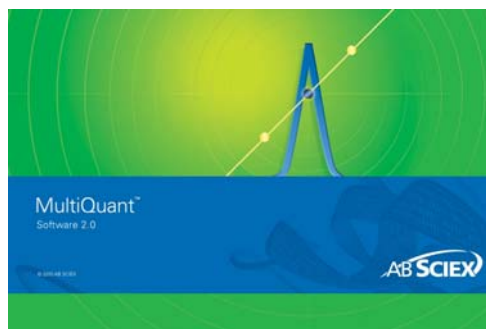


MultiQuant™ Software 2.0 with the SignalFinder™ Algorithm

The Next Generation in Quantitative Data Processing

Quantitative analysis using LC/MS/MS has benefited from a number of advancements that have led to improved efficiency, increased sample throughput, and gains in overall speed. For example, automated sample preparation, the increased throughput of UHPLC, and multiplexing of chromatographic separations have increased the number of samples that can be analyzed in a given amount of time. Other improvements such as automated compound optimization (DiscoveryQuant™ Software¹) and the ability of modern triple quadrupole and QTRAP® systems to monitor hundreds of analytes (Scheduled MRM™ Algorithm²) have increased the number of compounds that can be analyzed per sample.

These improvements have placed great demands on data processing, which remains a significant bottleneck in the overall process. Ensuring peaks are integrated consistently and accurately is critical to the overall quality of an assay. However, peak integration



and especially peak review are time consuming manual tasks.

In this technical note, we describe powerful yet easy-to-use new features in MultiQuant™ Software 2.0 to increase the speed and accuracy of data processing while reducing the need for manual review. These features are the result of improvements in the software user interface and functionality, as well as fundamental improvements in the science of peak integration.

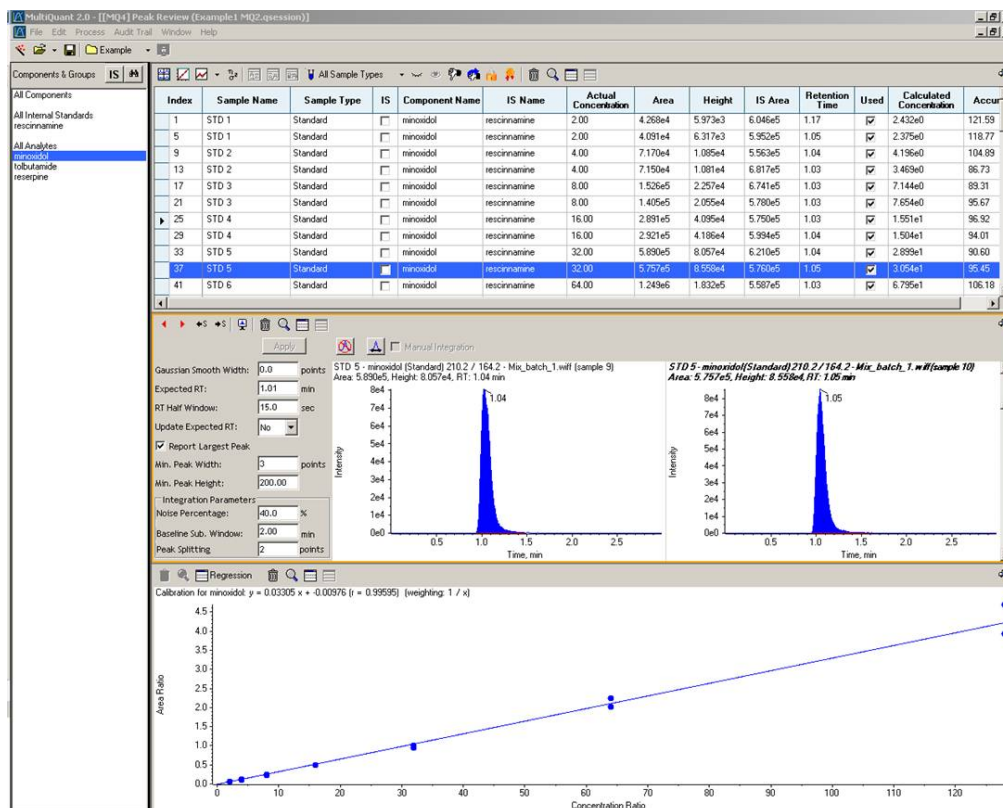


Figure 1. Easy-to-Use Interface. Intuitive user workspaces make data navigation easy, with dynamic linking of panes displaying results, analytes, integration and calibration curves.

