

TARGETED PROTEIN QUANTITATION



Biomarker & Omics Solutions

FOR TARGETED PROTEIN QUANTITATION



As the market leader in mass spectrometry quantitation for over 20 years, we understand that accuracy, precision and reproducibility are of the utmost importance, and AB SCIEX systems are engineered from the ground up with just that philosophy in mind. With optimized software, reagents and workflows, AB SCIEX Targeted Protein Quantitation solutions push the limits of analysis and help deliver breakthrough discoveries in protein and biomarker research.



Starting with the right tools

Powerful mass spectrometry systems are combined with innovative software and reagents, to create workflows that drive increased productivity, while saving time and money.

The market leading AB SCIEX Triple Quad™ and QTRAP® systems are designed to maximize the number of MRM/SRM transitions and deliver reproducible peptide quantitation at the lowest limits of quantitation (LLOQ) day after day. The unique TripleTOF™ system delivers powerful discovery data but is equally at home generating high-specificity quantitation using high-resolution MRM (MRM^{HR}).

MRM Assay Development



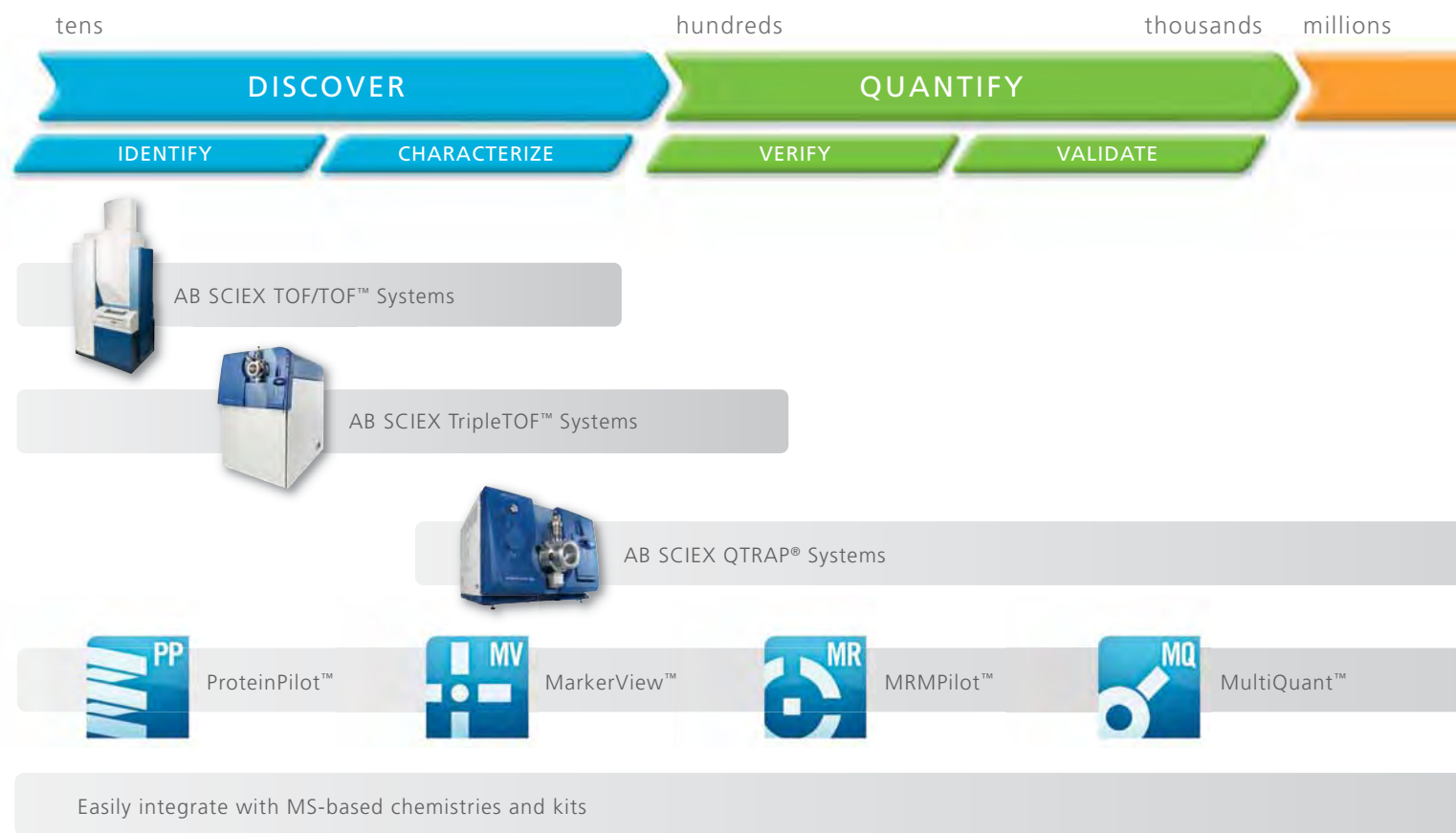
MRM Assay



