MassSpectator

Issue 5

Deconstruct and simplify mega-data from xenobiotic metabolite studies with PCVG

Counting needles in a haystack: Improving sensitivity and quantitation of low-level tryptic peptides

Is your lamb only dressed in sheep's clothing? Using the MIDAS workflow to authenticate meat.

What every GLP lab should know about software validation

How secure is your mass spectrometer?

AB SCIEX

For Research Use Only. Not for use in diagnostic procedures.

Contents

Technology focus 4-7
Deconstruct and simplify mega-data from xenobiotic metabolite studies with PCVG

Customer spotlight 8-11
Counting needles in a haystack: Improving sensitivity and quantitation of low-level tryptic peptides

Application focus 12-15
Is your lamb only dressed in sheep's clothing? Using the MIDAS workflow to authenticate meat

Customer support 16-18
What every GLP lab should know about software validation

Product highlight 20-23
How secure is your mass spectrometer?

RUO-MKT-09-1274-A

Denconstruct and simplify mega data from xenobiotic metabolite studies with PCVG

A powerful algorithm for automating comprehensive xenobiotic metabolite identification

Drug metabolism studies have traditionally relied upon compound-specific LC/MS/MS analyses to quantitate and identify metabolites during the drug discovery and development process. Early stage identification of all metabolites—including low-abundance products—is not always possible, and biotransformation scientists occasionally must backtrack, revisiting samples to unearth information on previously-unidentified compounds. If MS/MS spectra could be acquired upfront for all metabolites, both known and unknown, during the drug discovery process, the chances of overlooking an unpredicted metabolite would decrease, saving time and satisfying regulatory requirements more quickly. Novel, data-independent acquisition (DIA) strategies such as SWATH™ Acquisition[1,2] now make non-targeted analyses a reality in complex biological matrices and provide an overall snapshot of low-abundance, genotoxic, and major metabolites. Having complete coverage creates a richer, more detailed picture, but wading through the expanse of data—MS/MS spectra for every fragment of every precursor ion—can be daunting and time-consuming. Deconstructing this amassed data into interpretable results requires a powerful algorithm—principal components variable grouping (PCVG)—that effectively filters unabridged MS/MS data to extract comprehensive identification and quantitative information.

Generating complete metabolite fragment ion data sets using SWATH Acquisition

Recently, AB SCIEX scientists showed that PCVG algorithms could rapidly identify and quantify drug metabolites formed in complex biological matrices using DIA methods.[1] In these studies, all sample components were acquired in a single injection using SWATH Acquisition, an innovative, non-targeted LC/MS/MS data collection system on TripleTOF® 5600+ system, where an all-inclusive fingerprint is generated from MS/MS scans of every parent ion in the sample (Figure 1). SWATH Acquisition permits a full cluster of ions within a wide Q1 mass window to travel concurrently into the collision cell for fragmentation. Subsequent SWATH scans conducted during the same injection sequentially collect fragment ion information on incrementally increasing mass segments across the total mass range of interest (Figure 2). The resulting composite fragment ion data sets for each drug metabolite sample were laden with thousands of MS/MS spectra, rich in metabolic information. Additionally, a given MS/MS spectrum may be a combination of spectra for two or more metabolites, convoluted in such a way that interpretation of these raw, unprocessed spectra can be misleading.

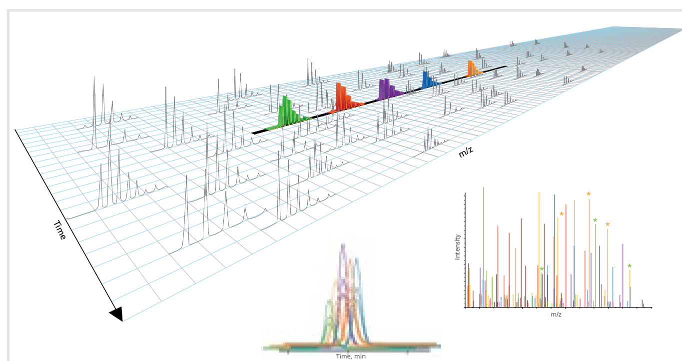


Figure 1: SWATH™ Acquisition of all fragment ions for all precursors results in complex data sets. The non-specific fragmentation data collection strategy generates an unbiased record of all fragments of all precursors within each of the multiple SWATH™ Acquisition isolation windows. Fragments that belong to the same precursor follow the chromatographic profile of that precursor.

PCVG deconvolution reduces and simplifies complex multivariate data sets

Advances in LC/MS/MS DIA methods have produced fragment ion data sets so vast that discovering the critical connections amongst correlated data points is challenging without further data processing. To simplify multivariate LC/MS data processing within metabolite identification workflows, AB SCIEX scientists have integrated a novel algorithm called principal component variable grouping (PCVG) into a research version of MetabolitePilot™ Software. PCVG reduces the dimensionality of complex data sets by combining correlated variables into new representative groups that are related to a particular peak in the LC/MS chromatogram, delivering data that is easier to manipulate and understand.[3] The PCVG algorithm uses an unsupervised method to assign related variables to groups, while also filtering out uncorrelated variables. Deconvolution by the PCVG algorithm proceeds in the following manner:

- The pre-processed, aligned, multiplexed fragment ion spectra (i.e., SWATH data) undergo principal component analysis (PCA).
- The variables are m/z values, the samples for the initial PCA are the raw, non-specific fragmentation spectra, and the resulting groups are the pure MS/MS spectra.
- PCVG analyzes the PCA loadings values to find correlated variables (fragment m/z values).
- PCVG automates data reduction, filtering variables that do not correlate with the target LC peak profile.

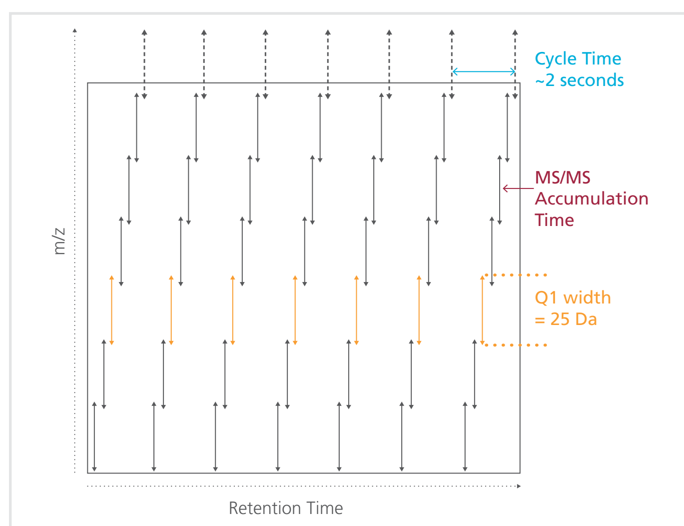


Figure 2: SWATH™ Acquisition sequentially collects MS/MS information for selected mass windows (*swaths*) across a total mass range of interest. Sequential Q1 isolation was stepped over the mass range of interest (e.g., 25 Da or user defined). The high speed of the TripleTOF® 5600+ system allows for full coverage of the selected mass range in an LC time scale and for high resolution XIC data for all fragment ions.

Key challenges of metabolite identification in complex biological matrices during drug discovery

- Missing, low-level drug metabolites in complex biological matrices such as bile, plasma, and tissue extracts
- Incomplete metabolite information leading to repeated sample analysis and decreased productivity
- Non-definitive metabolite identification and characterization due to inadequate MS/MS information
- Multiple, non-integrated software platforms complicate data processing, slowing metabolite ID and structure elucidation

Key benefits of SWATH™ Acquisition and PCVG algorithm for metabolite identification

- Comprehensive metabolite fingerprinting of irreplaceable experimental samples using SWATH Acquisition. Having a complete array of spectra (both MS and MS/MS scans) provides a digital archive of all analytes for samples with restricted availability (e.g., pediatric studies, expensive toxicological studies).
- The ultimate safety net with 100% MS/MS coverage is realized by capturing structural information for both predicted and unpredicted metabolites, including low-level and genotoxic products.
- MetabolitePilot™ is an all-in-one integrated software tool that helps rapidly identify and confirm metabolites with structural elucidation capabilities built-in without the need to switch between multiple software tools.
- Easy method development and retrospective data-mining
  - Requires no sample-specific method development

Key features of SWATH™ Acquisition and PCVG algorithm for metabolite Identification

- Selective MS/MS quantitation is achieved using single or multiple product ions that are summed from multiple transitions.
- A less complex MS/MS spectrum than traditional data-independent acquisition strategies due to PCVG-correlation of related peaks.
- Full retention of the isotopic pattern for each fragment due to a wider Q1 selection is ideal for stable-label drug studies (^{14}C- metabolism studies) and 100% MS/MS coverage for low-level metabolite/catabolite ID.
- PCVG algorithm enables simplified interpretation and data dimensionality reduction of complex metabolite spectra generated using data-independent acquisition.
- PCVG algorithm is a fast, robust, and reliable approach for the deconvolution of multi-component fragment ion spectra that is applied within a research version of MetabolitePilot™ Software.

- The smaller, deconvoluted data set facilitates spectral simplification, aiding in data interpretation.

PCVG processing correlates signals across the entire mass range examined, which, in turn, allows researchers to untangle complex relationships from among peaks of interest and to link information on isotopes, adducts, and fragments to related compounds.