Key Features of MultiQuant™ Software 2.0 for Fast and Powerful Data Processing

- Fast and user friendly interface with linked panes, custom queries and one click metric plots.
- The new SignalFinder™ integration algorithm, capable of more consistent peak integration with less user intervention.
- Saturation Correction for increased linear range.
- Predefined advanced queries for faster data review.
- Versatile functions such as external calibration tables and custom report generation.

		IntelliQuan			SignalFinder		
		Response	Each QC	Data Set	Response	Each QC	Data Set
		cps	%CV (n=20)	%CV (n=20)	cps	%CV (n=20)	%CV (n=20)
MRM #1	QC1	9.99E+05	3.3	3.2	9.97E+05	3.3	3.3
	QC2	7.58E+05	2.5	35.2	9.88E+05	2.5	2.5
	QC3	4.36E+04	2.2	33.9	5.71E+04	2.2	2.2
	QC4	9.38E+03	2.5	23.4	1.15E+04	2.4	2.4
	QC5	3.25E+03	3.8	52.0	3.61E+03	3.3	3.3
MRM #2	QC1	9.98E+05	2.9	6.7	1.01E+06	2.8	2.8
	QC2	8.79E+05	2.8	19.2	1.00E+06	2.7	2.7
	QC3	2.44E+03	3.0	19.9	5.78E+04	2.7	2.7
	QC4	5.05E+04	4.7	20.0	1.09E+04	4.4	4.4
	QC5	9.23E+03	8.3	17.8	3.24E+03	7.0	7.0

Table 1. Comparison of Integration Precision Across a Wide Concentration Range. SignalFinder algorithm performed significantly better when a *single* set of integration parameters was applied to the entire data set.

Speed and Ease-of-Use

MultiQuant Software has been designed from the ground up with an intuitive easy-to-use interface. During data processing, the user can quickly build a new processing method and review data in linked panes which automatically display the chromatographic peaks and integration when a sample is selected. In a single click, the user can view all analytes or a specific analyte. The results table and peak review panes are automatically updated. In addition, metric plots such as an internal standard trend can be generated instantly with a single mouse click. Custom queries can also be set to help evaluate data and allow the analyst to focus their data review efforts on specific samples. These elements have resulted in substantial time savings in real life quantitative ADME assays³.

MultiQuant Software 2.0 is capable of processing all types of MS data generated by AB SCIEX instruments such as MRM, MRM³, and extracted ion chromatograms from full scan MS or MS/MS data, providing a single easy to learn platform for all quantitative data processing.

SignalFinder™ Algorithm: Consistent and Accurate Integration

SignalFinder Algorithm is a new and highly innovative integration algorithm developed for improved LCMS peak integration. Based on mathematical peak modeling, the algorithm is capable of performing more consistent integration of chromatographic peaks with less operator intervention. This results in greater accuracy and data processing speed.

Implemented in MultiQuant Software 2.0, the SignalFinder algorithm is able to achieve more consistent integration by employing advanced peak modeling. This next generation approach uses the actual raw data to construct a mathematical model of the chromatographic peak as well as the associated noise. This allows more intelligent detection of the peak and in turn, more consistent integration across a wide concentration range without the need for operator intervention to adjust integration parameters (Table 1).

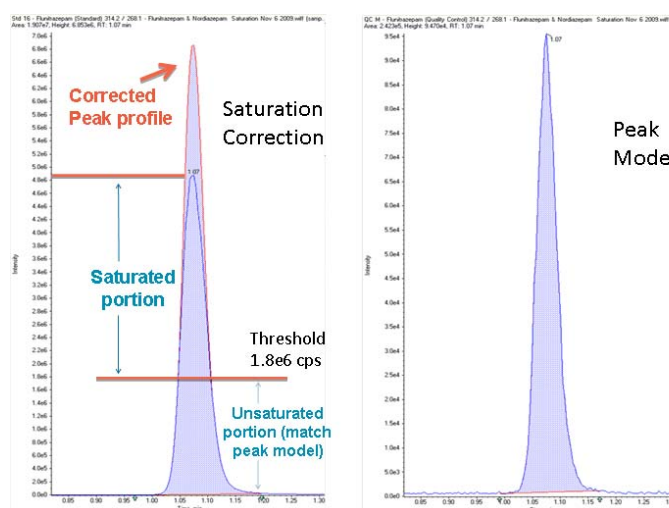


Figure 2. Saturation Correction. (Left) The unsaturated portion of the peak is used by SignalFinder to extrapolate the correct peak profile and intensity. (Right) Peak model from an unsaturated peak used to extrapolate the peak profile of the saturated peak.

