

# Simultaneous Quantitative Peptide Mapping and Host Cell Protein Detection in a Recombinant IgG1 Monoclonal Antibody Preparation using Data - Independent Acquisition



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## INTRODUCTION

Monoclonal antibodies (mAb) are major target-oriented biotherapeutics used to treat an array of human diseases. mAbs are typically produced in biological systems such as Chinese hamster ovary (CHO) or other cell lines. Any heterogeneity in purified IgG protein products, for example post-translational modifications (PTMs), sequence variants, degradation products, and contaminants (such as host cell proteins) must be characterized completely to understand the purity, stability and potency of the mAb product, and to avoid immunogenicity. Mass spectrometry provides superior analytical capabilities for the characterization of mAbs, both at the intact protein level and for peptide digests of antibody preparations. Conventional data-dependent approaches to automatic analysis of peptides in LC-MS/MS experiments are fundamentally stochastic. This is not ideal for analysis of biotherapeutics, where analytical consistency and quantitative accuracy across samples are of critical importance. Here we explore the application of an unbiased and deterministic, data-independent method for mAb analysis using SWATH™. This approach provides substantial benefits over other MS strategies because it captures comprehensive, quantitative MS and MS/MS information for every analyte in the sample, in every analytical run.



## MATERIALS AND METHODS

**Sample Preparation:** IgG1 mAb was reduced/alkylated and trypsin digested. A constant concentration of this digest was spiked with a range of Bovine Serum Albumin and Apomyoglobin digest concentrations representing varying levels of contaminating host cell proteins.

**Chromatography:** Samples were analyzed using the Eksigent ekspert™ 425 System. Varying amounts of digest were loaded onto a column (0.5 mm x 10cm ChromXP C18-CL 3µm 120Å) Elution gradients of 3-35% acetonitrile (0.1% formic acid) in 30 min were run.

**Mass Spectrometry:** Unmodified and spiked mAb digests were analyzed using a TripleTOF® 5600 system. An information dependent acquisition (IDA) LC-MS/MS method was used for initial peptide identification. SWATH™ data-independent acquisitions were subsequently performed in triplicate on each sample to obtain quantitative MS/MS chromatograms for every precursor ion between 400 and 1200 m/z, using a 20 Da Q1 window width. Peak areas of extracted ion chromatograms from each peptide were analyzed to provide a quantitative fingerprint of the protein product and its modifications. The TripleTOF® 5600 system's high sensitivity enables very fast MS/MS acquisition rates; as low as 20 ms accumulation time per MS/MS in Information Dependent Acquisition (IDA) mode. The IDA method consisted of a high resolution TOF MS survey scan followed by 20 MS/MS per second with a minimum accumulation time of 50 msec. Eluent from the column was sprayed using the Nanospray® III Source and heated interface.

**Data Processing:** Peptide mapping was performed using ProteinPilot® Software 4.5 searching a FASTA database that contained the sequence of the antibody and the sequences of the "host" cell proteome (Bos Taurus). Quantitative analysis was performed using the search results as a peptide library to inform SWATH™ peptide fragment ion chromatogram extraction using the SWATH Acquisition tool inside of PeakView® Software. MarkerView™ statistical analysis software was used to identify trends in the data, such as increasing or decreasing amounts of host cell protein peptides, or product peptide modifications, for example.

## MS/MS<sup>ALL</sup> with SWATH™ Acquisition

- What is it?
- MS/MS<sup>ALL</sup>
    - A data-independent workflow enabled by TripleTOF® system technology that acquires high resolution quantifiable MS/MS data for all detectable analytes in a complex sample, in a single run
- How does it work?
- SWATH™ Acquisition
    - Uses wide isolation windows stepped across a mass range, collecting high resolution composite MS/MS spectra in a chromatographic time scale
- What does this enable?
- Data processing by generation of post-acquisition fragment ion XICs at high resolution for quantitation with confirmation of identity
  - Quantitation and confirmation of everything in the sample
  - Digital record of everything in your sample
  - Single method for acquiring all your data

