MultiQuant™ Software Version 3.0

Improving Data Quality and Processing Throughput with Better Peak Integration, Quantitative and Qualitative Compound Review for the Analysis of Food, Drinking Water, and Environmental Samples

André Schreiber
AB SCIEX, Concord, Ontario, Canada

Key Features of MultiQuant™ 3.0

For laboratories analyzing food, water, or environmental samples for residues, contaminants and, pollutants data processing can be laborious and time consuming. The new MultiQuant™ software version 3.0 addresses some of the common bottlenecks laboratories face in data processing in order to improve quality and throughput:

- Full support of Windows XP, Windows 7 (32 and 64 bit) operating systems

- Processing of AB SCIEX triple quadrupole, QTRAP®, and TripleTOF® system data, including data generated using the *Scheduled* MRM™ Pro algorithm and *Scheduled* MRM[HR]

- Processing of UV, DAD, and ACD data

- Built-in queries for the calculation and flagging of:

  - Outliers in accuracy

  - Analytes below or above a target concentration

  - Ion ratios and ion ratio tolerances

- Easy result review using the display of ion ratios

- Side-by-side peak review to quickly compare the response of samples

- Peak review magnifier for easy review and adjustment of (manual) peak integration

- Additional result table columns to assess peak quality, including Asymmetry Factor, Tailing Factor, Slope of Baseline, Peak Width, Points Across Peak
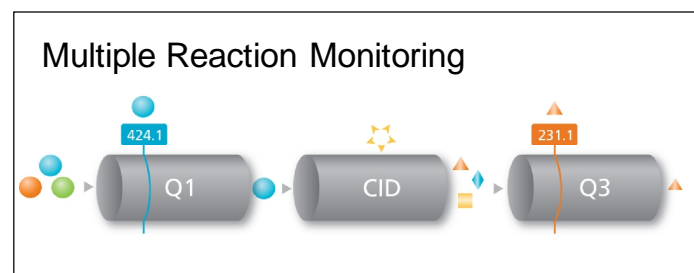
Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the quantitation and identification of chemicals in food samples and environmental samples. Triple quadrupole-based mass analyzers operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results (Figure 1a). Advancements in TripleTOF® technology also provide the ability to perform targeted quantitation with triple quadrupole-like performance (Figure 1b) and, at the same time, high confidence
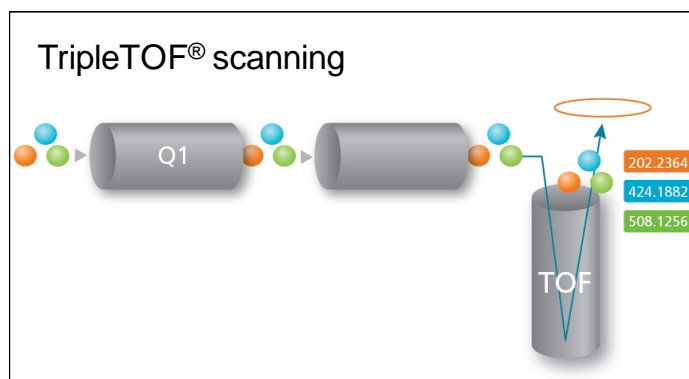

Figure 1a. Highly selective and sensitive quantitation using Multiple Reaction Monitoring (MRM) with triple quadrupole systems

Figure 1b. Highly selective and sensitive quantitation using narrow extracted chromatograms (XIC) of accurate mass TOF-MS ions generated using TripleTOF® systems

in compound identification based on accurate mass MS and MS/MS information, making accurate mass LC-MS/MS an interesting alternative for modern food and environmental laboratories.

The increase in throughput using simplified and automated sample preparation techniques and the ability to screen for hundreds of target compounds in a single analysis using the *Scheduled* MRM™ Pro algorithm and full scan accurate mass techniques has placed greater demand for faster data processing and review, which has remained a significant bottleneck. Peak integration, review of quantitative and qualitative results, and reporting are time consuming and labor intensive tasks.

MultiQuant™ software version 3.0 was designed for laboratories with the goal of improving data processing efficiency. Integrated within Analyst® software, the user has the ability to quantify and identify chemicals of interest in complex samples in data files generated on AB SCIEX triple quadrupole, QTRAP®, and TripleTOF® systems.

Here, innovative new features in MultiQuant™ software version 3.0 are highlighted which significantly improve the data analysis workflow for quantitation and identification of compounds of interest in food and environmental laboratories.

