeted metabolomics to pseudo-targeted analysis. The untargeted analysis collects metabolites and their product ions from NIST 1950 by data-dependent acquisition using Thermo Scientific™ Q Exactive™ Plus MS, then does peak picking of the metabolites with the targeted ion-pair for analysis on a Thermo Scientific™ TSQ Altis™ MS by selected reaction monitoring (SRM). The data were processed using Thermo Scientific™ TraceFinder™ 5.0 and Thermo Scientific™ Compound Discoverer™ 3.1 software.

Results: Using this workflow, 4,574 transitions for positive mode and 2,744 transitions for negative mode were determined for pseudo-targeted analysis. Then the method was applied to analysis by NIST SRM1950 More than 3200 and 1300 ion pairs were detected in positive and negative mode respectively.

INTRODUCTION

LC-MS-based metabolomics studies play an important role in life science and biomarker discovery currently, and for investigation of a wide range of metabolite classes present in plants, natural products, food, and environmental exposures, etc. Metabolomics can be roughly classified into two subtypes: un-targeted profiling analysis mainly using HRMS, and targeted analysis of limited known metabolites mainly using triple quadrupole mass spectrometers. How to combine the advantages of both targeted and untargeted methods remains a challenge for metabolomics researchers, especially for the wide range of metabolites present in unknown samples.

To resolve these questions, we developed a workflow to transform untargeted profiling with the Q Exactive Plus instrument to pseudo-targeted analysis with the TSQ Altis MS, in order to improve the metabolites coverage and support a deeper metabolomics study.

MATERIALS AND METHODS

Sample Preparation

The commercial plasma was purchased from NIST(NIST SRM1950). Metabolites were extracted by addition of methanol at a ratio of 3:1 (methanol:sample). After centrifugation, the supernatant containing the metabolites was evaporated. Then the dried metabolites were resuspended in Methanol:water=1:9.

Liquid Chromatography / Mass Spectrometry

UHPLC Conditions

Reversed phase chromatography separation was carried out on a Thermo Scientific™ Vanquish™ UHPLC system by using a Thermo Scientific™ Hypersil Gold™ C18 column (2.1 x 150mm, 1.9μm) at a 300 μL/min flow rate and column temperature of 40 ° C. Mobile phase A was water with 0.1% formic acid and mobile phase B was methanol. The RP gradient is described as follows: The injection volume was 2 μL.

Table 1. RP gradient

Time(min)	A(%)	B(%)	Flow rate(mL/min)
0	100	0	0.3
5	70	30	0.3
9	2	98	0.3
16	2	98	0.3
16.1	100	0	0.3
20	100	0	0.3

Q Exactive Plus Conditions and TSQ Altis Conditions:

Untargeted profiling was performed with a Q Exactive Plus mass spectrometer using the Full mass/ddMS2 mode. The data were acquired with 70-1050 Da at 70,000 resolution for MS1 followed by Top 8 data dependent MS/MS at 17,500 resolution. Ionization conditions were operated as follows for Q Exactive Plus and TSQ Altis:

Table 2- Scan Parameters- Ion source and SRM condition

Ion Mode	Postive & Negative
Spray Voltage:	3200V(POS)/2800V(NEG)
Vaporizer Temperature	350℃
Ion Transfer Tube Temperature	320℃
Sheath Gas:	40
Aux Gas:	10
Q1/Q2 Resolution（TSQ Altis）	0.7/1.2Da
Cycle Time（TSQ Altis）:	0.8s
CID Gas (mTorr, TSQ Altis))	2

Data Analysis

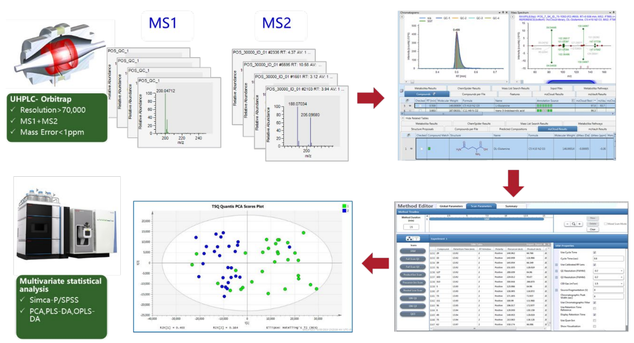
The data were processed using Thermo Scientific™ TraceFinder™ 5.0, Simca 13.0 and Compound Discoverer™ 3.1 software.