PCVG-filtering finds and correlates related metabolite peaks

Motivated by the successful reduction of the dimensionality of other LC/MS multivariate data sets obtained for proteomic[2] and drug metabolite discoveries,[4] AB SCIEX scientists applied PCVG to xenobiotic metabolite data generated by SWATH™ Acquisition to derive a fingerprint of all parent-related compounds, creating a more easily interpretable data set that equaled and—for some analytes—surpassed the results obtained using information-dependent acquisition (IDA) methods. After deconvoluting the SWATH Acquisition data, researchers confirmed that the PCVG-filtered MS/MS spectra included identical peaks at similar intensities as those obtained by IDA.[1] Shown here, SWATH Acquisition of nefazodone metabolites resulted in full retention of isotopic fragment ions and accurate mass information (Figure 3). The PCVG filters maintained fidelity of the raw data and revealed a minor characteristic peak that correlated to the nefazodone (m/z 317) structure. In similar experiments, Biogen Idec scientist, Natasha Penner, used SWATH Acquisition to systematically identify metabolites for a drug candidate[5] (Table 1). When comparing SWATH Acquisition data with results obtained using more conventional IDA techniques,

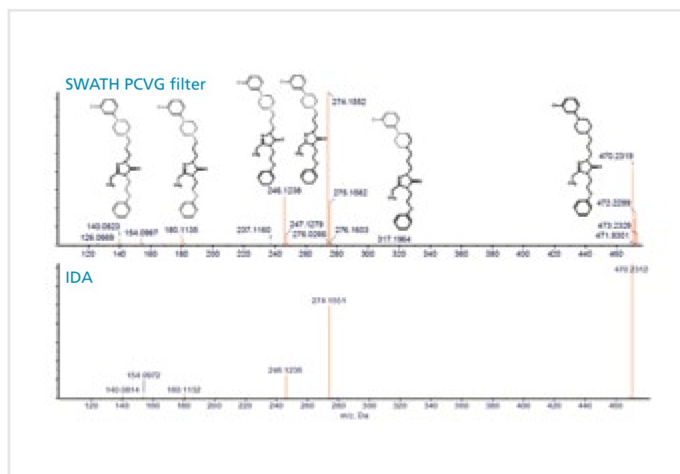


Figure 3: TOF MS/MS spectra of nefazodone metabolites collected with IDA and SWATH™ Acquisition methods.[1] SWATH Acquisition with PCVG-filtering result in MS/MS spectra that contain the full isotope pattern for fragment ions. Both background subtract and PCVG-filtering strategies yielded accurate mass information enabling a more confident structure proposal. In the PCVG-filtered TOF MS/MS spectrum, an additional minor peak (m/z 317) that corresponds to a direct substructure of nefazodone was recovered from the raw data.

Dr. Penner observed an increased number of PCVG-filtered metabolite peaks—13 out of the 13 known metabolites—with complete MS/MS coverage when using SWATH Acquisition, surpassing results achieved with generic TOF MS data-dependent acquisition. Additionally, each fragment in the MS/MS data filtered by the PCVG algorithm, retained the full isotopic pattern and compared favorably to samples analyzed using traditional MS/MS approaches (Figure 4). Taken together, these data validate and confirm the versatility and accuracy of the PCVG method compared to well-established IDA methods.

Metabolite	Formula	RT (min)	(M+H)+	Generic TOF MS	SWATH HS
Parent	C14H15ClN6O	3.89	319.1068	√ (MS/MS)	√ (MS/MS)
Oxidation-1	C14H15N6O2Cl	3.63	335.1016	√(MS/MS)	√ (MS/MS)
Oxidation-2	C14H15N6O2Cl	3.72	335.1016	√ (NO MS/MS)	√ (MS/MS)
Oxidation-3	C14H15N6O2Cl	3.81	335.1016	√ (MS/MS)	√ (MS/MS)
Oxidation-4	C14H15N6O2Cl	4.11	335.1016	√ (NO MS/MS)	√ (MS/MS)
Dechlorination & loss of CH2	C14H16N6O2	3.27	301.1405	X	√ (MS/MS)
Demethylation	C13H13N6OCl	3.45	305.0914	√ (MS/MS)	√ (MS/MS)
Internal Hydrolysis	C14H17N6O2Cl	3.47	337.1174	√ (NO MS/MS)	√ (MS/MS)
Loss of C5H2N5Cl+Oxidation	C9H13NO2	0.99	168.1024	√ (NO MS/MS)	√ (MS/MS)
Loss of C9H11NO	C5H4N5Cl	0.98	170.0232	√ (MS/MS)	√(MS/MS)
Di-oxidation	C14H15N6O3Cl	3.48	351.0967	√ (MS/MS)	√ (MS/MS)
Ketone Formation	C14H13N6O2Cl	4.11	333.0861	x	√(MS/MS)
Dechlorination,loss of CH2+Internal Hydrolysis & Di-Oxidation	C13H16N6O4	3.43	321.087	x	√ (MS/MS)
Loss of Cl Di oxidation	C14H16N6O3	2.2	317.1354	x	√(MS/MS)
Total Metabolite coverage				9/13 (70%)	13/13 (100%)
Total MS/MS coverage				5/9 (55%)	13/13 (100%)

Table 1: Metabolite Coverage for Biogen Idec Drug Candidate BIIB021 in Complex Biological Matrix Using Generic TOF MS IDA and SWATH™ Acquisition[5]

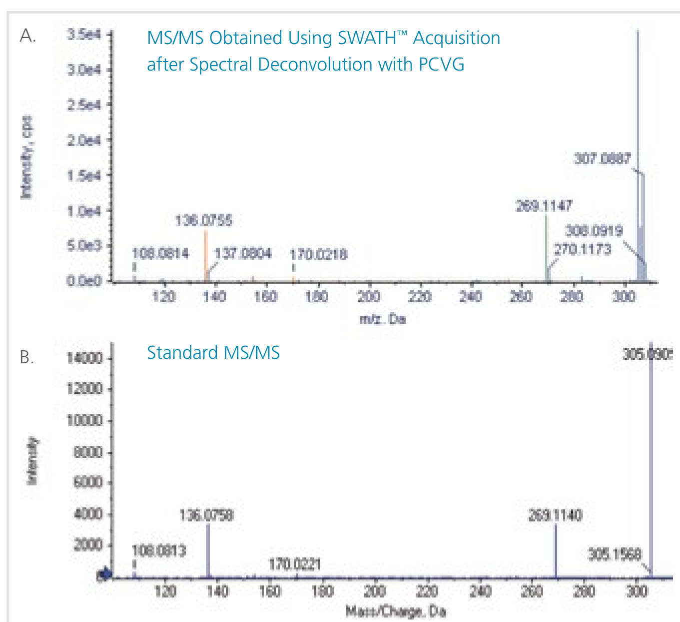


Figure 4: MS/MS obtained using SWATH™ Acquision and retention of the full isotopic pattern for a fragment from Biogen Idec drug candidate, BIIB021.[5] MS/MS data were deconvoluted (A) with PCVG-filtering and (B) without PCVG-filtering.

Advantages of metabolite discovery with SWATH™ Acquisition and PCVG-filtering

In combination, SWATH Acquisition and the PCVG algorithm provide a powerful method for confident metabolic structure assignment and offer many advantages when processing complex MS/MS fragment ion data sets for quantitation and identification of metabolites. The following benefits allow for improved selectivity and specificity when pinpointing drug-related material:

- The ultimate safety net is realized by capturing both predicted and unpredicted metabolites. Having a complete array of spectra (both MS and MS/MS scans) provides researchers a digital archive of all analytes, so that data corresponding to unexpected products can be retrospectively probed without having to re-acquire a sample.
- Retention of full isotope pattern for each fragment is possible due to the relatively wide SWATH window, which significantly aids in the designation of metabolite structures and elemental composition during metabolite discovery. This isotopic data along with 100% MS/MS spectra provides sufficient structural information for the identification and quantitation of low-abundance metabolites.
- A less complex MS/MS spectrum is generated by MS/MS^{ALL} with SWATH Acquisition than other DIA techniques. PCVG correlates related peaks, and the relevant spectra make it easier to decide which parent ion goes with which fragment, resulting in higher quality data even with complex data from plasma or bile samples.
- Easy method development allows researchers to focus on data analysis instead of compound-specific methods. Because MS/MS^{ALL} with SWATH Acquisition is a data-independent scan providing quantitative information on all analytes, there is no need to create specialized, data collection strategies for a particular drug candidate; this saves time and unnecessary consumption of limited samples.
- Multicomponent quantitation is possible with multiple fragment ion transitions captured simultaneously during SWATH Acquisition in a single injection. This adds an additional layer of confidence to quantitative data by allowing multiple product ions to be summed.

In summary, the PCVG algorithm provides a fast, robust, and reliable approach for deconvoluting non-specific fragmentation data from drug metabolism studies obtained using SWATH Acquisition. PCVG-filtering diminishes the complexity inherent in large, multivariate data sets, while still creating a global picture of xenobiotic drug metabolites and generating highly-interpretable spectra for comprehensive metabolite identification and quantitation. Used during the preliminary stages of the drug discovery process, SWATH Acquisition coupled with the powerful PCVG algorithm (incorporated into MetabolitePilot™ Software) delivers complete metabolite coverage—even of minor products—eliminating the possibility of a missed or underestimated metabolite quantity, thereby streamlining the development of new drug candidates and furthering the understanding of their biotransformation pathways.

Eva Duchoslav[1]; Gordana Ivosev[1]; Ignat Shilov[2]; Hesham Ghobarah[1]; Lyle Burton[1], Suma Ramagiri[1]
[1]AB SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada;
[2]AB SCIEX, Redwood City, CA, USA

Special thanks to Natalia Penner, Biogen Idec, DMPK, Cambridge, MA for providing SWATH data examples on Drug Candidate BIIB021

[1]Duchoslav E, Ivosev G, Shilov I, Ghobarah H, Burton L. "Automated metabolite identification and profiling in non-specific fragmentation high-resolution accurate MS data." Poster session presented at: the 61st annual conference of the American Society for Mass Spectromtery; 2013 June 9-13; Minneapolis, MN.

[2]Gillet LC, Navarro P, Tate S, Röst H, Slevsek N, Reiter L, Bonner R, Aebersold R. "Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis." (June 2012) Mol. Cell Proteomics. DOI 10.1074/mcp.0111.016717

[3]Ivosev G., Burton L., Bonner R. "Dimensionality reduction and visualization in principal component analysis." (July 2008) Anal. Chem. 80: 4933-4944.