## SWATH™ Leverages High Resolution MS2 for High Fidelity Quant

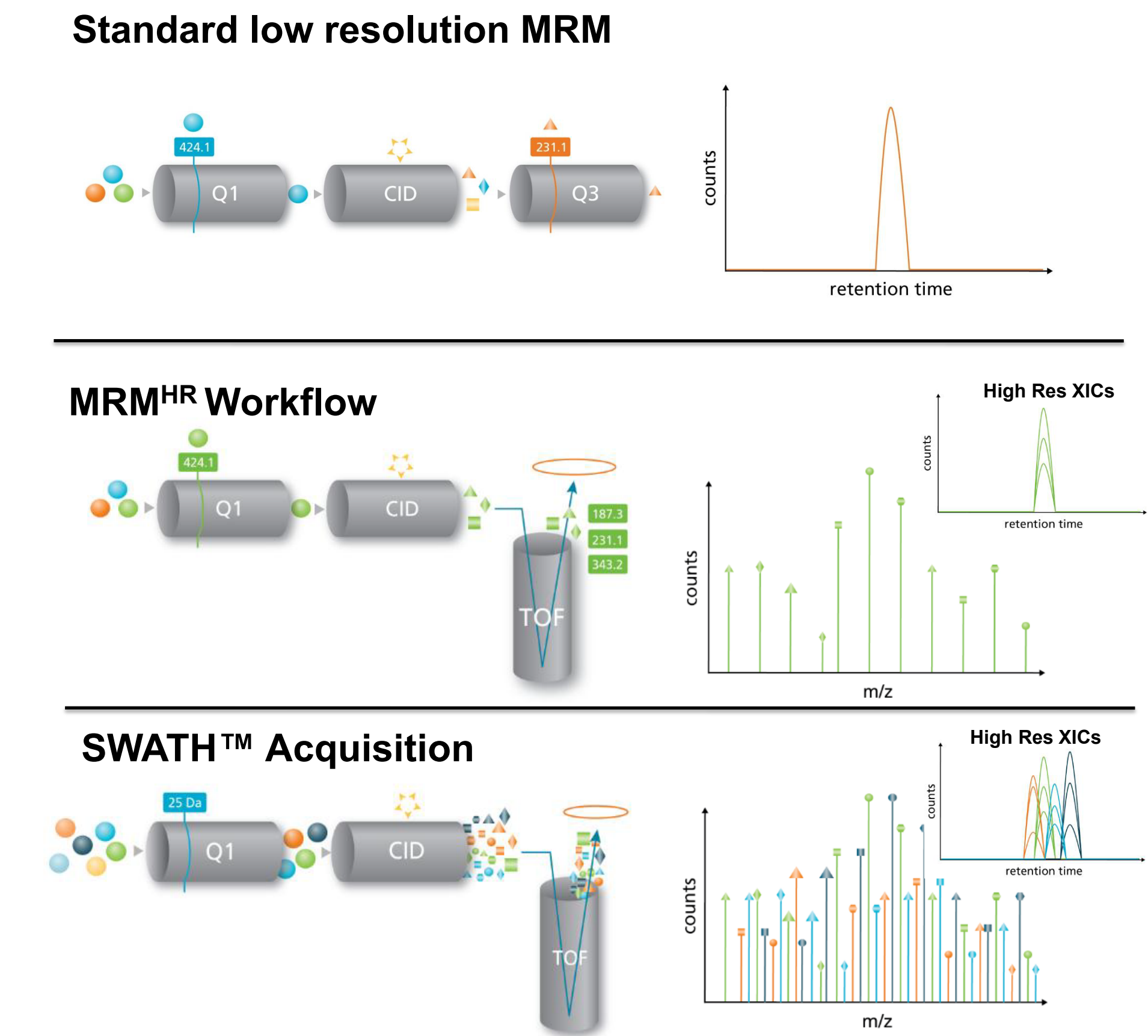


Figure 2. SWATH™ Scan Modality Overview. Standard MRM quant has 0.7 Da isolation on both quadrupoles. MRM<sup>HR</sup>, available on the 5600, still scans Q1 at a width of 0.7 Da but fragment ion chromatograms can be extracted from high resolution (>30K) MSMS scans at much narrower widths (0.007 Da) enabling higher selectivity. SWATH™ technology maximizes the use of MSMS resolution, extracting narrow fragment ion chromatograms from fragmentation of a wide isolation in Q1 (~25Da).

