Establishment of a global metabolomics method for Arabidopsis thaliana using high-sensitivity MS/MS on the ZenoTOF 7600 system

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ABSTRACT

Metabolomics analysis is an important part of systems biology. Data derived from metabolomics experiments can more closely match an organism's phenotype than data derived from either genomics or proteomics experiments. Plants rely on many types of metabolites, such as primary metabolites for plant growth and development and secondary metabolites related to plant disease and stress resistance. Given the diverse functions of metabolites in plants, analyses that can be applied to many metabolites are ideal. Typically, either non-targeted or targeted approaches are used to collect metabolomics data. Here, a non-targeted metabolomics analysis was performed to identify metabolites in *Arabidopsis thaliana* using the ZenoTOF 7600 system. These discovery data were then used to develop a targeted assay for high-throughput analysis using the QTRAP 6500+ system.

INTRODUCTION

There are over 200,000 metabolites in plants, including primary metabolites that are necessary for maintaining plant life activities, growth and development and secondary metabolites that are closely related to plant disease and stress resistance. This large number and wide variety of plant metabolites requires a comprehensive metabolomics approach. Non-targeted metabolomics and targeted metabolomics are the 2 main applications of metabolomics, and each has its own advantages and disadvantages. Combining these 2 approaches provides a high-coverage and high-precision metabolomics method that enables the advantages of both. This combined approach is known as widely targeted/quasi-targeted metabolomics.
The experiment described here adopted a non-targeted method on the ZenoTOF 7600 system using data-dependent acquisition (DDA) and dynamic background subtraction (DBS) with the Zeno trap activated. SCIEX OS software was used for data collection and analysis of Arabidopsis thaliana and identification of its metabolites. After locking the retention time of the identified metabolites and the detected ion pairs, the results were then quantified using a targeted assay on the QTRAP 6500+ system with the Scheduled MRM algorithm. Using the ZenoTOF 7600 system together with the QTRAP 6500+ system ensures comprehensive coverage and accuracy, making this method a uniquely useful metabolomics approach.

MATERIALS AND METHODS

HPLC conditions:
An Exion LC AD system with a Phenomenex Luna Omega Polar C18 column (100 x 3.0 mm, 3.0 µm) at 40ºC was used. Mobile phase A was water with 0.03% formic acid, and mobile phase B was 50:50 (v/v) acetonitrile/methanol with 0.03% formic acid. The flow rate was 300 µL/min and the injection volume was 2 µL.

MS/MS conditions:
Samples were run on the ZenoTOF 7600 system using DDA. The TOF MS precursor ion scan was performed with an m/z range of 50-1,500 and with an accumulation time of 0.1 s. Twenty candidates ions were selected for fragment ion scans with an m/z range of 30–1,500 and an accumulation time of 25 ms. DBS and the Zeno trap were on. Each sample was injected twice, in positive and negative polarity separately.

The QTRAP 6500+ system was used with a Turbo V ion source and an electrospray ionization (ESI) probe.
Each sample was injected once using positive and negative polarity switching. The Scheduled MRM algorithm can intelligently allocate dwell time to achieve the best reproducibility (Figure 1).

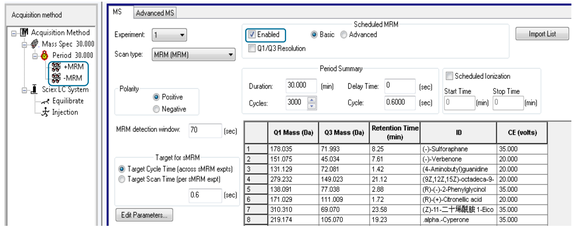

Figure 1. Detection of 538 compounds using the QTRAP 6500+ system in a single injection (positive and negative polarity switching) using the *Scheduled MRM algorithm for the best reproducibility.*

After obtaining high-resolution data using the ZenoTOF 7600 system with DDA and DBS, and with the Zeno trap activated, the samples were quickly, accurately and comprehensively identified using SCIEX OS software combined with a secondary local database. The database was established and converted into MRM transitions. Batch sample data were collected using the Scheduled MRM algorithm of the QTRAP 6500+ system.