[4]Hopfgartner, G. "High-resolution mass spectrometry for integrated qualitative and quantitative analysis of pharmaceuticals in biological matrices." (March 2012) Anal Bioanal Chem, 402(8): 2587-96

[5]Penner N. (July 2013) "High throughput metabolite ID: Are we there yet?" AB SCIEX Mass Spec Webinar Series. Retrieved at: http://www.absciex.com/events/webinars/high-throughput-metabolite-id-are-we-there-yet

Counting needles in a haystack: improving sensitivity and quantitation of low-level tryptic peptides

Improving sensitivity and quantitation of low-level tryptic peptides

Carmen Fernández-Metzler, president of PharmaCadence Analytical Services, believes that quantitation should not be the limiting factor in biological studies. She took this mantra to heart when determining the basal levels of the membrane-bound isoforms of UDP-glucuronsyl-transferase (UGT), a major enzyme in the phase II-elimination of over 200 xenobiotic drugs and endogenous metabolites. Having a good understanding of UGT's role in drug metabolism, by correlating both the activity and absolute protein levels, provides a handle on effective dosages for clinical trials. But, these calculations require exact protein quantitation at very low cellular concentrations—around 2-100 pmol/mg of microsomal tissue. When tackling this issue with the UGT family of enzymes, Dr. Fernández-Metzler was presented with a challenging situation. "We didn't have a pure UGT protein standard at the time, so the difficulty was in quantitation of overexpressed recombinant protein. Additionally, peptide concentrations did not always agree with each other due to variable digestion efficiency and recovery," explained Dr. Fernández-Metzler. To address these problems, a strategic workflow was devised: isotopic dilution of signature peptides, tightly-controlled tryptic digestions, and analysis using the sensitive AB SCIEX QTRAP® 6500 System and highly-reproducible chromatographic separations using the Eksigent microLC System to achieve quantitation of six endogenous UGT isoforms in a complex matrix (Figure 1).

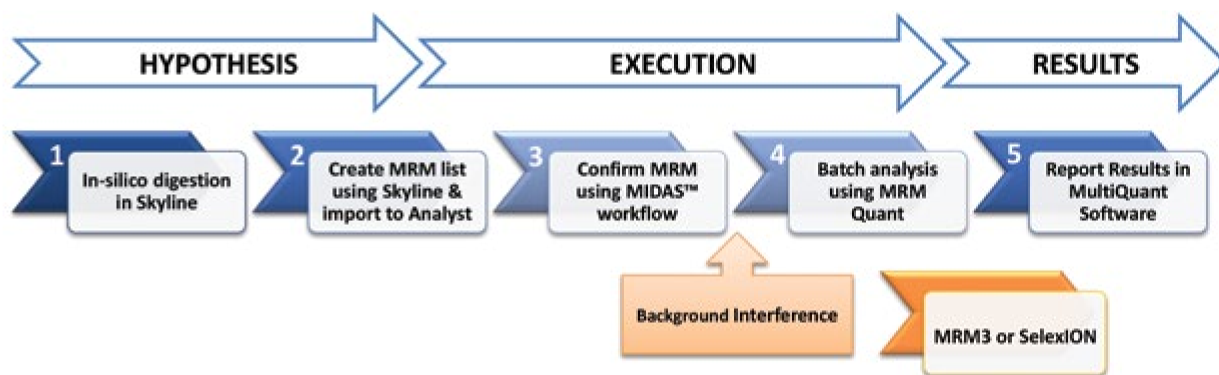


Figure 1: Overview of UGT tryptic peptide quantitation using the MIDAS™ Workflow[1]

1. After in silico digestion of the target protein, Skyline Software computed ideal representative (or signature) peptides based on charge sites, MS/MS fragment ions, and resistance to post-translational modification.
2. MRM lists are computed in Skyline Software for each UGT isoform using pre-validated peptides prior to transfer of the MRM lists to Analyst® Software.
3. Predicted signature peptides sequences were verified using microflow separation followed by analysis using the MIDAS™ workflow. Eluting peaks corresponding to MRM transitions of signature peptides triggered full-scan MS/MS. All fragments were captured and then scanned out of the trap on a UHPLC time scale, providing additional in-depth peptide structural and quantitative information, as well as selectivity.
4. Standard curves generated using rUGT microsomes in the presence of rat liver microsomes and stable isotope-labeled surrogate peptides were used to quantify UGT levels in human liver samples using the MIDAS workflow. A batch analysis of various UGT isoforms was enabled, capturing multi-MRM transitions for each sample.
5. The resulting data for each isoform was imported into MultiQuant™ Software for data processing and results reporting.
6. For peaks with background interference, SelexION™ technology or MRM^3 assays provided an additional filtering step.

Consistent peptide release and quantitation

The first hurdle in the quantitative strategy was to develop optimal tryptic digest conditions for the protein standard (recombinant human UGT (rUGT) expressed in insect cells and prepared as microsomes), an essential step towards consistent peptide release and quantitation. The signature peptide method makes a key assumption—that the tryptic digest of the parent protein will go to completion, liberating one peptide from every instance of that protein—a uniformity that was not consistently maintained between peptides from the same isoforms. Optimizing tightly-controlled tryptic digest conditions, including the timing and concentration of all components, made the assay come together, producing effective standard curves. As Dr. Fernández-Metzler explained, "When you have reproducibility in the digest, you will have reproducibility in the mass spectrometry data."

With digestion conditions optimized, Dr. Fernández-Metzler then further refined peptide separation and quantitation techniques, working closely with AB SCIEX application specialists. Faced with measuring numerous human liver samples, PharmaCadence needed a high-throughput application for separating low-abundance peptides, methods traditionally handled by more sedate nanoflow techniques. Combining divergent chromatographic conditions from small molecule and proteomics studies, Dr. Fernández-Metzler devised a microflow-based separation using an Eksigent microLC 200 System, which generated higher resolution data by using wider columns and faster flow rates than traditional nanoflow regimens. Sensitivity and reproducibility were not compromised under these conditions, and coupling microflow LC with the improved detector dynamic range of the QTRAP® 6500 system yielded a 3–9-fold elevation in raw signal and 2–5-fold improvement in S/N ratios of UGT peptides compared to microflow conditions on the QTRAP 5500 system (Figure 2).

Quantitative and qualitative assessment at once

Even with reproducible digestions and chromatographic separations, distinguishing low-abundance UGT peptides from the multitudes of other tryptic peptides remained a nuanced process; very small amounts of analyte needed to be selectively plucked from the sample milieu while still retaining a meaningful signal. Dr. Fernández-Metzler's team quantitated the UGT signature peptides of interest using multiple reaction monitoring (MRM) methods on the QTRAP 6500 system, screening tryptic digest peaks through two mass filters. Multiple peaks for the same MRM signal are the norm, not the exception, necessitating an additional discovery step—enhanced product ion (EPI) analysis. In this scan type, precursor ions are fragmented by true collision-induced dissociation, and then the fragments are collected, concentrated and scanned from the linear ion trap at speeds much more rapid than are possible using traditional triple quadrupole instruments. This process is called an MRM information-dependent acquisition (IDA)-based method (MIDAS™ workflow), enabling both quantitative and qualitative assessment of peaks in the same run. Confirmation of a precursor peptide's identification can then be derived from these information-rich product ions.

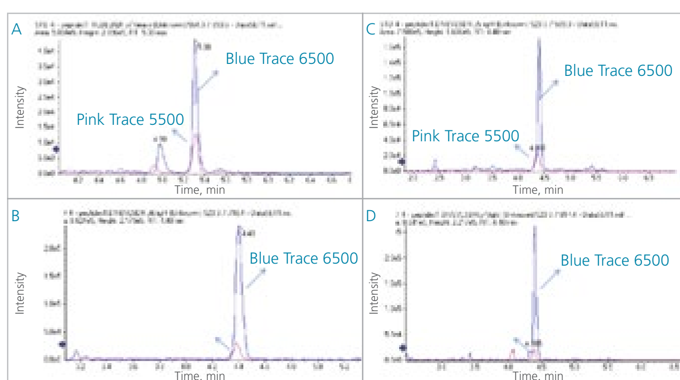


Figure 2: Sensitivity improvements on the QTRAP® 6500 versus the QTRAP 5500 for MRM detection of UGT tryptic peptides following microflow separation.[2] A) An UGT tryptic peptide corresponding to MRM transition 554.3/893.5 showed 3.2-fold raw signal and 2-fold S/N improvements for fragments detected on the 6500 (blue trace) versus the 5500 QTRAP system (red trace). Similar data were obtained for three other MRM transitions: B) MRM 523.3/589.3 showed a 5-fold raw signal and 3-fold S/N improvements. C) MRM 523.3/718.4 showed a 9-fold raw signal and 5-fold S/N improvements. D) MRM 523.3/817.4 showed a 9-fold raw signal and 4-fold S/N improvements. Each sample was acquired n=4 times on two different QTRAP 6500 systems (3.3–7.5% CV) to show reproducibility.

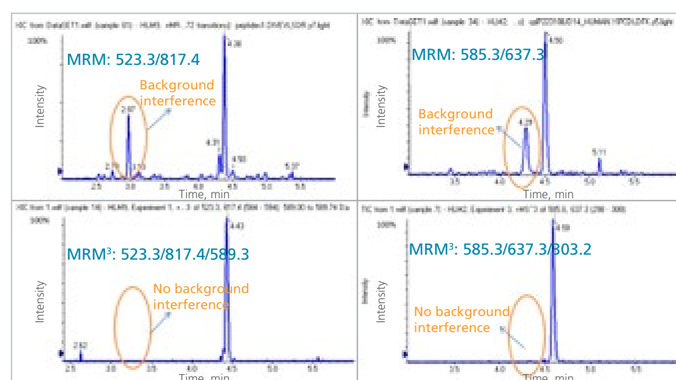


Figure 3: Elimination of tryptic background interferences from UGT signature peptides using MRM^3.[3] A) For y1 tryptic peptide, DIVEVLSDR, the MRM-based chromatogram for the signature transition shows background interference peaks at 2.97 min and at 4.31 min. B) The MRM^3 chromatogram for DIVEVLSDR (Figure A) completely eliminated the interference peak and improved peak integration for a better %CV. C) For y6 tryptic peptide, YIPCDLDFK, the MRM-based chromatogram shows an interfering signal at 4.29 min, which was 40% of the area of the parent peak (4.50 min). D) The MRM^3 chromatogram for YIPCDLDFK (Figure C)

The AB SCIEX QTRAP® 6500 System's approximate 20-fold improvement in detector dynamic range provided the necessary sensitivity for detection of low-abundance UGT peptides; but, most importantly, the QTRAP 6500 system's fast linear ion trap scan speeds allowed rapid MS/MS analysis while still providing 10 data points across the peak for optimal quantitation.

