A Qualitative and Quantitative Metabolomics Analysis of Plant Phytohormones.

*Plant Metabolomics Analyses on the SCIEX X500R QTOF system*

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**Introduction**

The application of mass spectrometry (MS) to the analysis of the plant metabolome enables a global based investigation into a plant’s particular cellular state. This allows the user to observe quantitative based responses to a variety of environmental factors¹ allowing particular positive physiological attributes such as drought resistance to be selectively bred creating new crops with faster growth characteristics and higher yields.

A new QTOF mass spectrometer employed for metabolomics analyses was used to explore the capabilities for quantitative analysis by using high resolution accurate mass. The X500R QTOF system is powered by the new SCIEX OS acquisition software, this intuitive new software enables on the fly automated mass calibration as well as simplified data acquisition and processing in a single interface allowing all types of users to run the platform with ease.

Here we evaluated the X500R QTOF system for a metabolomics application specifically for a series of major plant phytohormones. As the speed of the instrument allows for qualitative and quantitative analysis within a single injection these were evaluated in the case of plant phytohormones.

**Benefits of the X500R QTOF Qualitative and Quantitative Workflow for Plant Metabolomics**

- The X500R QTOF allows high resolution qualitative and quantitative analysis on a single platform all within a single injection!
- The qualitative data allows for confident identification of plant phytohormones when matched against a library
- The quantitative data collected can be used to calculate concentrations and LDR of the assay
- SCIEX OS acquisition software based around a simplified user interface enables new users to mine data with minimal training

![Figure 1. Linear Dynamic Range on the X500R QTOF System. Quantitative performance for Aabscisic acid showing 3.5 orders of linear dynamic range in full scan using MS1.](image-url)
**Experimental Information**

**Sample Preparation:** All phytohormone standards were purchased from Sigma-Aldrich and were diluted to create the calibration curve using mobile phase A.

**Chromatography:** The samples were analyzed using a SCIEX EXION AD LC system with a Phenomenex Kinetics EVO C18 Column 100 x 2.1 mm, 1.7µm with a 10 µL injection volume. An elution gradient of water and acetonitrile over 13 minutes using a flow rate of 0.6 mL/min. (Figure 2)

**Mass Spectrometry:** We applied a SWATH® Acquisition method which uses a combination of a full-scan TOF spectrum (100-1000 Da in 100 milliseconds) with TOF MS/MS fragmentation using fixed Q1 transmission windows. In this experiment 10 fixed SWATH windows of 25 Da were used to span across the precursor range of 100-350 Da each requiring an accumulation time of 30 milliseconds for data independent MS/MS analysis. The combined SWATH approach required a total cycle time of 0.47 seconds. (Figure 3)

**Data processing:** The SWATH Acquisition experiments were acquired on a X500R QTOF System. Data was processed using the Analytics portion of the SCIEX OS acquisition software. Quantitative calibration lines were extracted from the SWATH precursor TOF MS scan using an extraction width of ±0.05. The associated SWATH product ion data was also processed within Analytics to provide compound identification based upon matching the MS/MS fragmentation profile to a known library entry.

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**Figure 2. Chromatographic Conditions.** EXION AD LC gradient conditions optimized for the screening of known plant metabolites and the plant growth hormones

**Table:**

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<th>Precursor ion stop mass (Da)</th>
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**Figure 3.** Example SWATH method showing the precursor ion SWATH windows used for phytohormone screening.
Data Overview

The chromatographic performance of the method was assessed using a standard phytohormone mixture of 100 ng/mL. The extracted precursor ion chromatograms from the full scan (TOF MS) are shown in Figure 4. Six phytohormones were evaluated namely Salicylic acid, gibberellic acid, indole-3-propionic acid, abscisic acid, indole-3-butyric acid and gibberellin A4.

![Figure 4. Metabolomics MS workflow explored for comparing traditional data dependent approach for untargeted metabolomics applications versus a data independent acquisition approach.](image)

The resolution of the system is >30,000 is maintained across the mass range and both is the full scan (TOFMS) and in the MS/MS scans too, without dropping sensitivity (Figure 5a and 5b).

X500R for Quantitation

To determine the quantitative performance of the X500R system a series of solvent standard calibrators were created from 0.5-1000 ng/mL. Figure 6 shows the TOF MS level quantitation statistics for Abscisic acid and Gibberellic acid with the associated reproducibility statistics based upon triplicate injections.

![Figure 6. Example SWATH quantitative performance for Abscisic and Gibberellic acid showing 3.5 orders of linear dynamic range](image)

X500R for Identification

SWATH Acquisition allows collection of MS/MS data for all the precursor ions detectable in a given sample which can be processed in the Analytics portion of SCIEX OS using targeted libraries. This enables the user to screen samples in a targeted manner to identify compounds within complex mixtures. The MS1, isotopic distribution, retention time and the MSMS are used to confidently identify the metabolite with confidence by using a scoring system which can be weighted in favor of any one of these criteria. The output of a phytohormone SWATH screening experiment is shown in Figure 7.

The Analytics results table provides the operator with a simple table enabling a traffic light reviewing system, A green check box is used when a compound is identified, yellow means to be reviewed and red means no match. Predicted elemental formulae and an in-silico predicted fragmentation from the ChemSpider database can also be employed to enable a higher level of confidence by matching any experimental data to the theoretical. The results table generated in the Analytics portion of SCIEX OS is linked to the original data file so each of the identifications to the library can be quickly reviewed (Figure 8).
The peak review pane provides the user with the information on the identified compounds firstly the chromatographic performance is assessed using the extracted ion chromatogram and associated LC retention time. Then the MS spectral quality is investigated where the accurate mass (<1 ppm error) precursor ion data is used to predict an elemental formula and then a co-responding isotopic pattern is matched to the experimental data. Finally the MS/MS information is compared to the library and the combination of all three provides confidence in identification.

Conclusions

Using the X500R QTOF system with SWATH Acquisition provides a full quantitative and qualitative digital profile of the phytohormone samples in a single injection. SCIEX OS provides an easy to use and intuitive platform for the acquisition and subsequent processing high resolution accurate mass data. Here we show the quantitation and identification of the phytohormones, but the provided LC gradient and the SWATH Acquisition process can be applied for global plant metabolomics.

References