## Comprehensive Quantitation

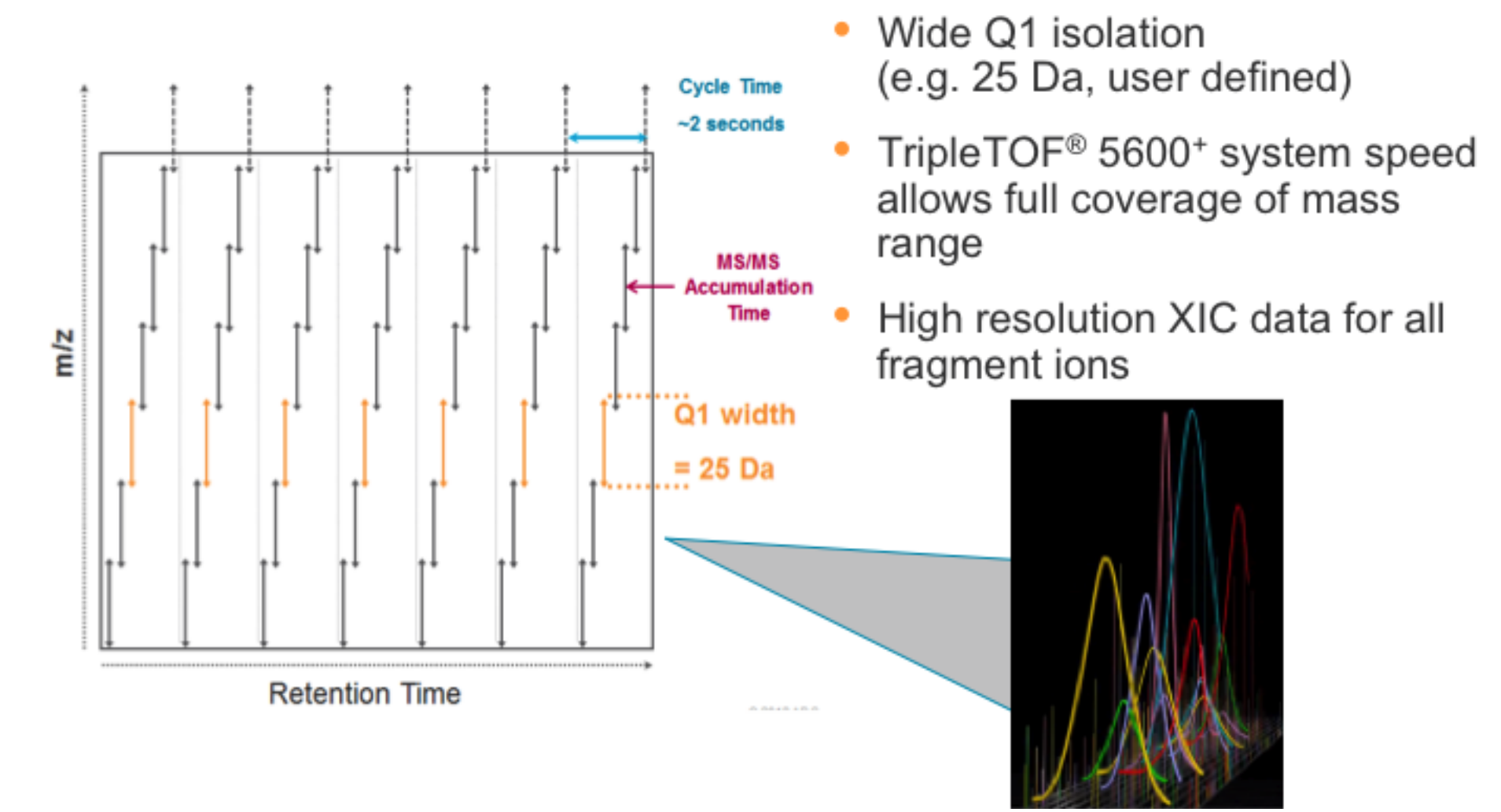


Figure 3. SWATH™ Scan Obtains MSMS data on all ions. By stepping the mass range in 25 Da increments, fragment ion chromatograms of all observed ions are collected.

## RESULTS

Peptide mapping sequence coverage was 100% for Light Chain and 99.5% for Heavy Chain(Figure 4). Several deamidation sites and oxidation sites were observed and quantified relative to the unmodified forms. Comparison of SWATH™ peak areas from replicate analyses revealed consistency typically within 5%CV(Figure 8). Critically, the data indicate that we can detect our model "host cell proteins" at levels down to 10 PPM contamination (wt/wt). Using the quantitative SWATH methodology, in a single sample run we can acquire MS/MS chromatograms on every fragment ion from every precursor peptide ion between m/z of 400 and 1250. By examining the data retrospectively, we can quantify the extent of PTM heterogeneity and host cell protein contamination with MRM-like fidelity and sensitivity, without any up-front method development or foreknowledge of the PTM or contaminating protein. Additionally, a SWATH™ data file can be mined retrospectively if a contaminating protein is discovered in a subsequent lot.