Even with the selectivity of the MIDAS™ workflow, co-eluting contaminants and closely-overlapping isobaric peaks constrained optimal peak integration conditions for a number of the UGT peptides. To remove interferences from the peptide spectra without introducing additional chromatography or sample preparation steps, the QTRAP system offered an additional advantage—the MRM^3 scan. During the EPI scan, when the fragments of the precursor peptide are assembled in the trap, a selected ion is further isolated and fragmented. This secondary fragmentation process produced additional ions for further structural analysis and high-resolution quantitation of UGT signature peptides, removing background interferences (Figure 3). "After MRM^3, only one peak remained in the chromatogram, and it was really easy to process the data, as the automated integration routines worked more reliably with the MRM^3 data," noted Dr. Fernández-Metzler, who added that the "MRM^3 method is very clean, very selective, but requires a lot more work to set up."

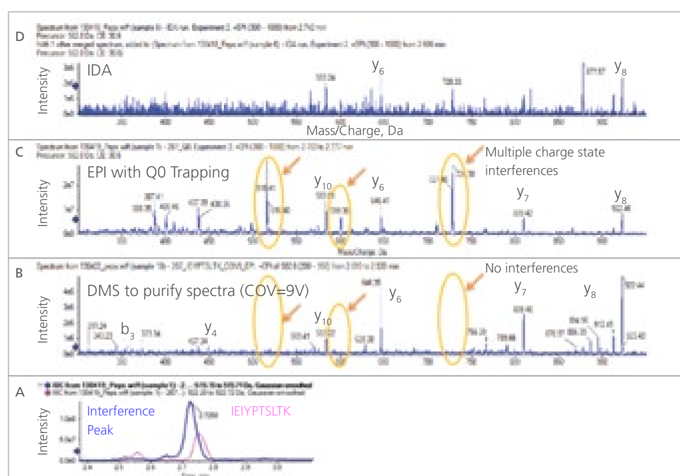
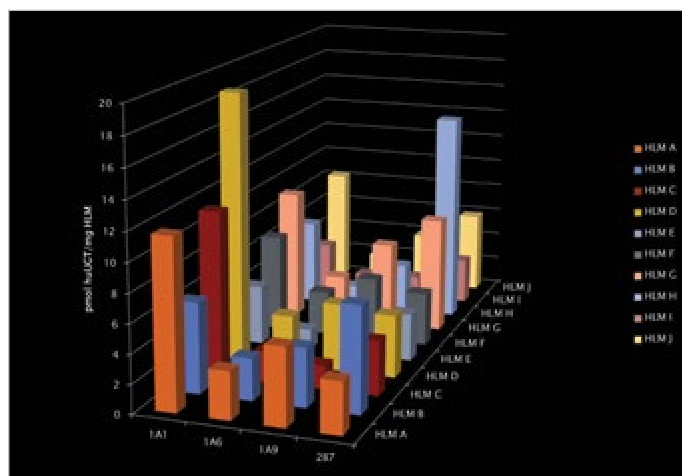


Figure 4: Elimination of co-eluting multiply-charged interferences from UGT signature peptides using SelexION™ Technology.[4] A) An enhanced product ion (EPI) scan of UGT-2B7 tryptic peptide, IEIYPTSLTK (fragments labeled in pink) was captured on a QTRAP 6500 system, which enhanced sensitivity without loss of qualitative sequence information. B) An EPI scan with Q0 trapping of IEIYPTSLTK shows that a co-eluting, interference (fragments circled in yellow) produced a chimeric spectrum. C) The interfering peaks were removed by DMS filtering using SelexION technology, yielding a clean MS/MS spectrum for quantitation. D) Overlays of the tryptic peptide chromatograms for UGT-peptide, IEIYPTSLTK (pink) and the interference (blue) are shown.

Reduced matrix interferences

If method development time is limited or if the second generation fragments are either not specific enough or are too low, differential ion mobility separation (DMS) based on SelexION™ technology can provide an additional degree of selectivity. This technique exploits an ion's mobility through a set of plates with high and low energy fields applied to quickly resolve isobaric species and single and multiple charge state interferences on a timescale compatible with UHPLC and MRM acquisition. For certain UGT signature peptides, these background interferences from overlapping peaks were problematic, complicating peak integration. To acquire a clean spectrum, interfering ions were essentially tuned out of the instrument using DMS, significantly improving the MS/MS spectrum for UGT-specific peptides that were previously muddled by overlapping peaks (Figure 4). Furthermore, SelexION technology reduced the matrix interferences, effectively boosting the signal to noise and sensitivity of the UGT assays.



UGT ISOZYME	ISOFORM CONCENTRATION (pmol huUGT/mg HLM)	
	Mean	Range
UGT 1A1	18.10	4.32 - 45.14
UGT 1A4	10.98	3.84 - 31.23
UGT 1A6	4.02	1.17 - 7.95
UGT 1A9	5.72	1.22 - 12.77
UGT 2B7	19.94	3.78 - 79.5
UGT 2B17	4.86	ND - 25.82

Figure 5: Concentrations of UGT isoforms in 10 individual human livers. Quantitation of each human isoform in liver samples was conducted as described1 using rUGT-infused rat liver microsomes and stable-label signature peptides to create standard curves for the assessment of UGT isoform levels in human liver samples. A) The range of concentrations obtained for four UGT isoforms 1A1, 1A6, 1A9 and 2B7 from ten separate human liver samples are graphically displayed here, visually representing the quantitative variability. B) A table summary of mean concentrations and ranges of all six UGT isoforms in 50 individual human liver microsomes.

After perfecting the experimental process, Dr. Fernández-Metzler obtained highly accurate quantitative data on the basal levels of UGT isoforms by creating high quality calibration curves under a stringent assay optimization process where tightly-controlled digestions gave rise to extremely reproducible conditions[1] (Table 1). When constructing the standard curves, occasionally the concentrations of different peptides from the same proteins did not correspond well because of poor signal to noise. After processing the results in MultiQuant™ Software, even peptides such as UGT 1A1 with high background produced good quality standard curves with accuracies within 15% CV and showed precision within 15-20% CV, an appropriate range for a discovery assay. Other UGT isoforms produced three closely corresponding peptide concentrations (such as those from isoform 2B7) yielding calibration curves with a % CV less than 10%. In the end, was all this work worth the effort? "Yes!" confirmed Dr. Fernández-Metzler. "This study will help design a clinical trial that will hopefully lead to a better medicine one day."

[1] Fernández-Metzler C. (August 2013) "Peptide Quantification on the QTRAP® Mass Spectrometers with MicroflowLC: Bridging the Best of Small Molecule and Proteomic Analysis." AB SCIEX Mass Spec Webinar Series. Retrieved at: http://www.absciex.com/events/webinars/peptide-quantification-on-the-qtrap-mass-spectrometers-with-microflowlc – bridging-the-best-of-small-molecule-and-proteomics-analyses.

[2] "UGT Family of Enzymes: Quantification of Tryptic Peptides. Part 1 of 3: The QTRAP® 6500 Platform and MicroLC Provide the Combination of Sensitivity, Specificity and Robustness for the Quantitation of UGT Enzymes." (White Paper) AB SCIEX. Accessed November 2013. Retrieved at: www.absciex.com

[3] UGT Family of Enzymes: Quantification of Tryptic Peptides. Part 2 of 3: Accelerating MRM3 Workflows on QTRAP® 6500 System for Enhanced Selectivity in Complex Matrices like Tryptic Digests." (White Paper) AB SCIEX. Accessed November 2013. Retrieved at: www.absciex.com

[4] UGT Family of Enzymes: Quantification of Tryptic Peptides. Part 3 of 3: Using SelexION™ Technology for Additional Selectivity by Separating Multiple Charge State Ions in Tryptic Digests." (White Paper) AB SCIEX. Accessed November 2013. Retrieved at: www.absciex.com

Identifying meat authenticity by mass spec

Testing real food samples for targeted meat markers using the AB SCIEX QTRAP® and the MIDAS™ workflow

Is it LAMB?

Meat consumption is one of the highest of all food types across the globe, and food producers are sometimes looking to find more economic and cost effective ways to produce assorted meat products for human consumption.

In 2013, news was made when beef was adulterated with horse meat, which has some potentially hazardous side-effects for human consumption (since horses are sometimes treated with medicines that are toxic to humans). Additionally, there was news in Asia of mutton rolls being adulterated with duck and even rat meat, and 'fake lamb' meat was caught being sold in supermarkets (but the product was actually comprised of other meat species). Besides consumer safety, there are ethical and religious concerns related to consumption of some meat products. For example, consumption of pork is of high concern for Islamic and Judaic communities.

Labs are now trending to find new approaches to test meat products for authenticity and adulteration to identify exactly what species are present.

Meat speciation in the laboratory has traditionally been performed using either PCR (polymerase chain reaction) or ELISA (enzyme-linked immunosorbent assay). Both techniques are limited in accuracy, sensitivity, and specificity, making the search for alternative analytical approaches to verify meat authenticity very important to food testing laboratories. LC/MS/MS provides an excellent alternative to traditional methodologies to identify and confirm targeted peptide markers associated with different meat species more accurately and reliably than existing methodologies.