Saturation Correction

Among the most innovative features of MultiQuant™ Software 2.0 with SignalFinder™ Algorithm is Saturation Correction. This feature can compensate for saturation of detector response at higher intensities, which allows for a wider quantitative linear range and reduces the need to perform dilutions and re-injections.

Using the peak model, SignalFinder Algorithm performs saturation correction by analyzing the chromatographic peak and using the unsaturated portion to extrapolate the actual response (Figure 2). Saturation correction can result in significant extension of an assay linear range when detector saturation has caused the calibration curve to deviate from linear response.

Advanced Peak Deconvolution

Peak deconvolution in SignalFinder allows the software to consistently integrate poorly resolved peaks across the concentration range (Figure 3). By modeling the actual peak shape from the raw data,

the software can more reliably define the peak boundaries and provide accurate integration. Traditional algorithms which only attempt to identify where the peak starts and ends require more operator intervention to integrate these types of peaks consistently.

Queries

MultiQuant™ Software 2.0 provides powerful query functionality to aid in data review. For example, queries can be constructed with user defined tolerances to identify poorly integrated peaks (Figure 4). This allows the analyst to focus on peaks and samples that require manual review leading to significant time saving during data processing.

Queries are versatile and can also be used to quickly spot failed standards or QC's. The failed samples are automatically flagged and a reason is provided. Queries are fully customizable and can even consist of a collection of sub-queries to evaluate the statistical acceptance of a data set based on multiple criteria. The user can select which sub-queries to apply to each data set.

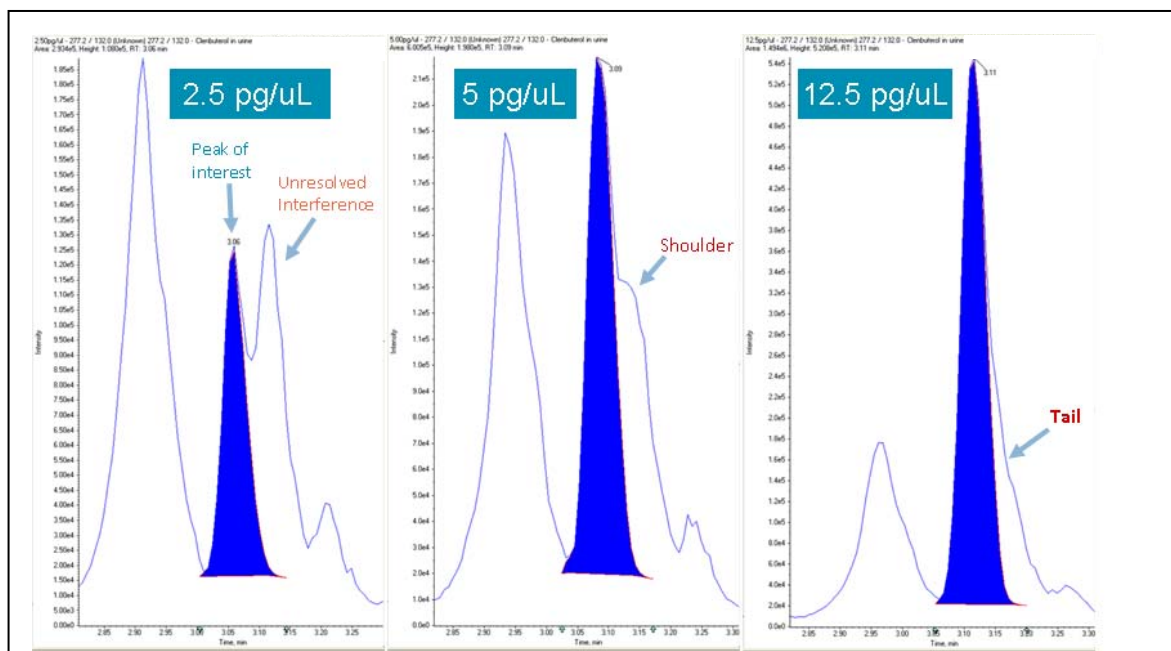


Figure 3. Advanced Peak Deconvolution. Peak modeling allows the peak of interest to be integrated correctly in the presence of an interference. No parameter adjustments were required for different peak intensities from 2.5 pg/μL to 12.5 pg/μL.