## Protein Pilot® Search Results

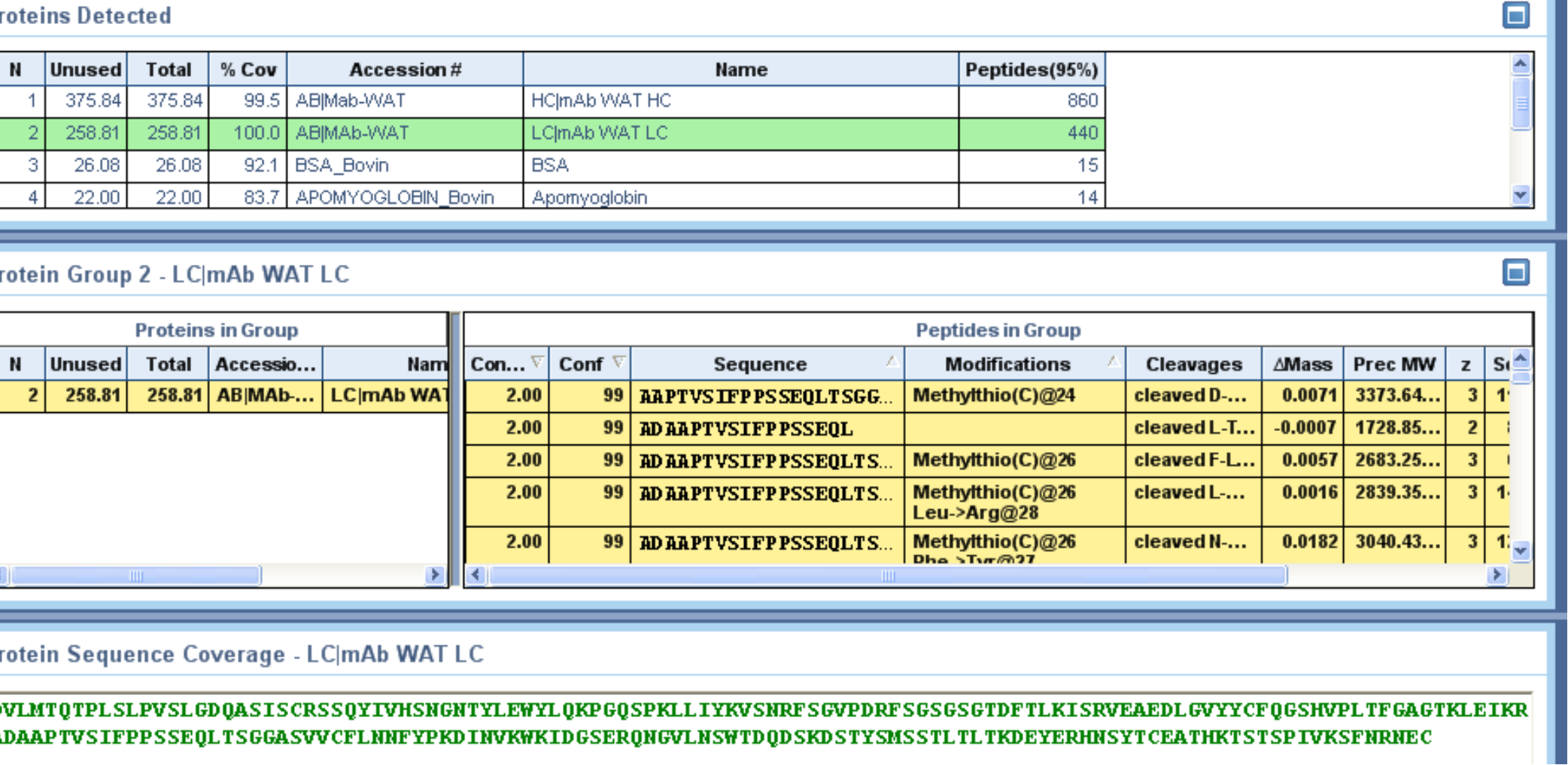


Figure 4. Search Results. Protein Pilot is capable of searching for over 350 modifications and all sequence variants in a single search, without the liability of generating large numbers of false positives, as occurs with an error tolerant search.