In this article, we will describe a two-pronged approach using LC/MS/MS to first identify the unique protein markers specific to a meat species using the TripleTOF® 5600, then utilizing the MIDAS workflow (MRM-initiated detection and sequencing, Figure 1) on the QTRAP system to perform survey scans targeting the MRMs of targeted peptide markers of multiple meat species of interest then triggering the acquisition of an EPI spectrum for added peptide confirmation.

This approach has been successfully applied to the detection of horse, duck, chicken, beef, pork, and rat meat contamination in food samples ranging from ground meat to mutton rolls (which should only contain lamb meat) purchased from local supermarkets in China. The approach has also been applied in the speciation of gelatin used in candies and pharmaceutical capsules.

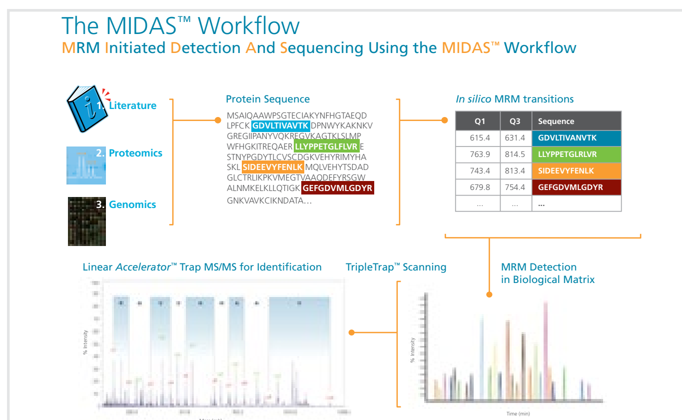


Figure 1: The MIDAS™ workflow utilizes MRM-initiated detection and sequencing.

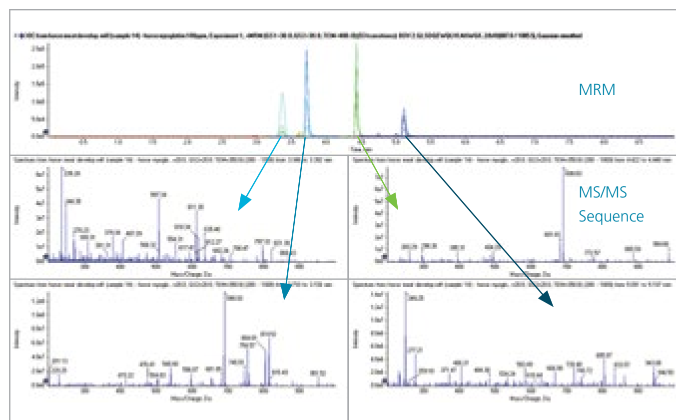


Figure 2: For the detection of horse peptides, MRM initiated acquisition of MS/MS spectra was used to sequence characteristic proteins for added selectivity and specificity in the analysis.

The results show that this LC/MS/MS workflow can be successfully applied to testing food products and accurately identifying what meat species are present in those samples to verify their authenticity and integrity. The result is a more accurate, reliable, and direct approach to determining meat authenticity than traditional analytical testing methodologies.

Materials

Duck, chicken, lamb, beef, pork, horse, and rabbit meat tissue were purchased commercially. Rat tissue was harvested from rat leg muscle that had been used in previous toxicological experiments. Food samples such as beef, lamb, mutton rolls, gummy bears, and others were purchased from local supermarkets (Europe and China).

Sample preparation

Details of the sample preparation for different samples types can be reviewed in the assorted reference publications and application notes[1-4], which can vary slightly depending on the matrix. In short, homogenized food samples are extracted with a buffered extraction solution then undergo a tryptic digest. In some cases, the digested extracts are purified using solid-phase extraction prior to analysis by LC/MS/MS.

Identifying peptide targets for each species

Initial identification of species-specific peptides from the tryptic digests was done using a shotgun proteomics approach on the TripleTOF® 5600 system coupled to an Eksigent nanoLC or ultraLC system. In these survey scans, an information dependent acquisition (IDA) method is used to trigger the acquisition of TOF-MS/MS spectra associated with all peaks detected in the TOF-MS survey scan. For the gelatin speciation experiments, principal component analysis using MarkerView™ Software was performed to identify the specific markers for each tissue type[4]. For the experiments testing mutton rolls for authenticity, ProteinPilot Software was used to identify all characteristic proteins and peptides for each meat species.

We targeted the highest abundance proteins for each species to ensure best sensitivity and accuracy. Detailed comparisons of proteins and peptides for each species were made, especially since some species have close genetic relationships (such as chickens and ducks or sheep and cows) and have proteins and peptides that differ by only a few amino acids.

Final results were confirmed through a blast on the NCBI website to ensure that each identified peptide was totally unique to that meat species to ensure highest specificity for the analysis.

Analysis of meat peptide MRMs using the MIDAS workflow

Once the peptide fragments for each meat species were identified, the MIDAS workflow (Figure 1) was used to create MRM detection methods for analysis using the QTRAP® system. Detailed method conditions can be found in the reference documents below, highlighting the methods for the analysis of horsemeat contamination in beef[1, 2], meat species analysis in commercial mutton rolls[3], and gelatin speciation in gummy bear candies[4].

Using QTRAP technology, the mass spec was set-up for electrospray ionization (ESI) utilizing an MRM-triggered EPI method, enabling us to collect full MS/MS spectra associated with each MRM for the highest selectivity in sequence identification. Figure 2 shows the MS/MS spectra collected for horse peptides, which allow for sequencing of the peptides for added specificity and selectivity in the analysis.

Figure 3: The NCBI database is incomplete for duck proteins, resulting in duck samples being identified as chicken in the ProteinPilot search. The MIDAS workflow allows these 2 species to be distinguished (Figure 4).

A chicken or duck conundrum

The close genetic relationship between chickens and ducks, and the incomplete NCBI database for duck proteins, resulted in database search results in ProteinPilot™ identifying both duck and chicken samples as chicken proteins (Figure 3). From this initial analysis using the database, we were unable to unequivocally identify if the exact fowl species was chicken or duck.

However, through the use of the MIDAS™ workflow to selectively identify unique peptide markers for each, we are able to use MRM detection to selectively detect and accurately identify both species (Figure 4). A key benefit to using LC/MS/MS for meat speciation is the selectivity for the mass spec to identify even small differences in amino acid sequences to enable the reliable identification of even closely-related meat species.

Speciation results in commercial samples

Many commercial samples, from beef, to mutton rolls, to candies, were analyzed using our LC/MS/MS speciation methods to identify what species of meat tissue were present in these samples, and, in some cases, verify how authentic the product was based on its label claims.

Figure 5 shows the analysis of a lamb purchased at a local supermarket in China (top panel). The peptides detected in that sample were consistent with the peptide markers for duck meat, suggesting that the samples were falsely labeled as lamb but actually consisted of approximately 50% duck tissue.

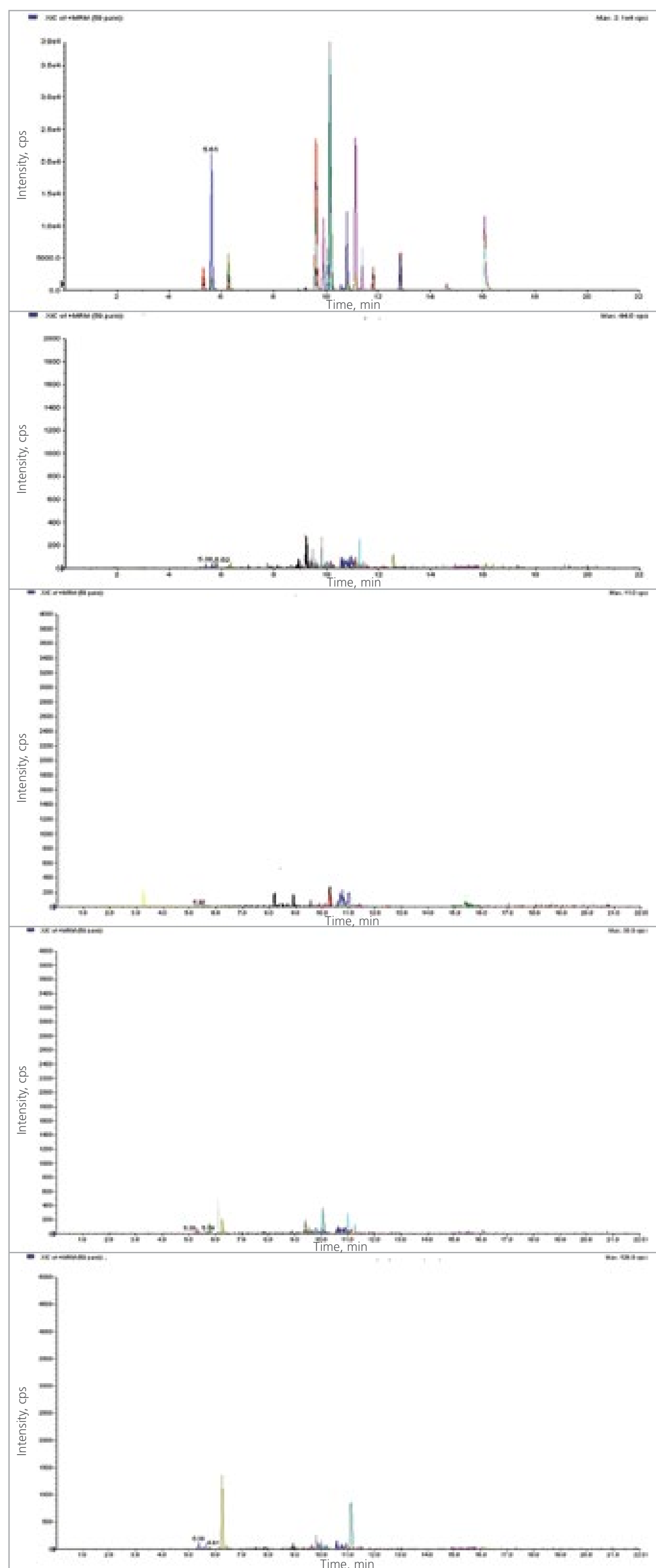


Figure 4: The LC/MS/MS MIDAS workflow enables us to identify peptide markers for each meat species, and allows us to selectively identify even closely related species. Here we show the analysis for detection of duck peptides, which we detect in the duck sample (top panel) but not in any other meat tissue sample tested, showing the high specificity of the analysis.

