Introduction

Although quantitative analysis has proven adequate to determine concentrations of environmental contaminants, the additional need to target and tentatively identified compounds has led to coupling Quantitative Analysis with Unknowns Analysis (QA).

Quantitative uses a prescriptive extraction method, and is well-accepted, but its assessment in concert with TentaSting Analysis, which offers deconvolution and library search algorithms, is not well-documented.

The study goal is to test a seamless joining of Unknowns Analysis to the Quantitation workflow, and to evaluate the degree to which these methodologies can provide both target and unknowns information from one scan.

Target and non-target analyses in the complex trace pesticide batch with overlapping matrix peaks were evaluated by comparing spectral scores and ion peak shape metrics.

Experimental

• The fruit juice extracts were prepared using the Agilent standard QuEChERS protocol. The extracts were evaporated to dryness.

• The pesticide standards were prepared in acetone at 0.01, 0.1, 1.0, 10.0, and 100.0 μg/mL, with two IS standards, 0.4-μg (EMQ) and Tert-Phenyl phosphates at 0.5 ng/mL. Each standard was spiked with 50% of the concentrated juice extract and analyzed in 3 replicates.

• The samples were analyzed by full-scan GC/MS using the instrumental arrangement as follows: 7890 GC with 5977A MSD utilizing the Rapid Universal GC/MS Bethesdata-I (G1512A) and the 5977B FID (G1458B) FID-PFP columns (p/n 5295-4537). The oven program was 30°C (2 min), 2°C/min to 160°C (3 min), 2°C/min to 280°C (5 min), 8°C/min to 290°C with constant flow of helium carrier at 1.3 mL/min.

• GC/MS analysis was performed by MassHunter Quant and Unknowns Analysis software.

Results and Discussion — Target Analysis

• Quant Analysis follows the steps of signal extraction, signal integration and quantitation using calibration curves to measure the concentration of targets in Quant the target ion is integrated for consumption sensitivity and the qualifier ratio serves as a confirmation metric.

• Unknowns Analysis displays the deconvoluted ion peaks as well as the ‘closest’ spectra compared to the library spectra to assist in evaluating the hit. Deconvolution may find more components than targets present. Reducing the list of components to isolate the tentatively identified compounds is the key operation.

• A mixture of 47 pesticide standards (targets) at 4 different concentration levels with 2 ISTD (top tablet) were spiked in fruit juice matrix and analyzed by GC/MS in replicate. There are 3 overlapping target pairs among the 96 where the TIC trace appears almost just a single peak and their retention times (RT) differences are less than 4 seconds. The figure above shows two of these overlapping target pairs where the retention time differences are both less than 1 second.

• Both approaches were used for target analysis: 1) Quant ion extraction where the targets were confirmed based on retention time and qualifier ratios; 2) Unknowns Analysis where the data were processed by spectral deconvolution and library search against a pesticide library containing 107 entries. The targets were the primary hits with match scores over 50 and retention time differences to the library hit to be less than 0.5 sec.

• At 10 ng/mL, Quant confirmed 40 out of 47 targets, but deconvolution only identified 25 targets. Loss of 12 is both retention and signal, 10 ng/mL, reflects the high influence of the matrix extraction at low concentration.

• Deconvolution detects more targets as concentration increases. At 2000 ng/mL, both Unknowns Analysis and Quant confirmed 46 targets.

Figure 1. (Left) Total Ion Chromatograms (TICs) of the pesticide mix at 5 conc. levels with overlapping matrix. (Right) Two chromatographic views of the overlapping target pairs along with the TIC at the 2000 ng/mL sample.

Results and Discussion — Non-Target Analysis

• Target Match step identifies both Target Hits and Non-Target Hits. Hits that are not target matched are labeled as non-target Hits. Estimation of constituent concentration leverages the Quant target response factors (RF) which are applied to Non-Target Hits. Estimation of response factors is flexible and can be adjusted to suit the particular analytical requirements.

For example, the RF of the closest target or ISTD in retention time can be used to estimate the concentration of any hit.

A list of 6 non-targets were identified in all samples by deconvolution. The extracted concentrations of each compound at different concentration level samples were calculated. The non-targets maybe the contaminants from the fruit juice matrix.

• The right figure displays the chromatograms of the six non- target compounds at 100 ng/mL. target 180 is highlighted in the top plot of the figure.

• The component information and the peak centers of the non-targets are listed in the linear table.

• The non-target compound, Cn-1.2,3-bis(trihydroxybutyl)ide, is highlighted in Compound. Its component spectrum along with the assigned library list is shown in the middle plot of the figure.

• The ion pairs displayed in the lower part of the figure shows the peak shapes. The Molecular Structure is drawn for visual confirmation.

Conclusions

• The Quant — Unknowns Analysis workflow matches the library hits with the known targets as the user can focus on the Non-Target Hits. The workflow offers the opportunity to employ a number of criteria to speed up the classification of non-targets to Tentatively Identified Compounds.

• Batch review of both quantitative and unknowns results on one scale meets the occupying industry demands of fast analysis as well as other industries facing more products in their tests. This workflow with DataID’s flight (10%) scan success along with derivate advanced of scan speed and mass accuracy ensures that precision.