The protein research pipeline

Protein research is a continuous pipeline. Typically beginning with a small number of samples, hypotheses are tested and key proteins are identified and characterized. These proteins can then be verified and validated in greater numbers of samples to account for biological variation. Finally, assays are developed for routine quantitation of the proteins.

Protein research pipeline



samples

ASSAY

HARDWARE

SOFTWARE

REAGENTS

Solutions for Quantitation

Powerful hardware

Establish the foundation for high-quality protein quantitation with the world-class performance and walk-up usability of our QTRAP® 5500 and TripleTOF™ 5600 systems with Eksigent LC technologies.

Timesaving software

Develop assays to many proteins and quantify across hundreds of samples using MRMPilot™ and MultiQuant™ software, and make use of the innovative SignalFinder™ and *Scheduled MRM*™ Algorithms.

Optimized strategies for internal standards

Maximize analytical reproducibility of your assay, correct for experimental inconsistencies, and enable normalization and comparison across many samples, instruments, and sites.

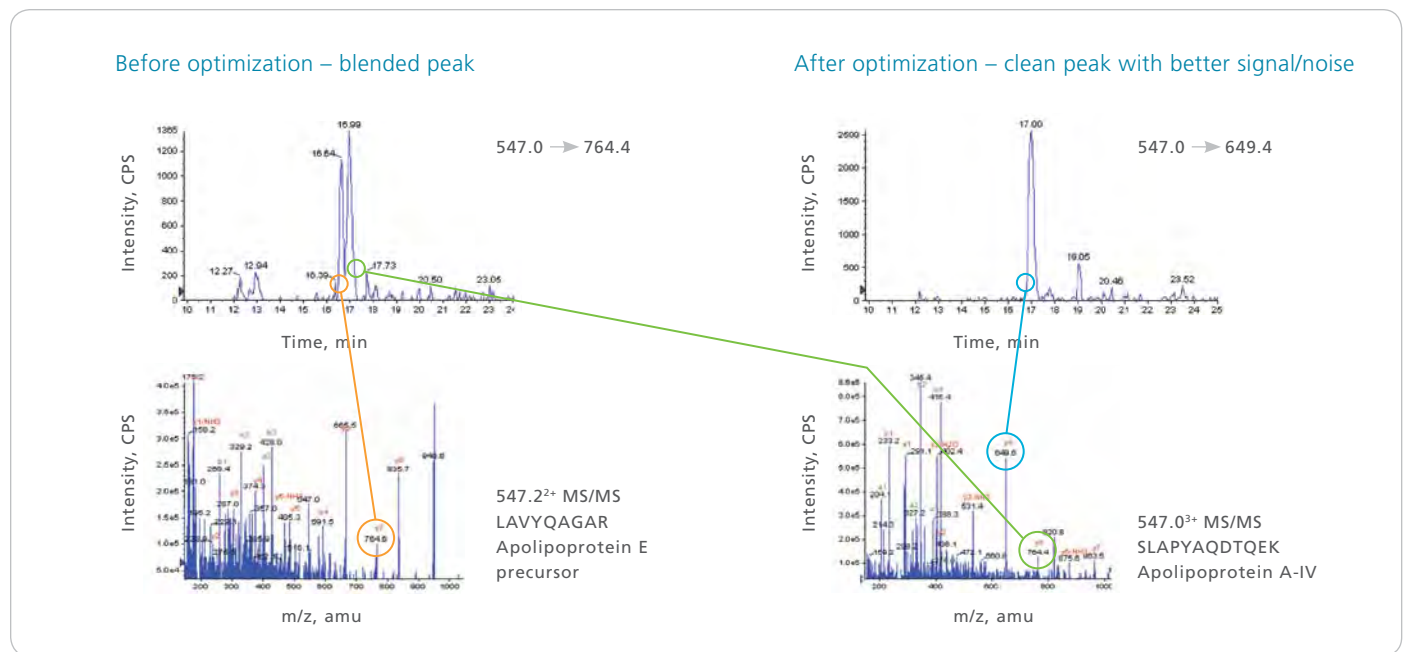
It all starts with MRM assay development