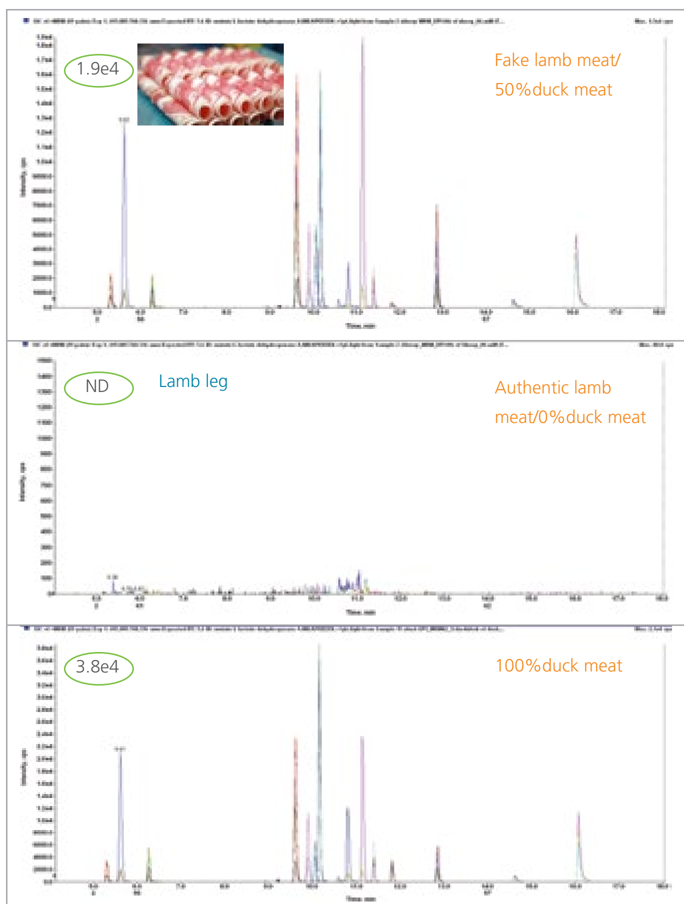


Figure 5: Lamb samples purchased from the supermarket were tested for a number of meat species including chicken, duck, and others, to verify their authenticity. Here we show the analysis for duck meat markers in a store-bought sample (top panel), an authentic lamb leg (middle panel), and in an authentic duck tissue sample (bottom panel). Results showed that the store-bought samples were 'fake lamb', and contained approximately 50% duck meat.

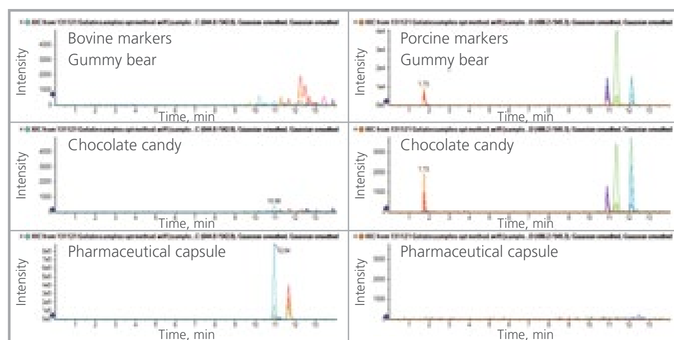


Figure 6: The meat speciation approach also works well for gelatin speciation. Here bovine and porcine gelatin markers were analyzed in gummy bears, chocolate candies, and pharmaceutical capsules. While pork gelatin markers were detected in the candies, only bovine markers were detected in the capsules.

Conclusions

Meat authenticity and integrity is a very hot and controversial topic in the news, both for the safety implications but also for the ethical implications associated with consuming falsely-labeled food products. LC/MS/MS is emerging as a proven technique to enable labs to speciate meat in assorted food and consumer samples. This article gives a brief overview of how LC/MS/MS can be used in meat speciation, and shows a few key examples of this approach being performed on real samples purchased in local supermarkets.

References

Get more details on these speciation applications:

[1]Von Bargen, et. al., J. Agric. Food Chem, 2013, 61 (49), 11986. http://pubs.acs.org/doi/abs/10.1021/jf404121b

[2]Can LC/MS/MS be used in horse meat detection?
http://www.absciex.com/Documents/Downloads/Literature/Horse-Meat-Detection_AB%20SCIEX_QTRAP5500-microLC_7580213-01.pdf

[3]Using LC/MS/MS and the MIDAS workflow for fake lamb meat detection (application note in Chinese)
http://www.absciex.com/Documents/Downloads/Literature/Fake-lamb-detection-QTRAP4500_Chinese_final.pdf

[4]Are pork extracts present in my gummy bears? Gelatin speciation by LC/MS/MS
http://www.absciex.com/Documents/Downloads/Literature/Pork-in-gummy-bears_QTRAP-4500-TripleTOF-5600_ABSCIEX_09270214-01.pdf

Answers to software validation questions for every GLP lab

Stacy "Dene'" Nelson & Patrick Quinn-Paquet

The AB SCIEX compliance team provides ongoing validation support for Analyst® and MultiQuant™ Software systems, working closely with regulated GLP compliant laboratories world-wide to document software security, reliability and fitness on mass spectrometry instruments. Software applications must be maintained in a validated state throughout their operation, and when changes occur (such as operating system or software version upgrades), the AB SCIEX compliance team delivers effective resources to ensure that currently-validated software remains compliant and that validation procedures are updated. This article describes four of the most important, current topics regarding mass spectrometry software validation facing GLP labs today:

1. Will Analyst and MultiQuant Software need re-validation when Windows XP support is discontinued?
2. What level of software validation is sufficient after installing an updated version of Analyst Software?
3. Can virtual machines be validated for use as processing workstations?
4. What is the best way to prioritize software validation risks?

This article includes insights from the AB SCIEX compliance team that answer these important questions for laboratory managers responsible for maintaining a regulated GLP environment.

Does upgrading to Windows 7 require re-validation of Analyst and MultiQuant Software?

The anticipated loss of support from Microsoft for Windows XP on April 8, 2014 has many lab directors questioning whether Analyst or MultiQuant Software will need to be validated after migrating to a new Windows operating system. AB SCIEX is ready to assist GLP labs with a clear support strategy, and new, improved versions of Analyst and MultiQuant Software have been designed to contend with any changes generated by the transition to the Windows 7 operating system.

Because Analyst and MultiQuant Software are tightly integrated with Windows, the switch to Windows 7 will have a big impact on the validation state of this software, likely necessitating a full re-validation of the current installation. A conservative approach would be to migrate from Windows XP to Windows 7, and then upgrade to the latest version of Analyst or MultiQuant Software prior to a complete re-validation process. However, careful evaluation of the costs versus the benefits of re-validation needs to be considered before moving forward. A risk-based analysis is recommended by GAMP 5 (Good Automated Manufacturing Practice Guide, Version 5) to help GLP labs determine if full re-validation is needed and is built on the following questions:

- What are the risks associated with upgrading?
- Loss of validated security databases?
- Loss of project data?
- Problems with data compatibility with the new version?
- Loss of reports?
- Additional training requirements for the updated version?
- Does this change have a regulatory impact when operating the software? At a minimum, standard operating procedures (SOPs) will need to be updated to reflect the new operating system.
- Is a change control documenting the change sufficient?

While GLP laboratories are ultimately responsible for fulfilling software validation requirements, AB SCIEX believes that regulatory compliance can be maintained by re-validating when upgrading Analyst® Software from version 1.4.x or 1.5.x to 1.6.2. Because of the many new features, revisions, and fixes made during the development of Analyst 1.6.2, the updated configuration of the software must be confirmed, as well as the configuration of the updated operating system. Therefore, upgrades to Windows 7 and to Analyst 1.6.2 will likely necessitate full re-validation.

What level of software validation is sufficient after installing an updated version of Analyst Software?

GLP lab managers responsible for the regulatory compliance of all laboratory instrumentation are continually faced with documenting software system performance to ensure conformity to standards. The extent of documentation required is dependent upon the software's vendor classification, and AB SCIEX provides the decision-making support lab managers need to understand the records requirements for Analyst and MultiQuant™ Software validation maintenance. Analyst and MultiQuant Software are classified as GAMP 5 category IV software—or commercial-off-the-shelf (COTS) software—and COTS vendors must submit to an industry-standard audit to ensure the correct functionality and usability of the software prior to distribution. AB SCIEX provides comprehensive compliance documentation by submitting a postal audit reply and proof-of-software-testing certifications (such as ISO 9001 and ISO 13485) to their GLP lab software customers as evidence that the software operates as intended. However, on-site software configuration and validation are still required during the installation process, necessitating an additional round of compliance testing on-location.

GAMP 5 implements a risk-based approach to software validation that assesses how compliance testing impacts patient safety, product quality, and data integrity. The validation decision-making process is complicated by considerations related to implementation, timing, execution, and scope, turning compliance administration largely into an exercise in risk management. GLP labs needing support in their software validation risk assessment can work directly with the AB SCIEX compliance team, whose members can provide the necessary expertise for on-site validation requirements.

Upgrading to the Analyst Software 1.6.2 version illustrates how risks associated with validation are assessed and how the necessary steps for the compliance process are identified. The transition from Analyst 1.6 to 1.6.2 was not a major version change (unlike upgrading Analyst 1.5.x to Analyst 1.6.x); however, substantive changes in the 1.6.2 release include feature enhancements, additional peripheral device and instrument model support, and several software bug corrections that still require additional validation steps to comply with regulatory requirements.

