INTRODUCTION

The purpose of this work is to demonstrate a generic information-independent acquisition workflow to perform targeted quantitation and unknown screening of pesticides. Fruit and vegetable samples were extracted using QuEChERS approach, and then subjected to a generic RPLC separation. High resolution TDF MS data was acquired using a MS/MS™ with SWATH™ acquisition workflow. All experiments were conducted using AB SCIEX TripleTOF 5600+ LC-MS/MS system coupled to an Agilent 1200 LC system. The MS method employed a TOP MS full-exact survey, followed by targeted MS/MS analysis. The MS/MS data was acquired using a SWATH™ acquisition method enabled to obtain MS/MS fragments for all the components in a given sample, two characteristic fragments ions (quantifier and qualifier) were selected for each analyte. Peak area ratios of qualifier and quantifier were used to confirm the identity of analyte.

MATERIALS AND METHODS

A screening method was developed using SWATH™ acquisition on the AB SCIEX TripleTOF 5600+ LC-MS/MS system coupled to an Agilent 1200 LC system. The MS method employed a TOP MS full-exact survey, followed by targeted SWATH™ product ion scans using Q1 isolation windows of up to 25 Da.

Sample Preparation:

Fruit and vegetable samples were extracted using QuEChERS approach based on EN15662 method. Briefly, 10 g of blended sample was mixed with 10 mL of acetonitrile in centrifuge tube, then the tube was shaken vigorously. The partitioning and buffering soils were added to the tube, and the tube was shaken vigorously again, and then centrifuged at 8000 rpm for 5 min, 1 mL of supernatant was transferred to a 2 mL tube that contained D5PJE powder. The tube was shaken vigorously for 30 min and then centrifuged at 8000 rpm for 5 min. 500 µL of supernatant was mixed with 500 µL of water, filtered through a 0.45 µm filter, and transferred to autosampler vials for analysis.

Pesticide standards for targeted quantitation were obtained from AB SCIEX QConcept™ Standards Kit. 100 of which were diluted at concentration level of 0.1 to 1000 ng/mL, to make calibration standards.

HPLC Conditions:

An Agilent 1200 LC system with a Phenomenex Spheri-Flash 25-µm Fusion-RP column and guard column was used to perform separation. Mobile phase A was acetonitrile (90/10) containing 5 mM of ammonium formate; mobile phase B was water/methanol (10/90) containing 5 mM of ammonium formate. The flow rate was 0.8 mL/min. Column temperature was set at 40 °C. A gradient reversed phase separation shown below was used for all the separations.

MS/MS Conditions:

An AB SCIEX TripleTOF 5600+ LC-MS/MS system with Duplex™ ion source was used. Samples were ionized in ESI positive mode. Data was acquired using SWATH™ acquisition, which facilitated the acquisition of MS/MS data for every component in the samples.

Data Processing:

For targeted quantitation, data was processed using MultiQuant™ software, which automatically extracted high mass accuracy XICs, integrated peak areas, and reported the concentration based on calibration curves. Two characteristic fragment ions (quantifier and qualifier) were selected for each analyte. Peak area ratios of qualifier and quantifier were used to confirm the identity of analyte.

Unknown screening was performed using MasterView™ software. Data from both organic and non-organic samples were loaded, and either of them could be used as control to perform sample-control comparison. Any peak that was significantly different in those two samples could be unknown pesticide. After discovering the possible pesticide ions, empirical formula was calculated using the TOP MS data, and tentative structure was determined by Chemspider database searching.

RESULTS

SWATH™ Experiments Setting

A few factors needed to be considered and balanced in setting the MS method. 1) There should be around 10 (or more) data points across one chromatography peak to achieve balanced reproducibility and accuracy for quantitation. Under the LC conditions used in this study, a typical peak width (baseline) was around 15 s. Hence one cycle for MS method shall be around 5 s. 2) LC-MS method was composed of 600 pesticides suggests more than 50% of which are between 100-500 Da. Given the fact that mass of many herbicdes (both endogenous and for samples) are also within this range, single Q1 isolation window is preferred at this mass range to increase selectivity for quantitation, and reduce the workload needed for qualification. 3) To prove the SWATH™ MS method we selected in two different SWATH™ analysis settings. Method 1 was set with a general Q1 selection window of fixed width 35 Da across the range 100-1000 Da. This method contained 36 SWATH™ experiments, and the total cycle time was 1.05 s. Method 2 was set with variable Q1 selection window, i.e., Window width of 10 Da across the range of 100-500 Da, and then 25 Da across the range of 500-1000 Da. This method contained 60 SWATH™ experiments, and the total cycle time was 1.8 s. Both methods allowed enough data points to achieve good reproducibility and accuracy for quantitation. However, as can be seen from Figure 3, Method 2 provided better selectivity due to the narrower Q1 selection window in this study. Method 2 was used for sample analysis.

CONCLUSIONS

A generic SWATH™ acquisition method enabled to obtain MS/MS fragments for all the components in a given sample, whether known or unknown. With variable Q1 selection window settings, the method provided good selectivity for postulated components for in real samples. Acquired data was processed for targeted quantitation using high resolution XICs, integrating peak area and reporting concentration based on calibration curves. The method contained 60 SWATH™ experiments, and the total cycle time was 1.8 s. Both methods allowed enough data points to achieve good reproducibility and accuracy for quantitation. However, as can be seen from Figure 3, Method 2 provided better selectivity due to the narrower Q1 selection window in this study. Method 2 was used for sample analysis.

Targeted Quantitation

Among the 100 compounds analyzed, over 100 of them fell linear dynamic range of 2.5 orders of magnitude or high, with typical concentration range of 0.2-2000 ng/mL (1-400 µg/mL) in sample. This concentration range covered the maximum residual level (10 µg/kg), and was good enough for quantitation. It was also noticed that some of the analytes showed relatively low intensity or poor linear dynamic range. This could be attributed to the generic MS settings for SWATH™ acquisition, and some compounds were not analyzed under their best MS conditions.

Unknown screening

Due to the data-independent nature of SWATH™ acquisition, a permanent record of MS/MS fragment for all the components in sample have been obtained. Except for targeted quantitation, the data could also be used for unknown screening. Further more, retrospective quantitation analysis maybe performed if any previously unknown pesticides became detectable in this study, both organic and non-organic samples, apple, peas and tomato samples were analyzed. By using organic species as control, a few pesticides were identified in their non-organic counterparts. For example, buprofezin and pyraclostrobin (both were in the targeted list) were discovered and confirmed in tomato sample (Figure 5). The calculated level for these two were 50 µg/kg and 3.5 µg/kg.

Five "unknown" pesticides were also identified in samples. For example, XIC of 732.4532 showed significantly high peak in organic sample compared to its non-organic counterpart, suggesting this could be an unknown pesticide. Further analysis was done to reveal the possibility of this unknown pesticide. Comparison of retention time and mass of this compound was compared to the tentative structure with acquired SWATH™-MS/MS spectra, spinosyn A became the most likely hit (Figure 6). In fact, another ion (m/z 746.4484) which was tentatively assigned to spinosyn D was also discovered in the same sample. Spinosyn A and spinosyn D often come in pair under a common name spinosad, which is an approved pesticide in organic agriculture.

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