You need the right tools to create robust, reliable assays. QTRAP's are the best triple quadrupoles available, but with combined Linear Ion Trap technology, they offer so much more. And MRMPilot™ Software brings intuitive simplicity.

Powerful and unique instrumentation

The unique TripleTrap™ scanning capability of the QTRAP® system enables the MIDAS™ workflow—an amazingly powerful solution to create optimized MRM/SRM assays directly from your discovery data, and without the need to develop peptide/protein standards. The MIDAS Workflow triggers a very fast, high sensitivity full scan MS/MS spectrum in the linear ion trap that allows you to see the full sequence of the peptide you are analyzing. This feature enables facile optimization of MRM assays as well as powerful troubleshooting when interferences confound results. What's more, not needing to use peptide or protein standards initially during assay development means huge cost and time savings.

The AB SCIEX TripleTOF™ 5600 System has a similar capability to the MIDAS™ Workflow because the detection of fragment ions is in the TOF analyzer. By design, quantitation on the TripleTOF system is performed by acquiring fast, full scan MS/MS spectra of target peptides and performing high resolution extracted ion chromatograms on the fragment ions of interest (MRM^{HR}). All fragment ions for each peptide are acquired in every scan, greatly facilitating assay development and troubleshooting. Additionally, the specific fragment ions used for quantitation are selected post-acquisition, further simplifying the amount of up-front method development work required and improving downstream processing options and robustness.