After assessing all of the risks involved in the Analyst Software upgrade, the AB SCIEX compliance team created an informational packet entitled "Software Change Control for Analyst Software Version 1.6.2–Installation Operational Qualification (IOQ) Protocol" that addressed the impact of the upgrade on the validation state and identified the extent of re-validation required for the GLP laboratory. Contents of this resource include:

- Tests to ensure maintenance of the validated state
- A comprehensive risk evaluation describing the nature of each change
- An assessment of the change and the impact on the validated state (Table 1)
- A recommendation to validate per change control along with protocols for accomplishing this validation

Nature of the change	**Assessment of change and impact on validation**			
Evaluation of need for validation	Fix originally released	Is this a new feature?	Does this change have a regulatory impact in the use of operation of the software?	Recommendation to validate?
Support for Windows 7 (64-bit) operating systems.	1.6.2	Yes	No	Yes. This is tested by requirement R.CC1(R.44).
The Analyst® 1.6.2 Software supports all current triple quadrupole and QTRAP® Systems including support for SelexION™ Technology on the 5500 and 6500 series of instruments.	Software omponents for 6500 series instrument, 1.6.2	Yes, feature enhance-ment	No	No. This is included in vendor testing.

Table 1: Assessment of change and impact on validation

The AB SCIEX compliance team can collaborate with GLP labs during the software validation process, providing recommendations and software support, but ultimately the extent of validation rests with the GLP lab manager. AB SCIEX is committed to developing effective assessments to assist GLP labs with validation decision-making and provides tools, such as the change control below that evaluates the risks involved in the Analyst® Software upgrade:The following table shows a portion of the risk evaluation provided by the change control.

Validating clones

To expedite the software validation process, AB SCIEX has applied cloning technology to GLP laboratory workstations, developing an innovative approach to regulatory compliance that results in significant time savings. New software products, such as VMWare, enable the creation of "virtual machines," allowing for hardware systems to be more efficiently utilized by loading more than one virtual processing workstation onto a single physical computer. In order to accomplish this, virtual machines are cloned or copied, and this new technology allows for software validation to proceed more quickly.

A GLP laboratory in Quebec was the first to adopt the workstation cloning process developed by AB SCIEX for rapid mass spectrometry software validation. Rather than separately repeating the validation process on discrete workstations, a first-in-family (FIF) virtual machine was individually validated, and once the validation was complete, the FIF machine was then cloned six times. Each clone was renamed uniquely and checked to ensure the cloning process finished successfully, but the clones did not require further validation testing. What typically would have taken several days was accomplished in a matter of hours by condensing the validation process to just one virtual machine. This time savings realized by this novel validation approach opened a critical window of opportunity for the company, enabling the Quebec laboratory to successfully meet their service quota for that quarter.

Prioritize software validation risks using Fibonacci sequences

GAMP 5 recommends ranking risks associated with software validation into three categories: high priority, medium priority, and low priority. However, GAMP 5 does not provide guidance for prioritizing risks within each ranking. For example, the software validation risk assessment provided by AB SCIEX for Analyst Software identifies 73 risks, and 41 of the risks are classified as high priority. If there are 41 high priority risks, which ones are the most important and need to be mitigated first?

To answer this question, the AB SCIEX compliance team has devised a novel approach using partial Fibonacci sequences to rank all the risks into an ordered list of priorities. Then, to comply with GAMP 5 requirements, the steps of the software validation process were used to divide the fully-ranked list into the three GAMP 5 categories. By prioritizing the risks in this way, a much clearer picture of risks needing more immediate attention is obtained, taking the guesswork out of validation risk assessment for the GLP laboratory.

Conclusions:

To support GLP laboratories during the software validation process, the AB SCIEX compliance team develops innovative resources and novel software tools enabling compliance with the wide-array of government regulations in a straightforward and efficient manner.

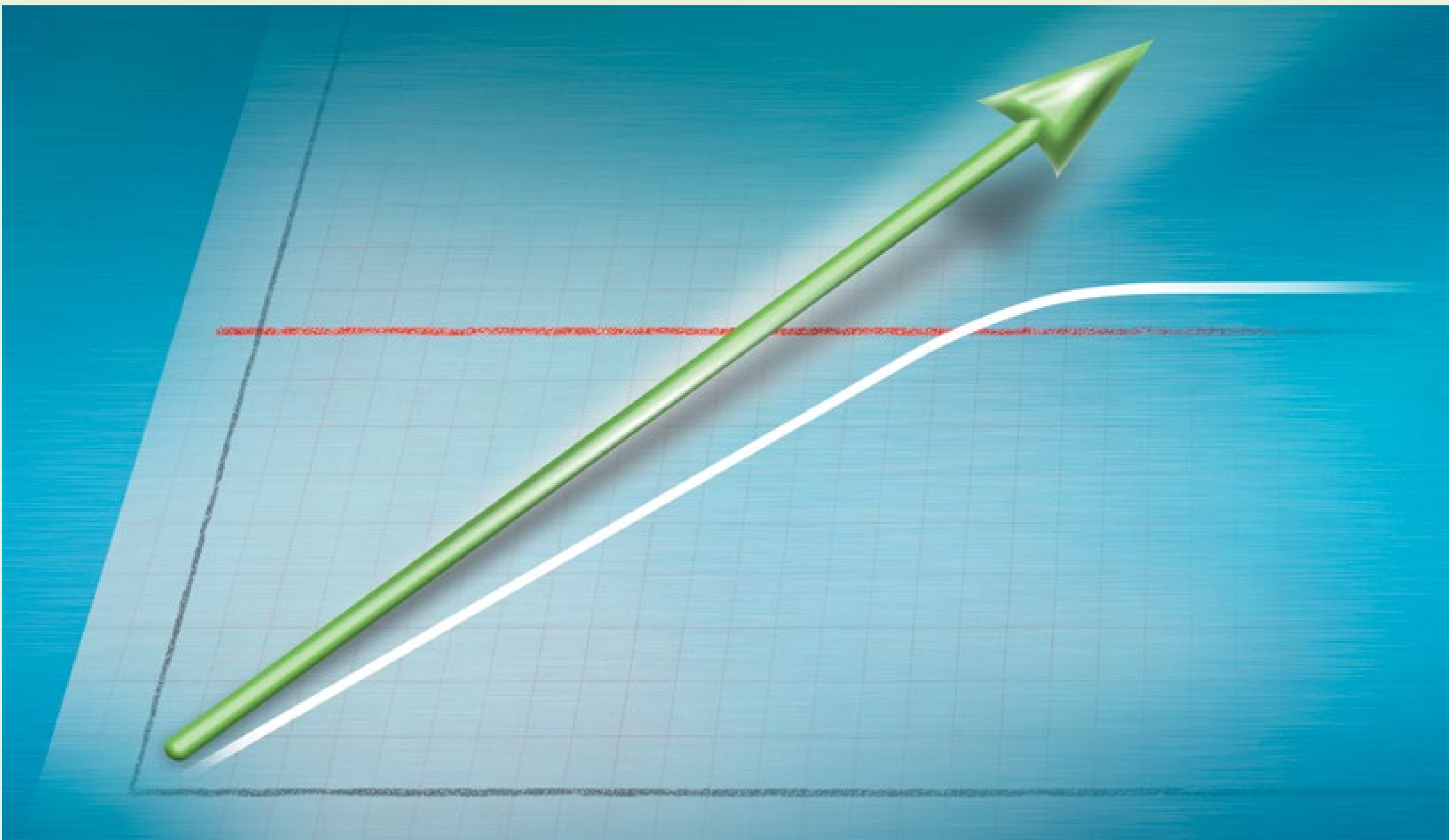
References

[1]http://www.microsoft.com/en-us/windows/endofsupport.aspx

[2]GAMP 4® to GAMP 5® Summary ISPE GAMP 5 2008

Calculate possible savings having greater sensitivity and linear dynamic range

Learn how much your lab can save with the extended linear dynamic range (LDR) and sensitivity provided by the AB SCIEX 6500 Series. Use our ROI calculator to compare your configuration with the 6500 system.



Equipped with an IonDrive™ high energy detector, the AB SCIEX 6500 LC/MS/MS Series delivers up to 6 orders of linear dynamic range. This advanced performance extends your upper limit of quantitation and reduces the need for reanalysis – saving you time and money.

Estimate out how much.

GO TO OUR **LDR ROI CALCULATOR**

Maintaining software security for your mass spectrometry system

Stacy "Dene'" Nelson

Software security controls in Analyst® Software ensure the highest standards in data protection for your AB SCIEX mass spectrometry systems. Safeguarding data security and integrity is of the utmost importance in maintaining quality control in any analytical testing laboratory, and specialized software security settings incorporated into Analyst Software fully protect essential, irreplaceable experimental records. Unfortunately, random cyber-attacks, targeted sabotage, results falsification, and accidental modifications happen all too frequently, and whether the reason for the security breach is deliberate is accidental, every lab can benefit from additional tools for maintaining the security of mass spectrometry records. Furthermore, regulations (including 21 CFR Part 11) require that automated systems be properly secured to prevent unauthorized access and to prevent corruption, loss, or falsification of data collected during the quality control process. Therefore, lab managers and system administrators are finding a secure and reliable computing environment is critical when collecting and storing mass spectrometry data.

To help tackle the issues of data integrity, AB SCIEX offers a number of security features within Analyst Software[1] (on the Windows 7 operating system), which include:

- A strong password creation policy
- Account lockout controls
- Unalterable system clock settings
- Automatic screen saver configuration
- Specified user file privileges
- Assignment of distinctive user roles

This article discusses the benefits of the Local Security Policy in the Microsoft Management Console of Analyst Software and gives an overview of the software settings that can be engaged to boost data integrity. Additional details on the security advantages of AB SCIEX software can be found by accessing "Managing Security for Analyst® Software on Stand-Alone Windows 7 Workstations."

Implementing effective security settings in Analyst® Software customized password policy

Formulating strong passwords is a fundamental requirement for safe data collection and one of the first steps in developing a security policy that lowers the risk of a security breach. Effective password design must be complex enough to prevent brute-force hacking and password guessing, and a customized framework for strong password creation in the Local Security Policy (Figure 1) can be implemented allow control of your system security based on your lab's unique quality system needs:

- Enforce password history prevents the re-use of previous passwords.