## Peakview SWATH™ Analysis Tool

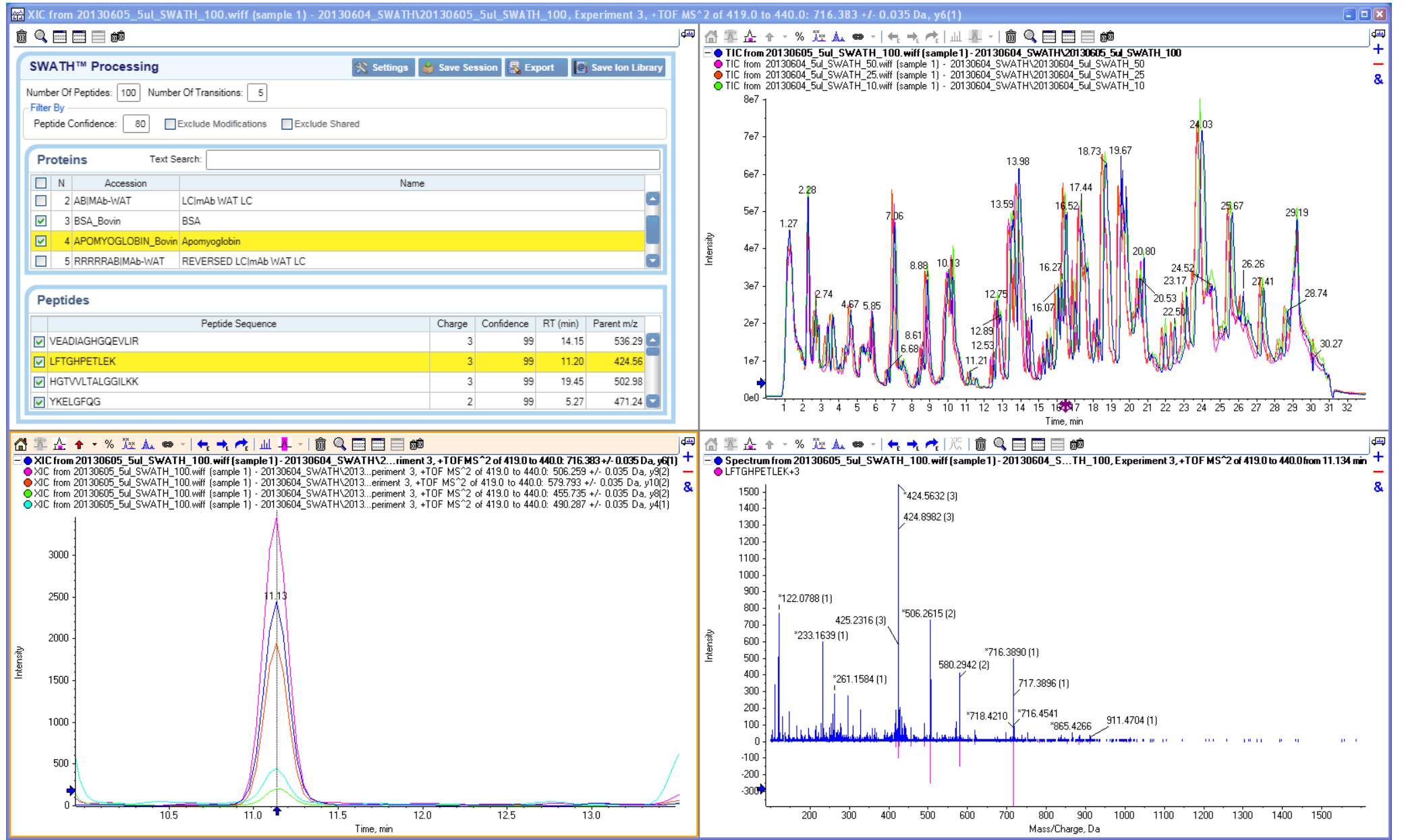
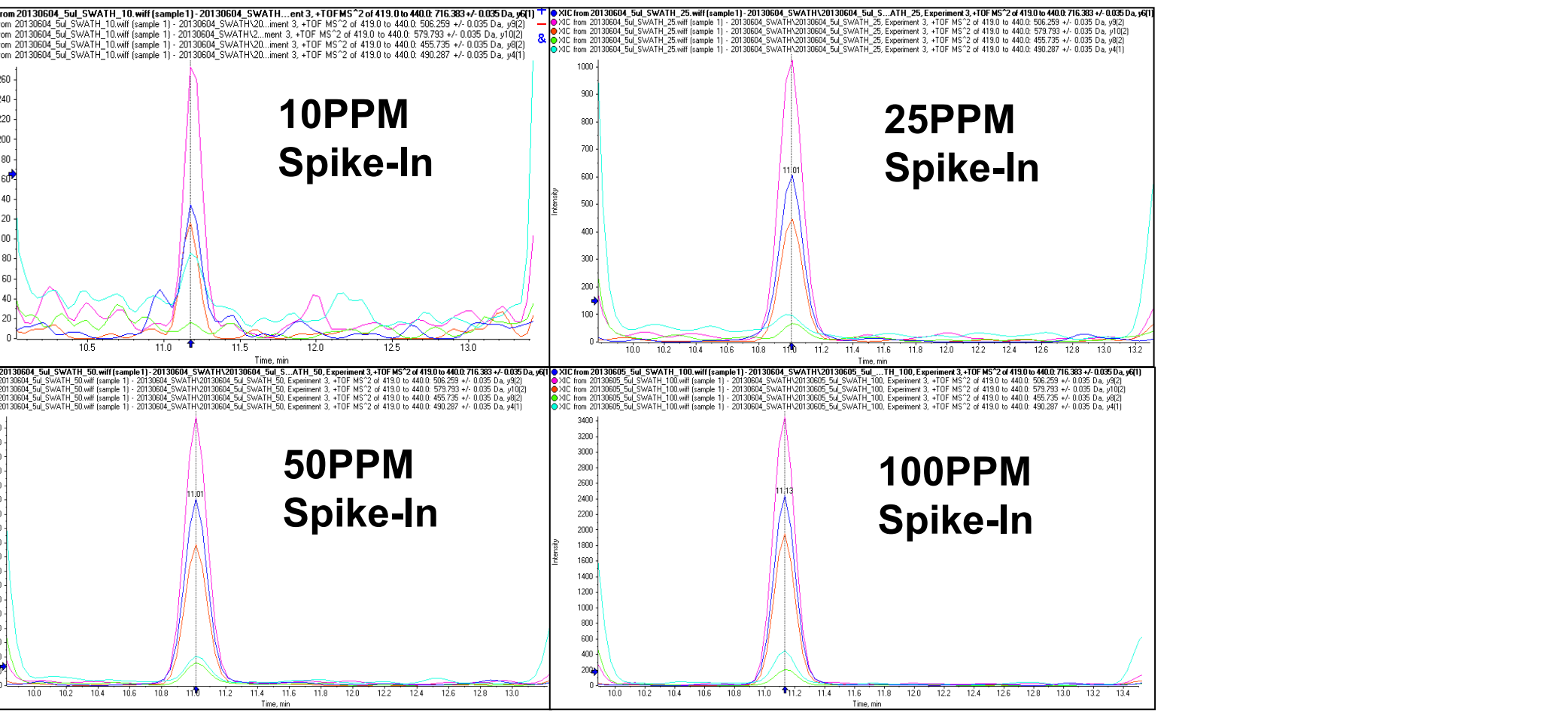