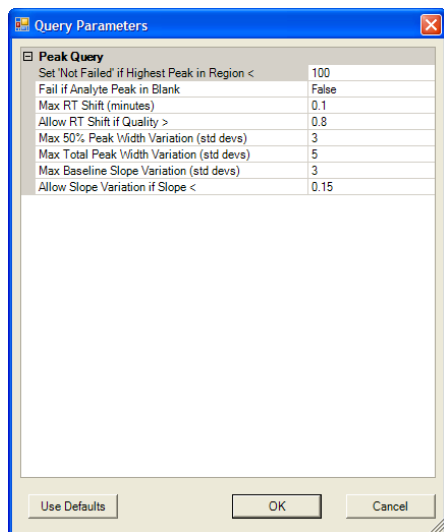


Figure 4. Automated Peak Query. Multiple criteria are used to evaluate peak integration. All tolerances are user defined.

External Calibration

MultiQuant™ Software provides versatile calibration options through the use of external calibration tables. This allows the quantification of one MRM transition against another. For example, a C-14 radiolabelled analyte can be quantified against a non-radiolabelled standard curve. Similarly, endogenous species can be quantified against a calibration curve constructed from an isotopically labeled analogue right from within MultiQuant Software.

Custom Report Generation

MultiQuant Software 2.0 includes powerful reporting functionality. Pre-configured templates are provided for the most common report formats and the templates are fully customizable through Microsoft® Word.

Processing of HTS and ADME Data with Multiple Rapid Injections Per Sample Data File

Rapid injection systems such as the Biocius RapidFire can perform more injections per minute for high throughput assays such as CYP inhibition and metabolic stability. It is advantageous to store the

data from multiple injections in the same data file. MultiQuant Software 2.0 makes it very easy to process this type of data using the multiple injections page of the method creation wizard (Figure 5).

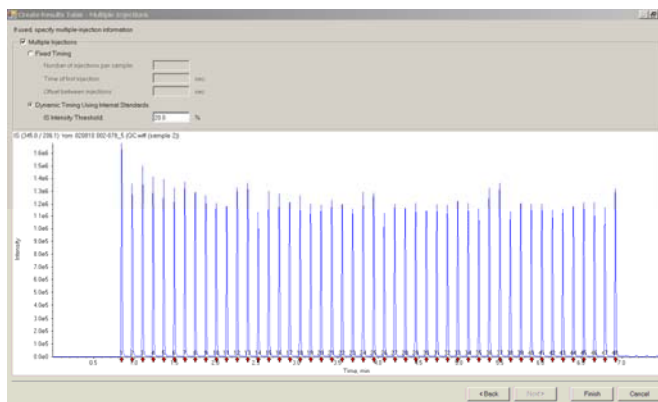


Figure 5. MultiQuant Easily Handles Multiple Injections per Data file. Dynamic timing is automatically applied using the internal standard signal.

Conclusions

- MultiQuant Software 2.0 is powerful yet easy to use software with the potential to significantly reduce the amount of time required for data processing and review.
- SignalFinder™ Algorithm is a novel integration algorithm capable of more consistent and accurate integration with less operator intervention.
- Saturation correction can compensate for saturation of detector response and extend the linear dynamic range of an assay.
- Advanced queries can be used to identify poorly integrated peaks and allow the analyst to focus their time on samples requiring manual review.
- The software is intuitive and easy-to-use which reduces the amount of time required to train new operators.
- With versatile features such as external calibration and processing multiple injections per sample data file, MultiQuant Software 2.0 meets the needs of wide range of quantitative ADME assays.

References

1. "DiscoveryQuant™ 2.0 Software: The Definitive Solution for LC/MS/MS Early ADME Workflows". AB SCIEX Technical Note, 2009.
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3. "Automation of Integrated LC-MS/MS Peak Quality Assurance and Analytical Run Quality Control," S. Crathern, K. Geddes, L. Burton, R. King. The 57th ASMS Conference on Mass Spectrometry and Allied Topics, May 31 – June 4, 2009, Philadelphia, PA.
4. "MultiQuant™ Software 2.0: The New Standard in Productivity and Compliance for the Regulated Bioanalytical Laboratory". AB SCIEX Technical Note, 2010.
5. To download a trial version of MultiQuant™ Software please visit:
<http://licensing.absciex.com/download/MultiQuant/2.0/>

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