Experimental

Pesticide Residues in Fruits and Vegetables

Pesticides were quantified and identified in food samples after QuEChERS extraction with automated DPX cleanup automated cleanup using a GERSTEL MultiPurpose Sampler (MPS) 2XL. The AB SCIEX QTRAP® 4500 system was used with Turbo V™ source and Electrospray Ionization (ESI) probe. The *Scheduled* MRM™ algorithm was used to achieve best data quality while monitoring over 200 pesticides using two MRM transitions per analyte to allow simultaneous quantitation and identification based on the ion ratio.[1]

Glyphosate, Glufosinate and AMPA in Drinking Water

These pesticides were analyzed using automated FMOC-derivatization and LC-MS/MS using a GERSTEL MPS 2XL coupled to an AB SCIEX QTRAP® 4500 system. Water samples were injected directly into the LC-MS/MS system providing sufficient sensitivity to identify and quantify targets at sub 100 µg/L concentrations.[2]

PAH in Food and Water Samples

Polycyclic Aromatic Hydrocarbons (PAH) were detected by LC-FLD-MS/MS. A Shimadzu NEXERA UHPLC system with fluorescence detector followed by MS/MS confirmation with an AB SCIEX QTRAP® 5500 system was used for analysis.[3]

PPCP in Environmental Samples

Pharmaceuticals and Personal Care Products (PPCP) were quantified and identified using direct injection of water samples and TOF-MS and MRM^HR scanning techniques utilizing an AB SCIEX TripleTOF® 4600 system.

Results

Built-in Queries to Calculate and Flag Outliers

Built-in queries of MultiQuant™ software can be used to calculate and flag outliers in standard and quality control samples, as defined in the settings tab of the quantitation method editor (Figure 2).

An example of highlighted outliers is shown in Figure 3. This software feature enables easy data review and quick adjustments of integration parameters and calibration lines.

Figure 2. Query settings in the quantitation method editor of MultiQuant™ software version 3.0 to calculate and flag outliers, target concentrations, and ion ratios
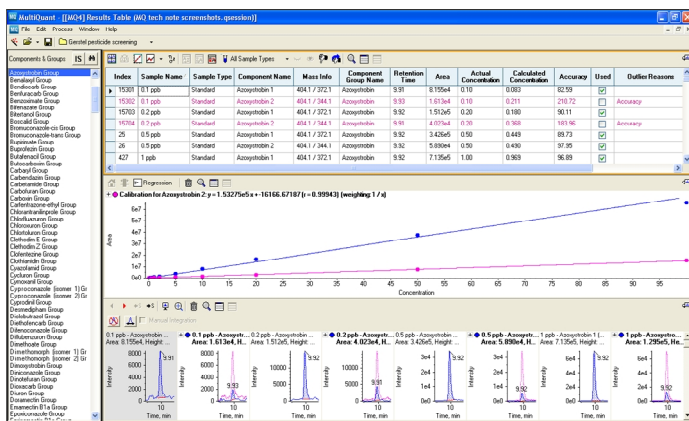
Figure 3. Azoxystrobin detected in a pesticide screening method using a QTRAP® 4500 system with highlighted outliers for the qualifier MRM transition at 0.1 and 0.2 ng/mL (calibration line of 0.1 to 100 ng/mL with r2 = 0.999 and %CV = 3.72% at 1 ng/mL, n = 10)

Built-in Queries to Highlight Analytes Below and Above a Target Concentration

Built-in queries of MultiQuant™ software can be used to highlight concentrations below or above a user specified value. The lower limit and upper limit of the calculated concentration can be defined in the method editor (Figure 2).

Examples of Spinosyn A detected in different food samples at a concentration higher than 5 µg/kg are shown in Figure 4.
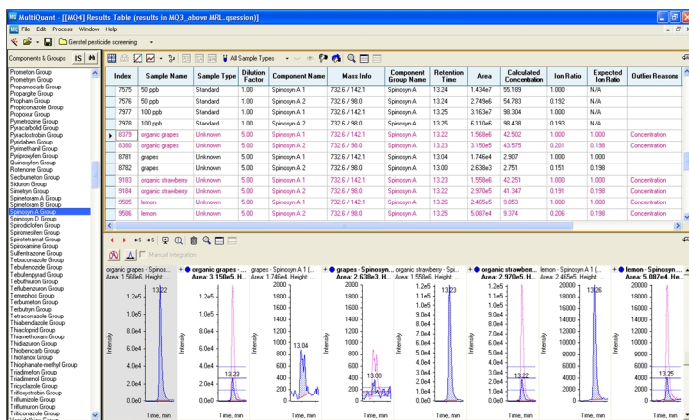