Figure 5. SWATH™ Analysis Software. Top Left: List of proteins and peptides from an ion library (in this case a Protein Pilot™ .Group file). Top Right: TIC chromatograms of each SWATH™ data file. Bottom Left: XIC chromatograms of six fragment ions from the selected peptide. Bottom Right: Mirror plot showing the MSMS Spectrum collected at the top of the chromatogram in the bottom left pane (Blue) over the Spectrum from the ion library (Pink).

## Bovine Apomyoglobin LFTGHPETLEK(3+)



## Bovine BSA AEFVEVTK(2+)

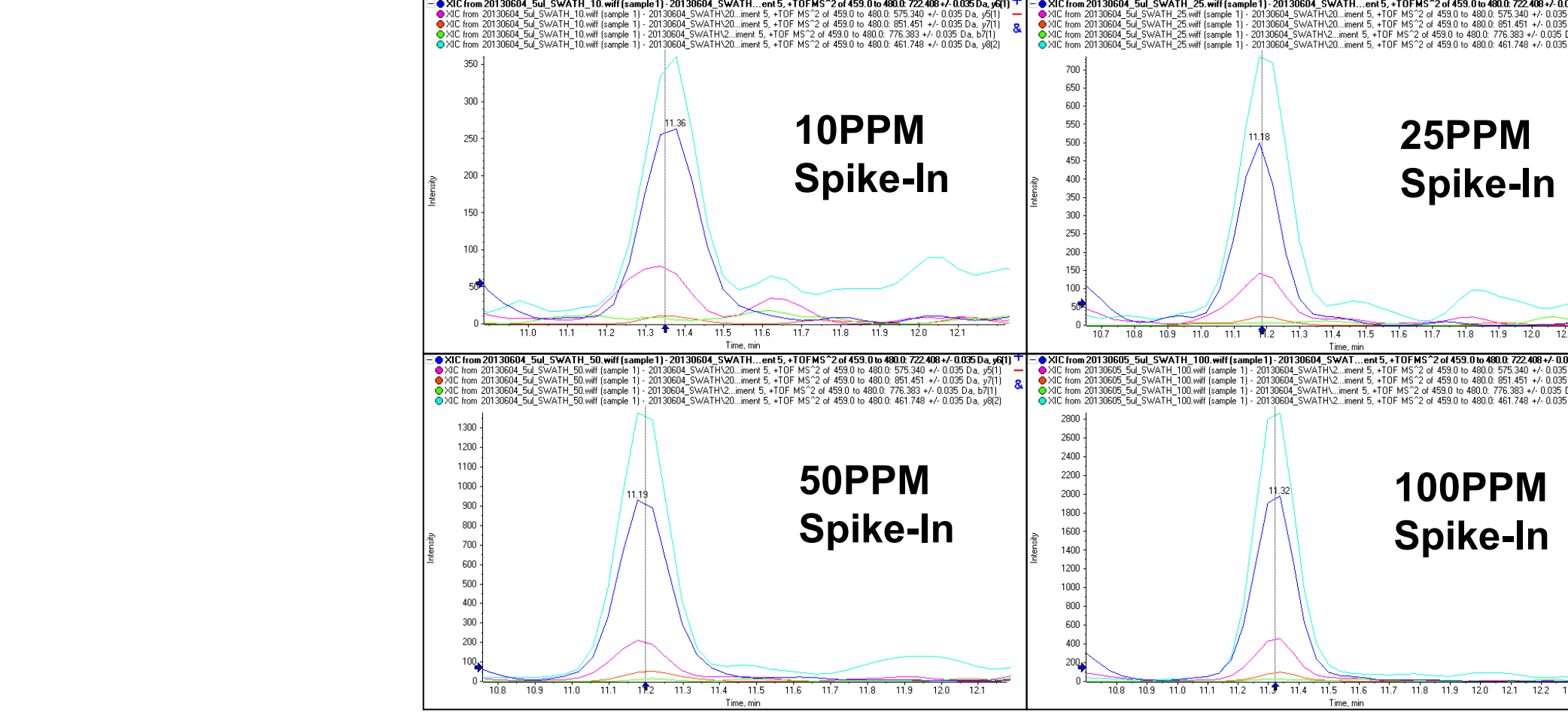


Figure 6. SWATH™ Data. SWATH data representing a single Apomyoglobin peptide (Top) and a single Each XIC is extracted from the 419-440 SWATH™. 10PPM wt/wt contaminant protein to protein product is easily detectable.