RESULTS

Workflow of transforming untargeted profiling to pseudo-targeted analysis

We defined a workflow to transform the untargeted metabolomics to pseudo-targeted analysis. First we acquired untargeted data using NIST 1950 SRM Plasma with Q Exactive Plus and processed by Compound Discoverer 3.1 software including peak picking and identification by searching against mzCloud, HMDB and KEGG databases. Then the compounds table was exported in TraceFinder format including the tentative annotation name, retention time, mass-to-charge ratio (m/z), charge, and product ions. The lists of corresponding information can be imported into the TSQ Altis method template and then acquired for as many compounds as possible by pseudo-targeted analysis.

Figure 1. The defined workflow to transform the untargeted metabolomics to pseudo-targeted analysis. This approach includes 3 steps: a). untargeted analysis of NIST SRM 1950; b) metabolites extraction and ion-pair selection; and c) pseudo-targeted analysis of the samples.



Transitions list determined by the workflow

The high data quality of the untargeted profiling analysis was attributed to superior HRAM capacity and structural annotation with high confidence. The defined workflow can provide an ideal transitions list with high coverage metabolites of the sample in a short time. Using this workflow, 4,574 transitions for positive mode and 2,744 transitions for negative mode including 2 internal standards were collected for pseudo-targeted analysis of the NIST SRM1950 samples. In a batch of 50 samples, more than 3200 and 1300 metabolite SRM transitions were detected by processing data with TraceFinder.

Application in serum samples

As a proof of the workflow, we applied the developed method in a study related to cancer. In addition, the QC samples were injected 3 times at the start of a run to condition the column, and then for every 10 samples throughout the run to assess instrument stability and data quality. The results show that TSQ Altis and the method performed well for CVs within-run QC's internal standards is less than 3.90% and 6.35% for positive and negative mode respectively.

The data acquired with TSQ Altis was compared with Q Exactive Plus. Principal components analysis (PCA) was performed on the two batches of data collected. As can be seen from the figure 2 and 3, the results of TSQ Altis are consistent with the profiling data processed by Compound Discoverer. The two results all shows that the normal group and the model group showed a clear separation trend, which means the workflow here we defined is suit for comprehensive metabolomics and lipidomics studies.

Figure 2. The PCA Scores plot for Q Exactive Plus data, the two groups of samples have a clear separation trend

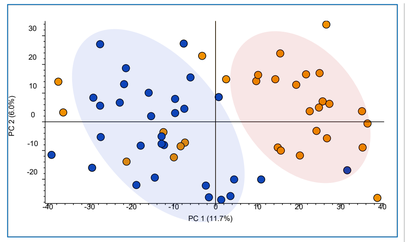
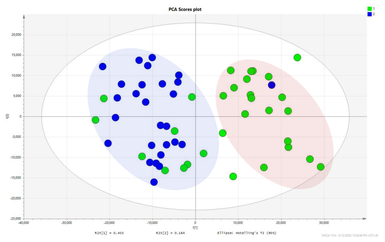


Figure 3. The PCA Scores plot for pseudo-targeted analysis data, the two groups shows a very similar result as Q Exactive Plus



CONCLUSIONS

This workflow provides a high coverage method which is reliable to transform untargeted profiling analysis to pseudo-targeted metabolomics.

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TRADEMARKS/LICENSING

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