Figure 4. Spinosyn A quantified in different fruit and vegetable samples at a concentration higher than 5 µg/kg with positive identification using the MRM ratio

Built-in Queries to Calculate Ion Ratios for Compound Identification

Despite the high selectivity of MRM detection, there is always a risk of false positive or negative findings due to interfering matrix signals. Accordingly, quantitative results have to be confirmed using additional qualitative criteria. Often a second MRM or accurate mass fragment ion is monitored per analyte and the ratio of quantifier to qualifier transition is calculated for each unknown sample and compared to the ion ratio of standards. Various guidelines such as the European Commission Decision 2002/657/EC and SANCO/12495/2011 define MRM ratio tolerance levels for compound identification.

Built-in queries of MultiQuant™ software can be used to calculate ion ratios and flag outliers. Ion ratio tolerances for each analyte can be defined in the quantitation method editor (Figure 2).

Examples of Thiabendazole identified in different fruit and vegetable samples with MRM ratios inside of defined tolerance levels are shown in Figure 5a. The ion ratio is also visualized using tolerance bars in the Peak Review pane. The calculated ion ratio and expected ratio can be found in result table columns.
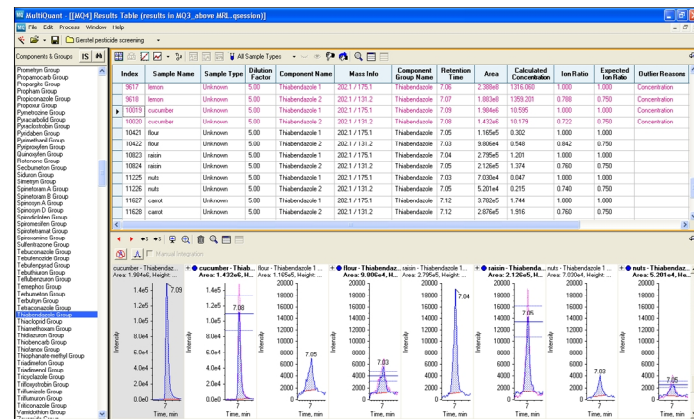

Figure 5a. Thiabendazole identified in different fruit and vegetable using the MRM ratio and compound dependent tolerance criteria, the ion ratio is also visualized using tolerance bars in the Peak Review

Figure 5b shows an example of reproducibility of ion ratios for identification detecting glyphosate, glufosinate, and AMPA in drinking water after automated FMOC-Cl derivatization using a GERSTEL MPS 2XL and LC-MS/MS.
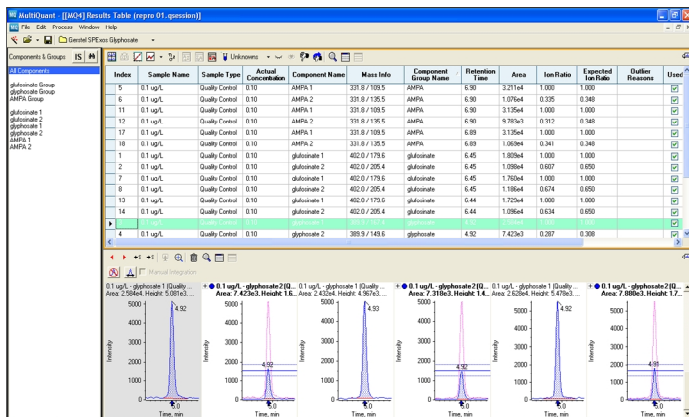
Figure 5b. Quantitation of glyphosate, glufosinate, and AMPA in drinking water at 0.1 µg/L with automatic ion ratio calculation for identification

Figure 6b. Buprofezin detected in a grape sample above the target concentration, the side-by-side peak review allows comparison of the response compound in different samples at a glance, the automatic calculation and visualization of ion ratios helped to identify a matrix interference causing the positive detection of Buprofezin

Side-by-Side Peak Review

MultiQuant™ software allows a side-by-side peak review of chromatograms to compare the response of a selected compound in different samples at a glance.