## SWATH™: Statistical Analysis Results

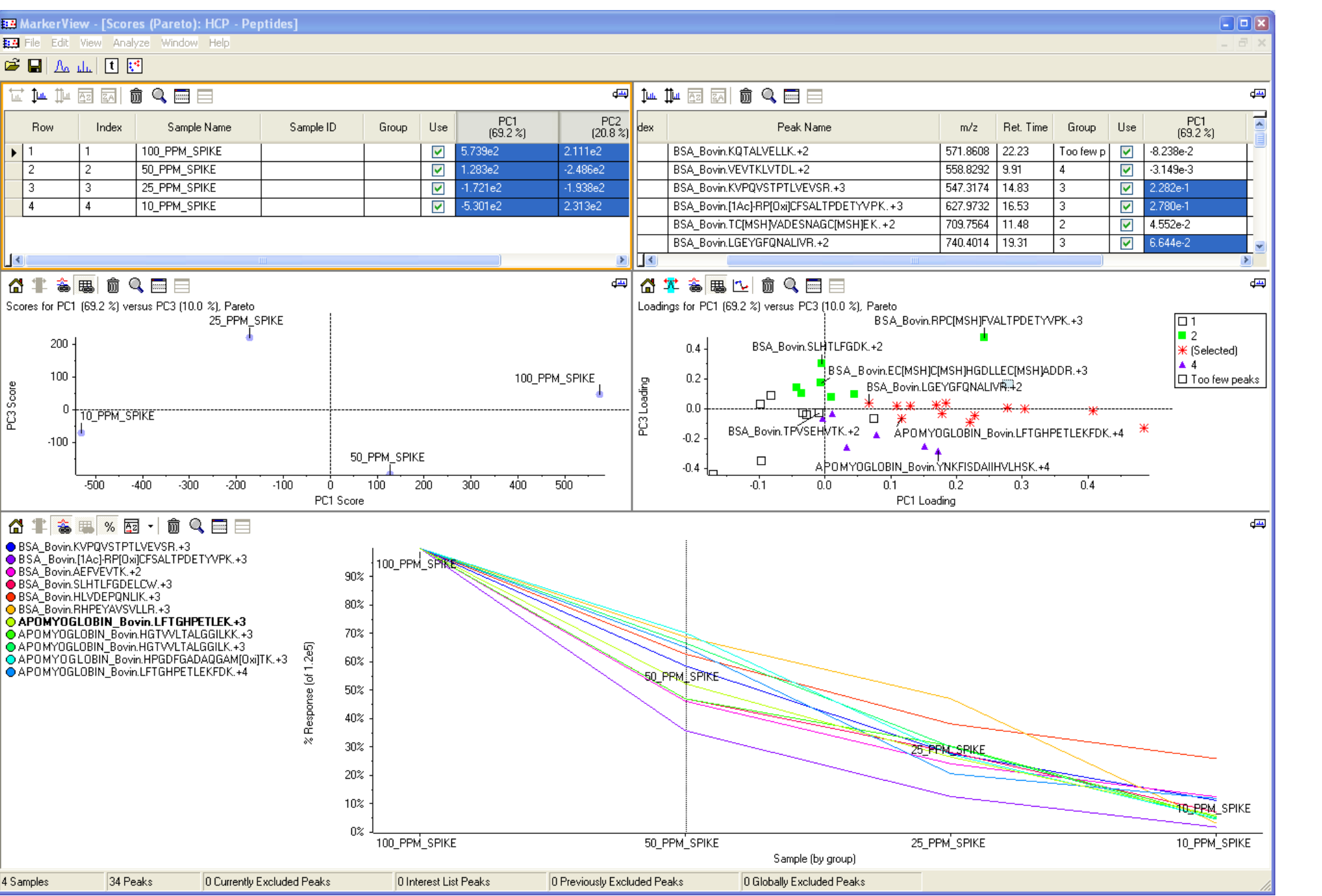


Figure 7. Principal Component Variable Grouping Analysis. Using MarkerView™ software, trends in protein concentration changes can easily be visualized and tracked through lots. In this case we wanted to identify all the peptides from our Spike-in experiment that behaved linearly and were the most robust indicators of "HCP" concentration.