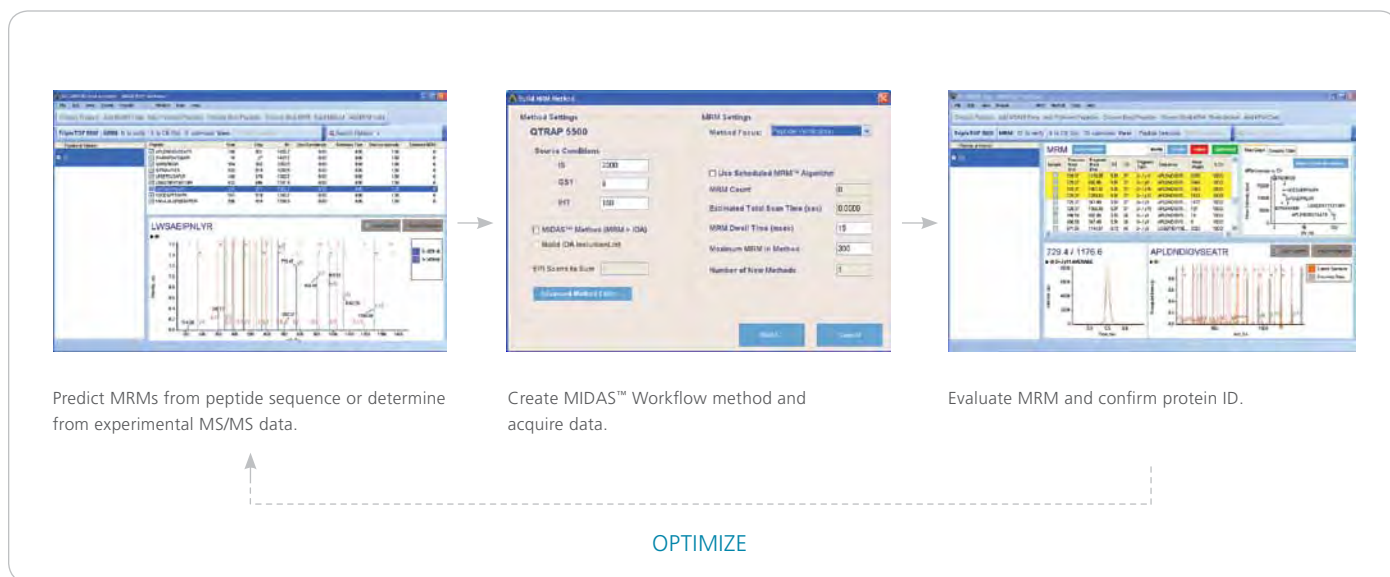


MRM assay specificity and robustness are maximized using the iterative design feature of the MIDAS Workflow on the QTRAP. TOP LEFT: The predicted transition based on discovery data generated a blended peak due to a common 547 fragment ion of Apolipoprotein E precursor (bottom left) and Apolipoprotein A-IV (bottom right). TOP RIGHT: After optimization using the MIDAS Workflow a different transition ion is selected, the interference is overcome, and the sensitivity is increased.

MRMPilot™ Software – simplified assay development

MRMPilot™ Software uses the power of the MIDAS™ workflow on QTRAP® systems and MRM^{HR} on TripleTOF™ systems to enable iterative development of MRM assays. With MRMPilot software, assay development is simple. Potential peptides and fragment ions for multiple proteins are determined from predictive methods or from existing MS/MS discovery data. For example, results from ProteinPilot™ software or other sources such as the MRMatlas can be seamlessly uploaded. MRMPilot software then automatically creates an MRM-based MIDAS workflow or MRM^{HR} acquisition method.

Once the data are acquired, the data files are opened back into MRMPilot software and evaluated for quantitative suitability and peptide identity using the powerful visualization tools in MRMPilot software. Additional iterations of the workflow can be used to further optimize transitions for all peptides and proteins, such as CE optimization and RT determination for *Scheduled* MRM™ Algorithm methods. In this way, high quality, robust and reliable assays are generated that can be stored for future use in MRMPilot's ProteinAssay catalog.



“We could not have developed the MRMatlas as quickly and productively without QTRAP® technology.”

RUEDI AEBERSOLD, PH.D., PROFESSOR, INSTITUTE OF BIOTECHNOLOGY AT THE SWISS FEDERAL INSTITUTE OF TECHNOLOGY

MRM Assays: Optimized precision, maximized capacity

For some assays it's very important to monitor hundreds or thousands of transitions all in a single run. With the *Scheduled MRM™* algorithm that's made possible without requiring time-consuming manual method creation. With the *Scheduled MRM* Algorithm, simply enter a few parameters such as the MRM transitions and their expected retention times and the algorithm does all the work for you. Methods are generated automatically

where each MRM transition is only monitored around it's expected elution time, thereby maintaining the analytical precision and accuracy of your assay and allowing a maximum number of transitions to be monitored in a single run. This unique strategy effectively reduces MRM concurrency to deliver gold-standard quantitation every time, whether you are using UHPLC to reduce run times or analyzing thousands of peptides in a run.

Scheduled MRM™ Algorithm provides better S/N and better %CV

A. No scheduling

B. *Scheduled MRM™* Algorithm data

C. Algorithm window

O1 Mass	O3 Mass	Time	ID	CE
738.9	487.3	30.3	APOC3.GWVTDGFSSLK.2y3 light	42
738.9	574.4	30.3	APOC3.GWVTDGFSSLK.2y4 light	42
738.9	778.4	30.3	APOC3.GWVTDGFSSLK.2y6 light	42
738.9	699.3	30.3	APOC3.GWVTDGFSSLK.2b5 light	42
742.9	491.3	30.3	APOC3.GWVTDGFSSLK.2y3 heavy	42
742.9	578.4	30.3	APOC3.GWVTDGFSSLK.2y4 heavy	42
742.9	762.5	30.3	APOC3.GWVTDGFSSLK.2y6 heavy	42
742.9	703.4	30.3	APOC3.GWVTDGFSSLK.2b5 heavy	42
619.7	527.3	21.8	APOC3.DALSSVQESQVAGQAR.3b4.36	
619.7	614.3	21.8	APOC3.DALSSVQESQVAGQAR.3b5.36	
929.0	440.3	21.8	APOC3.DALSSVQESQVAGQAR.2b3.51	

Duration: 5,000 (min) Delay Time: 0 (sec)
Cycles: 150 Cycle: 2,0000 (sec)

The *Scheduled MRM™* Algorithm enables the analysis of thousands of MRM transitions while still maintaining or improving the analytical precision. As shown in B, with scheduling, there are many more data points collected across each peak, improving the precision for all MRM transitions for each compound. In C, the algorithm window is displayed. A few simple parameters are input by the user and the method is built automatically.