Figures 6a and b show examples of detection of Boscalid and Buprofezin in different fruit samples. The side-by-side review with linked intensity axis of standard and samples allowed to quickly identify compounds above the target concentration of 10 µg/kg in the 5x diluted food extract.
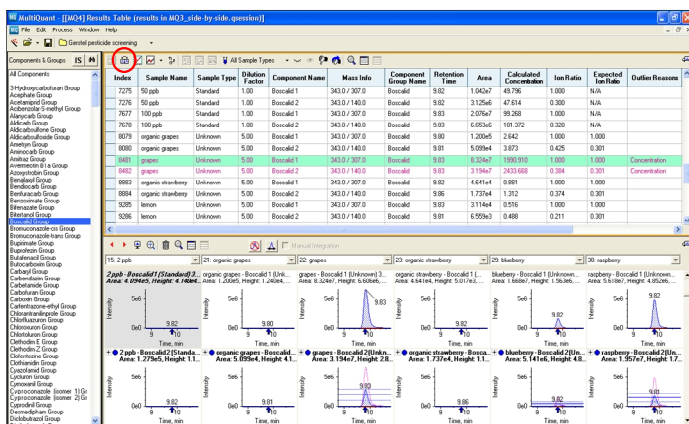
Processing of UV, DAD, ADC Data

MultiQuant™ software enables the use of UV, DAD, and other detectors via ADC channel for the quantitation. Method details are defined in the quantitation method editor (Figure 7).

An example of quantifying Benzo(a)pyrene using fluorescence detection (FLD) and MRM mode is shown in Figure 8.

Figure 6a. Boscalid detected in different fruit samples above the target concentration with positive identification using the MRM ratio, the side-by-side peak review allows comparison of the response compound in different samples at a glance, helping to quickly find samples of interest
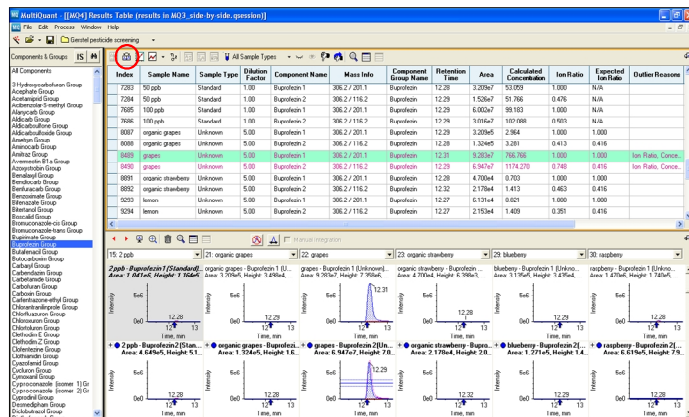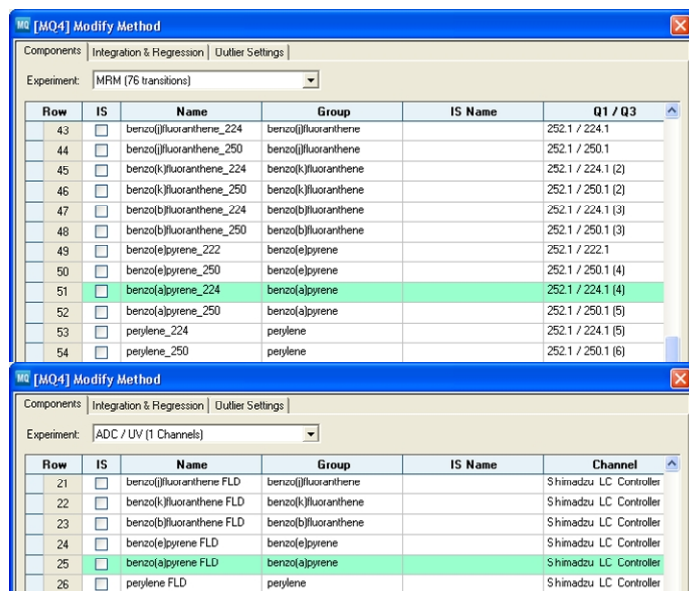

Figure 7. Method settings to process MRM and FLD data

Figure 8. Quantitation of Benzo(a)pyrene using FLD (left) and MRM mode (middle and right)

Easy Peak Review and Adjustments of Peak Integration

MultiQuant™ software offers a number of features to allow an easy peak review to correct peak integration of necessary. This includes the new 'Peak Magnifier' 'Peak Demagnifier'.