## MSMS Quantitation of all Product Peptides and Mods.

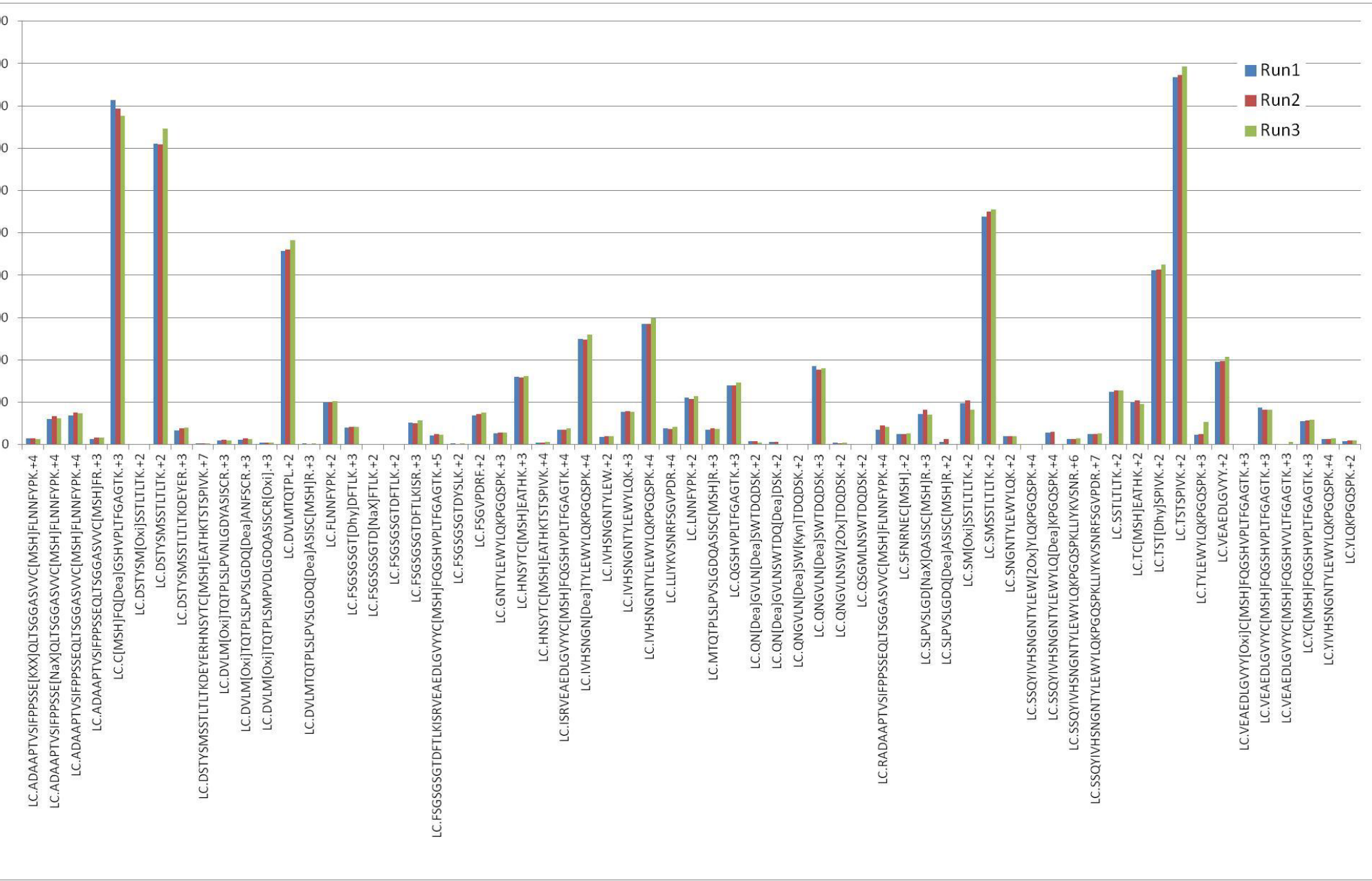


Figure 8. High Quality Quantitation. Extracted Ion Chromatogram peak areas of five fragments per peptide were summed to produce the above bar graphs. Triplicate measurements of each peptide and of its modified forms were all below 10% C.V.

## CONCLUSIONS

- MS/MS<sup>ALL</sup> with SWATH™ Acquisition is a novel data-independent acquisition strategy that provides:
  - Comprehensive high resolution MS/MS data for all detectable ions
  - High quality quantitation similar to MRM with no method development
  - Easy and retrospective data interrogation
- SWATH data can be processed by PeakView® Software and MarkerView™ Software or extracted for use with 3<sup>rd</sup> party informatics tools
- SWATH Acquisition is ideal for quantifying Protein Contaminants in Biologic protein products.
  - Quantitative sensitivity and fidelity rivaling ELISA without safety concerns of reagent preparation (not everything that produces a reaction in human produces a reaction in rabbit)
  - Captures a digital record of all fragments of all peptides in a protein product. This can be used to track changes over time and the data can serve as a digital archive of the current state of a sample at a given time. Can be retroactively mined for any protein contaminant concerns in the future.

## REFERENCES

- Nature Methods 6, 359-362 (2009).
- J. Proteome Res. 2008, 7, (9), 3661-3667.
- Electrophoresis, 20(18) 3551-67 (1999)

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