Interference happens, even using MRM

Interference in targeted quantitative assays can come from different sources and can degrade the quality of the quantitative data and ultimately the detection limits. The best way to increase the robustness of the assay is to avoid interferences all together through higher specificity methods.

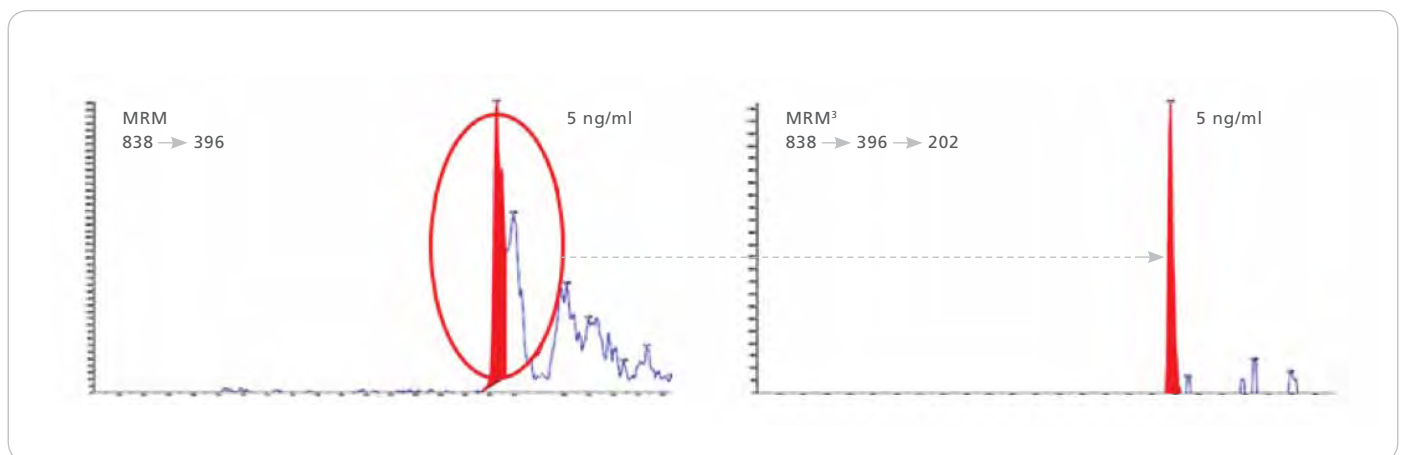
MRM³

MRM³ is a technique for increasing the specificity of your assay that is only available with the AB SCIEX QTRAP[®] 5500 System – the highest sensitivity triple quadrupole and linear ion trap, all in a single platform. It enables quantitation by enhancing normal MRM with a secondary product ion that is generated in the linear ion trap, and is an effective strategy for quantitation of analytes when high background or interferences make standard MRM quantitation difficult. Alternatively, MRM³ can be used to achieve similar LOQ's with less sample preparation and simplified or faster chromatography. MRM³ has been successfully applied to the detection and quantitation of small molecules, peptides, and protein biomarkers. The QTRAP[®] 5500 LC/MS/MS system delivers the most robust and sensitive MRM assays available on the market today, but can also provide users with this unique and powerful feature when you need it most.

MRM^{HR}

Because of its unique combination of high speed acquisition with high resolution, and 4 orders of linear dynamic range, the AB SCIEX TripleTOF[™] 5600 System enables ultra high specificity quantitation. MS/MS spectra are collected and fragment ions are extracted post-acquisition at high resolution to generate MRM-like data. The technique is sensitive and fast enough to enable quantitative performance equal to or better than many high end triple quadrupole instruments. In cases where there are interfering fragment ions, a significant improvement in LLOQ can be achieved by using higher resolution fragment ion extraction.

MRM³ – only available using QTRAP technology



Quantitation of the therapeutic peptide Exenatide in human plasma. MRM was not selective enough and suffered from large endogenous interferences in plasma. MRM³ completely eliminated the interferences and improved both the LOQ and the linearity.