The 'Peak Magnifier' allows increasing the size of the peak review for selected chromatogram to the entire window. This enables better peak review to verify and adjust integration parameters, manual peak integration, or using the 'Set peak not found' feature. The example chromatogram shown in Figure 9 is a magnified display of Boscalid detected in a blueberry sample (compare Figure 6a).
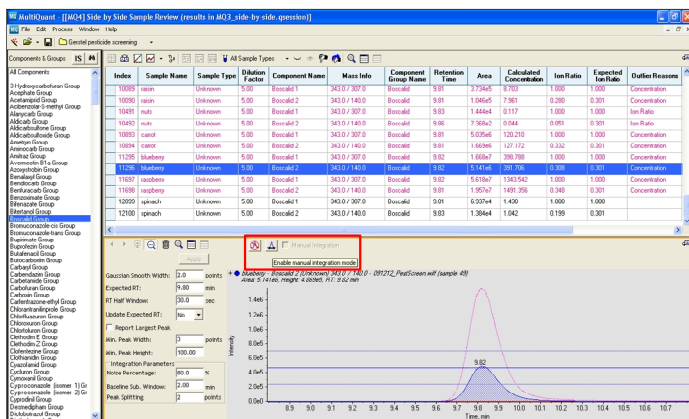

Figure 9. 'Peak Magnifier' to review a chromatogram of Boscalid detected in a blueberry sample, the peak integration can be adjusted quickly by changing integration parameters, manual peak integration, or using the 'Set peak not found' feature

Processing of TripleTOF® TOF-MS and MRM^HR Data

MultiQuant™ software can be used to process data generated on any AB SCIEX LC/MS/MS systems. TripleTOF® systems gain popularity for quantitative applications because of it triple quadrupole-like performance.

Figure 10 shows an example of quantifying Carbamazepine in a water sample spiked at 100 ng/L. The comparison of TOF-MS and MRM^HR data reveals the increased selectivity of the MRM^HR workflow. The different product ions detected in high resolution mode can also be used to calculate ion ratios automatically.
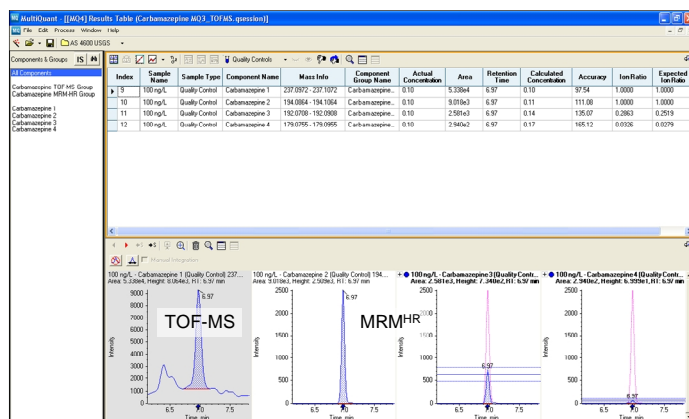

Figure 10. Carbamazepine at 100 ng/L quantified using TOF-MS and MRM^HR and identified based on ion ratio calculation

Summary

New features in MultiQuant™ software version 3.0, such as build-in queries, automatic ion ratio calculation, and side-by-side peak review with peak magnifier, significantly improve the data analysis workflow for quantitation and identification of food residues, contaminants, and environmental pollutants.

References

[1] André Schreiber et al.: 'Automated Sample Preparation and Analysis Workflows for Pesticide Residue Screening in Food Samples using DPX-QuEChERS with LC-MS/MS' Application Note AB SCIEX (2013) 8013613-01

[2] André Schreiber and Oscar Cabrices: 'Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Other Polar Pesticides' Application Note AB SCIEX (2013) 8013813-01

[3] Takeo Sakuma et al.: 'Analysis of Polycyclic Aromatic Hydrocarbons (PAH), Alkylated Derivatives, and Photo-degradation Products in Environmental and Food Samples using LC-FLD-MS/MS with Q TRAP® Technology' Application Note AB SCIEX (2011) 4520411-01

[4] André Schreiber et al.: 'Quantitation and Identification of Pharmaceuticals and Personal Care Products (PPCP) in Environmental Samples using Advanced TripleTOF® MS/MS Technology' Application Note AB SCIEX (2013) 7200213-02