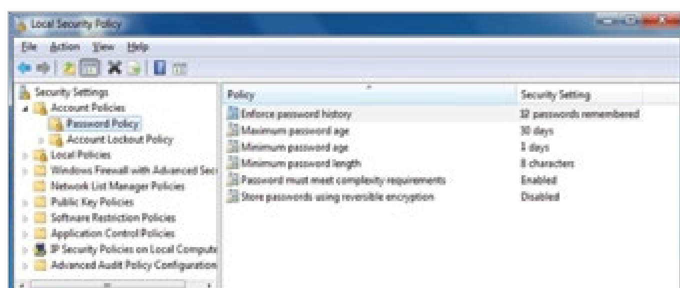


Figure 1: Password policy settings in the Local Security Policy in Analyst® Software.

- Minimum password age prevents users from changing their password repeatedly in rapid succession and blocks reuse of a favorite password. Users are also forced to change passwords periodically, typically within 30, 60, or 90 days.
- Minimum password length prevents password cracking tools from deciphering passwords and accessing your system. (Shorter passwords can be deciphered within days, sometimes hours, depending upon password complexity.)
- Passwords must meet complexity requirements calls for passwords that must exclude parts of a user's name, be at least 8 characters (preferably 12), and contain the following characters types: upper- and lower-case English letters; numerical digits (0-9); and special symbols (~ ! @ # $ % ^ & * ( ) _ + - = { } | \ : " ; ' < > ? , . /) [2]

Password hacking prevention

Hackers may repeatedly endeavor to access a data system with a known username by guessing the associated password. Within Analyst® Software security features, the Account Lockout Policy can prevent unauthorized system infringement by freezing further login attempts if unfamiliar password entry exceeds the specified threshold for login errors. Additionally, failed account login attempts are recorded in the Windows Security Event Log, which can be reviewed periodically and investigated as needed by system administrators.

System clock re-set restrictions

Altering the system clock can facilitate data falsification, and preventing changes to the system date, time, and time zone provides the necessary security to prevent data integrity violations. System administrators can adjust settings within Analyst Software so that access to the system clock is restricted to only selected individuals.

Inactivity-triggered system lockouts

Sensitive information can be disclosed to unauthorized individuals when sanctioned users leave workstations unattended even for brief periods of time. To protect against accidental system access during idle times, the Windows screen saver can be automatically configured to lock the computer after a period of inactivity (usually after 15 minutes), requiring users to re-enter login information before resuming work.

Restrictive user access controls

To validate and authenticate system-generated data, system administrators use Windows user groups to assign selected users to discrete data-access roles, which can limit or restrict a user's interaction with selected data processes. Individual users can be allocated to one of the four types of roles (listed in Table 1) in Analyst Software and the associated Windows user group, so that access to information can be well-regulated.

Role	Description	Windows User Group
Administrator	Analyst® Software administrator	Analyst_Administrator
Analyst	Analyst Software user who creates methods, acquires, processes, and reports data	Analyst_Analysts
Operator	Analyst user who operates instrument and acquires data. Does not create or modify methods, process or analyze data	Analyst_Operators
QA Reviewer	Quality assurance representative who reviews data	Analyst_QA_Reviewers

Table 1: Analyst Roles

Accidental record-deletion protection

To prevent the devastating loss of valuable mass spectrometry data, whether accidental or deliberate, requires regulated access to a system's data files. File privileges can be set that restrict data folder accessibility for individual users, reducing the possibility of accidental overwrites or intentional deletions. In fact, 21 CFR Part 11 requires that electronic records be protected from any possible deletion events, mandating secure file system controls for data collection during quality control experiments. System administrators can assign file privileges using the Windows User Groups, and some suggested privileges for various Analyst Software user roles are shown in Table 1.

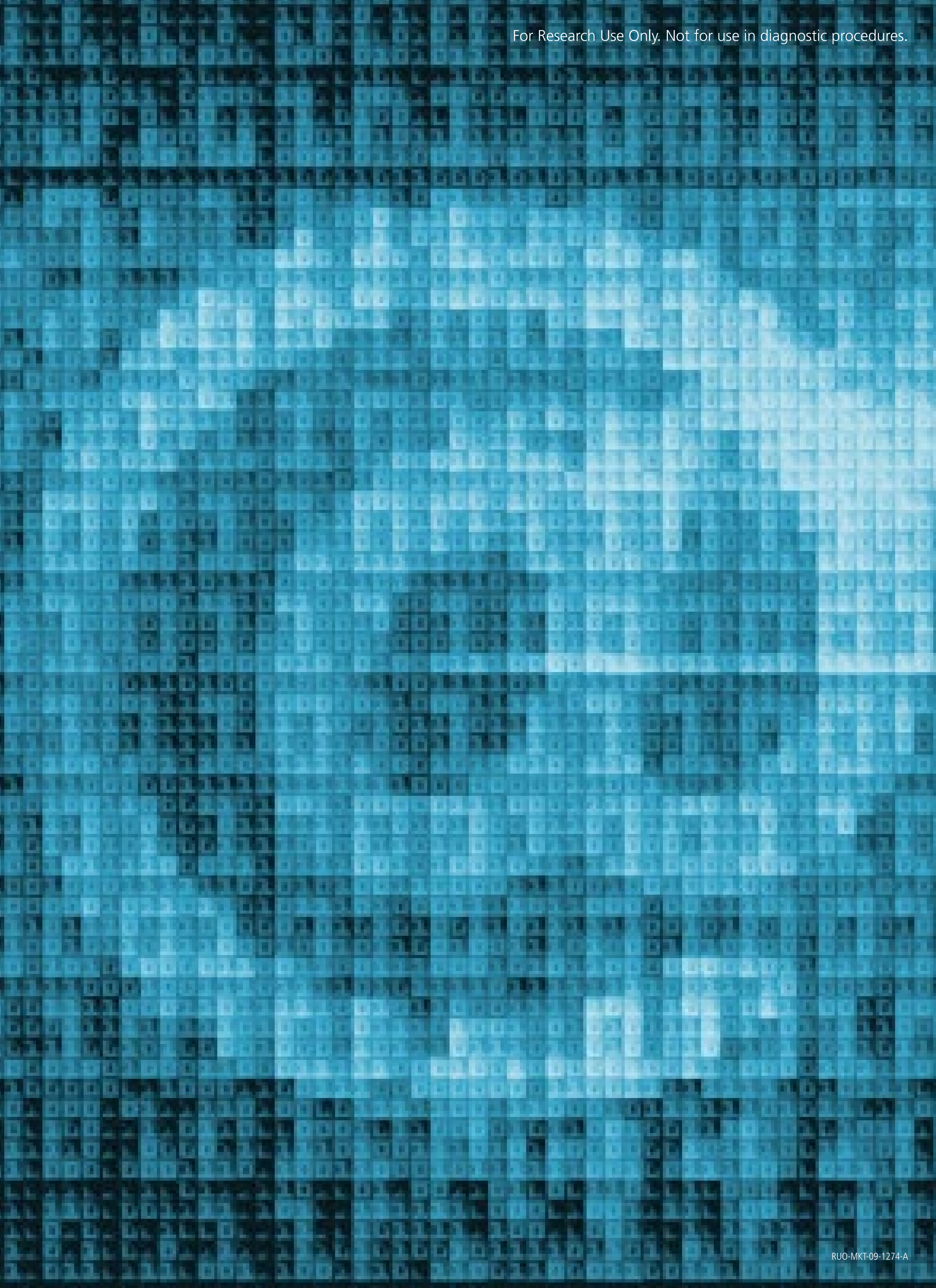
For more information on securing your mass spectrometry workstation to maintain data integrity and compliance, download "Managing Security for Analyst Software on Stand-Alone Windows 7 Workstations."

	Groups				
Permission	Analyst Administrators	Analyst Analysts	Analyst Operators	Analyst QA Reviewers	Everyone
Full control	•				
Traverse folder/execute file	•	•	•	•	•
List folder/read data	•	•	•	•	•
Read extended attributes	•	•	•	•	•
Create files/write data	•	•	•	•	•
Create folders/append data	•	•	•	•	•
Write attributes	•	•	•	•	•
Write extended attributes	•	•	•	•	•
Delete subfolder/file	•	Deny	Deny	Deny	
Delete	•	Deny	Deny	Deny	
Read permissions	•	•	•	•	•
Change permissions	•	Deny	Deny	Deny	
Take ownership	•	Deny	Deny	Deny	

Table 2: File Privileges by Role

REFERENCES

1. Analyst® 1.6.1 Laboratory Director's Guide, Release Date March 2012.
2. Strong Passwords. (2005). Retrieved from http://technet.microsoft.com/en-us/library/cc756109(v=ws.10).aspx.


For Research Use Only. Not for use in diagnostic procedures.

PUSHING THE LIMITS IN SENSITIVITY

Exceedingly sensitive.
Sharply focused.

THE 6500 SERIES WITH IONDRIVE™ TECHNOLOGY

See what couldn't be seen. Until now. The new AB SCIEX 6500 LC/MS/MS series with multi-component IonDrive™ technology is the world's most sensitive triple quadrupole, improving sensitivity up to 10X and detector dynamic range by 20X over competitive high performance instruments – with no compromise in mass range.

Unique QTRAP® linear ion trap technology and optional SelexION™ differential ion mobility technology help enhance data quality and improve throughput while reducing the need for sample preparation. With ultra-sensitivity and unrivaled robustness the 6500 Series enables scientists to push the limits in LC/MS/MS sensitivity and achieve the lowest limits of quantitation for today's most challenging quantitative assays.

The new AB SCIEX 6500 Series. It's the farsighted successor to a long line of leading AB SCIEX mass spec systems.

Explore visionary sensitivity at www.absciex.com/6500

© 2014 AB SCIEX. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB SCIEX Pte. Ltd. or their respective owners.