Higher reproducibility quantitation using chips

The Eksigent cHiPLC®-nanoflex system

The cHiPLC-nanoflex System is an innovative and flexible solution for highly reproducible chromatography with chip to chip reproducibility of less than 2% for standardizing your workflows. The patented chip-to-world connection provides the lowest dead-volume, leak-free connections every time and maintains the highest data quality.

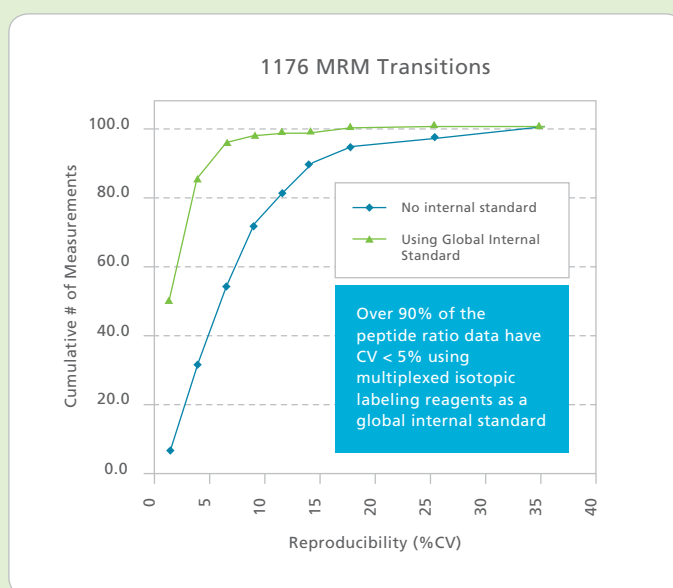
When combined with the split-less Eksigent NanoLC-Ultra® UHPLC System the retention time reproducibility of less than 0.5% RSD makes protein and peptide quantitation more reproducible than ever before.

- Reproducible results from day-to-day and lab-to-lab
- Flexibility to switch between workflows and projects rapidly in multi-users labs
- Plug & play chip simplicity with the performance of a nano column



Compatible with all internal standard strategies

Internal standards are important in order to maximize the analytical reproducibility of your assay. They correct for experimental variation as well as enable normalization and comparison across many samples, instruments, and sites. Using a multiplexed isotopic labeling reagent, such as mTRAQ® reagents, a global internal standard strategy from a pooled reference can be employed that generates an internal standard for every peptide in the sample simultaneously, no matter how many there are. This strategy can be much more cost effective than using isotopically labeled synthetic peptide standards, especially when monitoring many peptides.



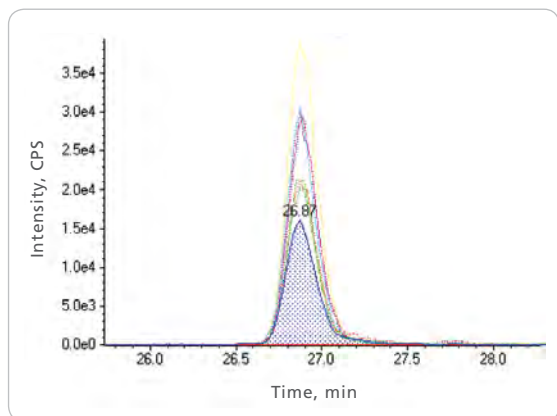
MultiQuant™ Software – automation drives productivity

Built upon years of experience in MRM quantitative processing, MultiQuant™ Software was designed to enhance and automate the processing of large peptide-MRM datasets in an intuitive, user-friendly workspace. It supports all internal standard strategies for both relative and absolute quantitation and provides query tools to minimize manual data review and data integration. Flexible reporting capabilities are available to create user-specific reports, and the data can be easily exported to Microsoft Excel®.