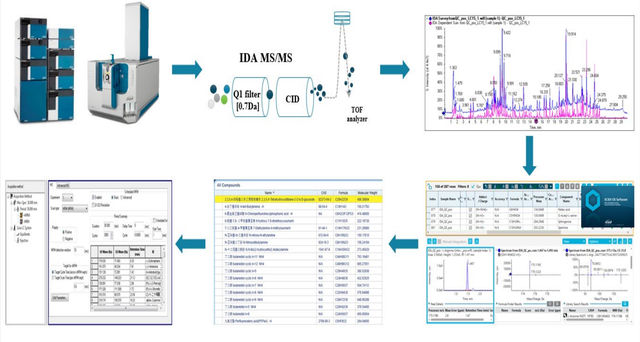

Figure 2. The process of establishing global metabolomics methods.

RESULTS

Non-targeted, semi-targeted and targeted data processing workflows were used to process the MS and MS/MS data acquired and to identify metabolites. The high-speed MS/MS acquisition on the ZenoTOF 7600 system permitted both high-resolution TOF MS and high-sensitivity MS/MS to be obtained in a single injection, which reduced the time for data acquisition and improved efficiency. A total of 538 metabolites were identified from the data acquired in positive and negative ion modes and a local database was established for these metabolites. The metabolites mainly included *Arabidopsis thaliana* glycosides, phenylpropanoid, plant hormones, phenols, amino acids and their derivatives, organic acids, sugars and glycosides.

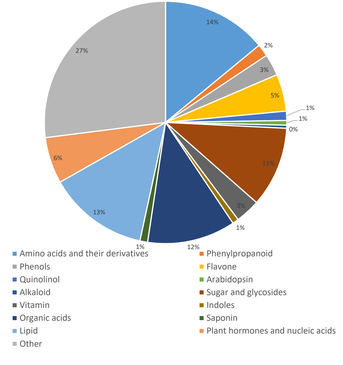

Figure 3. Overview of 538 identified metabolites categories in *Arabidopsis thaliana.*

Using the information obtained from the non-targeted analysis, a targeted MRM method was built to quantify these metabolites using the Scheduled MRM algorithm. A single method containing 538 MRMs with polarity switching was used to cover all the detected metabolites. Next, a batch was developed to analyze a large set of *Arabidopsis thaliana* samples with quality control acquisitions interspersed every 10 acquisitions. The quality control samples were obtained by mixing 5 µL of each of the 366 processed sample solutions. In total, 366 samples and 37 quality control samples were analyzed to assess reproducibility in this study. The observed retention times were stable, with peak variation of the internal standard compound within 0.05 min. The RSD of the peak area was 5.7%, which provides high-quality data assurance for omics research.

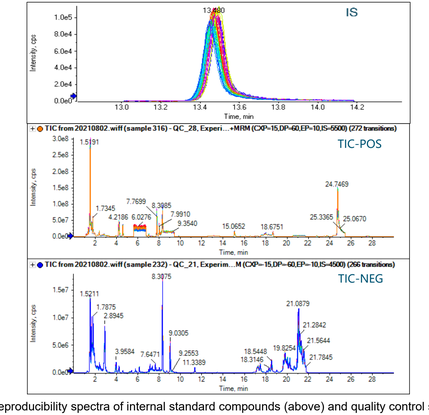

Figure 4. Reproducibility spectra of internal standard compounds (above) and quality control samples (below).

CONCLUSIONS

This study shows the advantages of using the ZenoTOF 7600 system combined with the QTRAP 6500+ system in the field of plant metabolism. Using the ZenoTOF 7600 system with DDA and DBS, and with the Zeno trap activated, allows for high-resolution and high-quality MS/MS data with a single injection, reducing the hassle of manually subtracting matrix background and collecting data multiple times, greatly shortening data collection time and improving efficiency. The Zeno trap enables low concentration compounds to still obtain high-quality MS/MS data for identification and analysis. The QTRAP 6500+ system provides the advantages of high sensitivity, high throughput and a wide linear range in the quantitation of metabolites in complex matrices. The 2 systems complement and validate each other, greatly improving the coverage and accuracy of omics research results, which is a development trend in omics research.

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