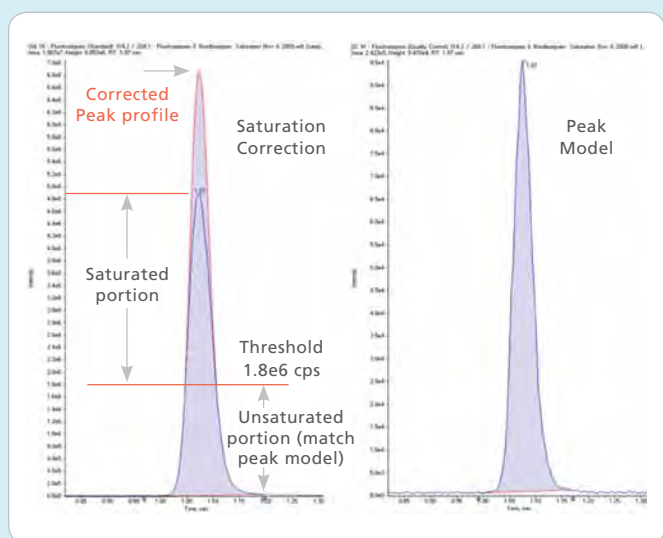
Powerful integration algorithms increase performance

The SignalFinder™ Algorithm integrates chromatographic peaks with exceptional consistency and accuracy – especially in cases of low level peaks and difficult baselines. Using the SignalFinder™ Algorithm, you can identify a single set of parameters that work for most if not all of the chromatograms in a sample set, while also eliminating manual integration and potential regulatory difficulties.

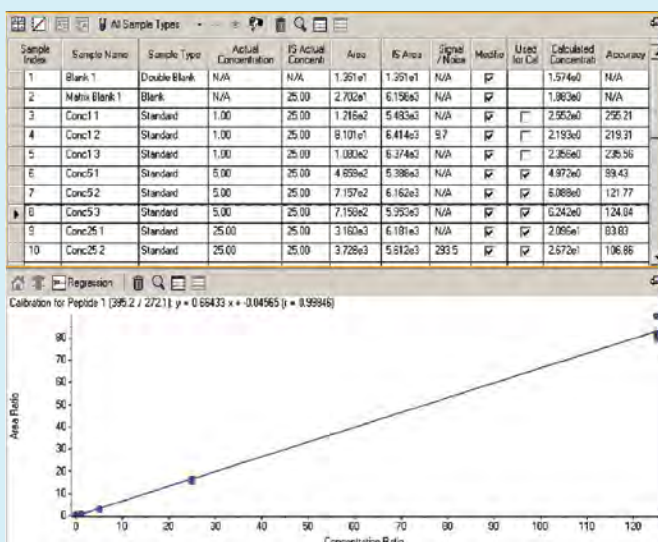
And that's not all. The SignalFinder™ Algorithm can optionally compensate for detector saturation at higher concentrations. This helps to extend the quantitative linear range and potentially reduces the need to perform dilutions and re-injections on higher concentration samples.



Multiple transitions for a peptide are graphically overlaid for instant comprehension of assay integrity.



Saturation Correction. (Left) When the 'Use Saturation Correction' option is enabled, the unsaturated portion of the peak is used by the SignalFinder™ algorithm 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.



MultiQuant Software works directly with your data. For absolute quantitation, a standard concentration curve is created from the analysis of known concentrations of a labeled version of a peptide of interest. To determine the amount of peptide in a biological sample, the MRM response is compared to the standard concentration curve. Then, peaks and peak integration can be visualized and displayed for manual data review if desired.

You invest in our technology. We invest in your success.

As the world leader in mass spectrometry, AB SCIEX solutions are backed by the industry's most extensive service and support organization. With a network of service professionals, experienced compliance specialists, and over 150 PhD application scientists worldwide, we are dedicated to supporting your technical needs and helping you get the most out of your AB SCIEX systems.

AB SCIEX service professionals are recognized as the most highly qualified in the industry. They are certified on our instrument platforms through a rigorous 4-step certification program, with re-certification occurring every two years. This award-winning program helps to ensure that you receive the most efficient, highest-quality, and most up-to-date service available for AB SCIEX products and technology. Choose from flexible service plans and a variety of services for the right level of support for your laboratory's needs and budget.

Our customer support network is available to provide expert assistance in the use and application of AB SCIEX products through a comprehensive range of services, including application support, technical service, and training.

Whether you access our service and support team by phone, email, on-site visits, or through our innovative remote monitoring technology, you can be confident that the AB SCIEX organization will be there for you.

For more information, visit www.absciex.com

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0930510-01 04/2011



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