

**Transfer of
Optimized
Acquisition
Parameters Between
Mass Analyzer
Types for Improved
Protein Identification
and Quantification**

ASMS 2009

Christine A. Miller, David Horn,
Shripad Torvi, Ning Tang and
Keith Waddell

MPA 015



Introduction

Putative protein biomarker candidates must be validated by performing targeted quantitative analyses with patient samples. The most common MS/MS approach has been to generate a highly specific and sensitive MRM-based assay for peptides seen in the discovery process. However, optimization of the selected peptides can be a time-consuming process. The traditional discovery approach has used ion trap based methodology, which can produce substantially different fragment patterns than triple quadrupole mass spectrometers. Using shared collision cell design, Q1 selection ability, nanoflow LC/MS interface and nanoflow-based chromatography, this work examines using information from a biomarker discovery experiment run on a Q-TOF to speed validation assay development for a MRM-based workflow on a triple quadrupole system.

Experimental

Instrumentation: All work was done on an Agilent 6420 Triple Quadrupole (QQQ) mass spectrometer (below left) or a 6520 Accurate Mass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer interfaced to an HPLC-Chip/MS system (below right) for routine, robust nanoflow LC/MS. The chromatography was done on a standard HPLC-Chip with a 40 nL enrichment column and a 43 mm x 75 μm nanocolumn packed with Zorbax 300SB-C18. Gradient chromatography was performed using A) 0.1% formic acid in water and B) 0.1% formic acid in 90% acetonitrile in water.

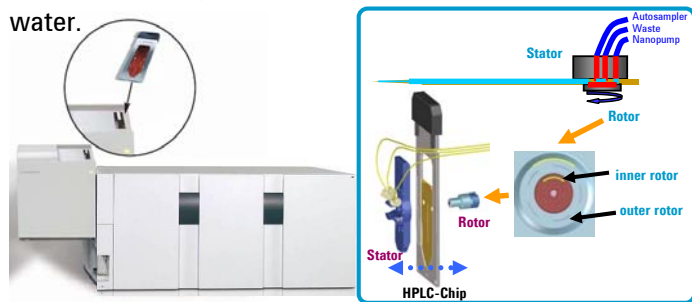


Figure 1: HPLC-Chip/MS system (left) and schematic of HPLC-Chip (right).

Collision Energy (CE) Optimization: The chromatography-based MRM parameters optimization was tested on synthetic peptides and on tryptic peptides from digestion of standard proteins. Acquisition parameter optimization focused on collision energy values as ion optic settings are generally the same for peptides. For each peptide tested, 3 transitions were preferred for each precursor with all transitions generally at a higher m/z than the precursor for optimal selectivity. For the synthetic peptides, 30 LC/MS analyses were done to examine collision energy in one-volt steps.

Automated MRM Optimization

An automated routine was developed to optimize the predicted transitions for each peptide without operator intervention. For peptide optimization, some of the key goals were:

- Minimize sample injections needed
- Allow optimization of multiple transitions and peptides with a single injection (limited only by sensitivity needs)
- Directly import or enter peptide sequences
- View predicted b and y fragment ions and select ions for optimization
- Optimize CE in MRM mode using CE setting determined from Q-TOF formula as a starting point

In the peptide version of Optimizer, charge states >1 can be selected and several charge states for the same peptide can be selected if the optimal precursor is not known. The details of the peptide sequences, charge state and results are stored as an Optimizer project.

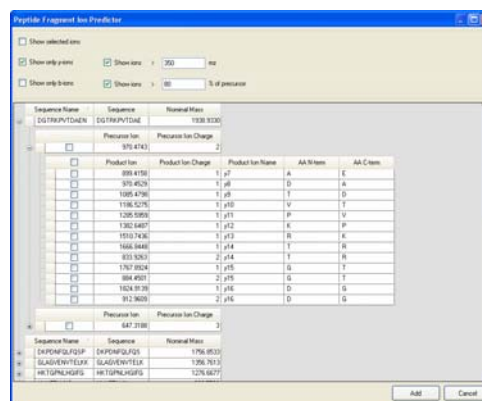


Figure 2: Selection of product ions for optimization based on fragmentation prediction for the imported peptide sequence

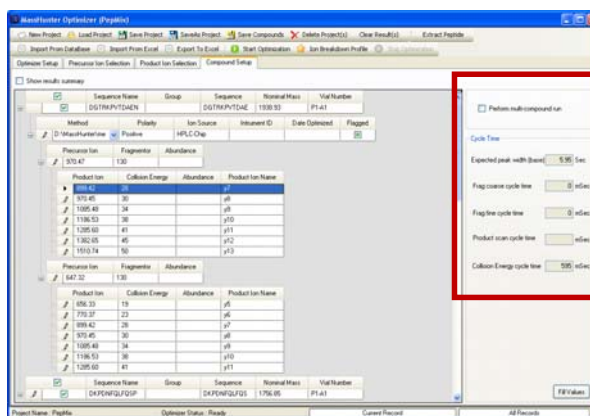


Figure 3: Selected transitions ready for optimization. Note that the panel on the right (red box) displays cycle time information. In this example, 28 MRM transitions with a dwell time of 5 ms results in a cycle time of 595 ms.

Manual CE Optimization

For a mixture of synthetic peptides, the normalized results of manual CE optimization in one volt steps are shown in Figure 4. From this data, it is clear at least 90% of the maximum response is achieved over a 5-10 V window. For some of the peptides, the optimal CE is different for the 3 transitions.

From this data, a linear regression was done for m/z vs. CE. The regression gave a slope = 3.6 and intercept = -4.8 using the equation:

$$(m/z / 100) * \text{slope} + \text{intercept}$$

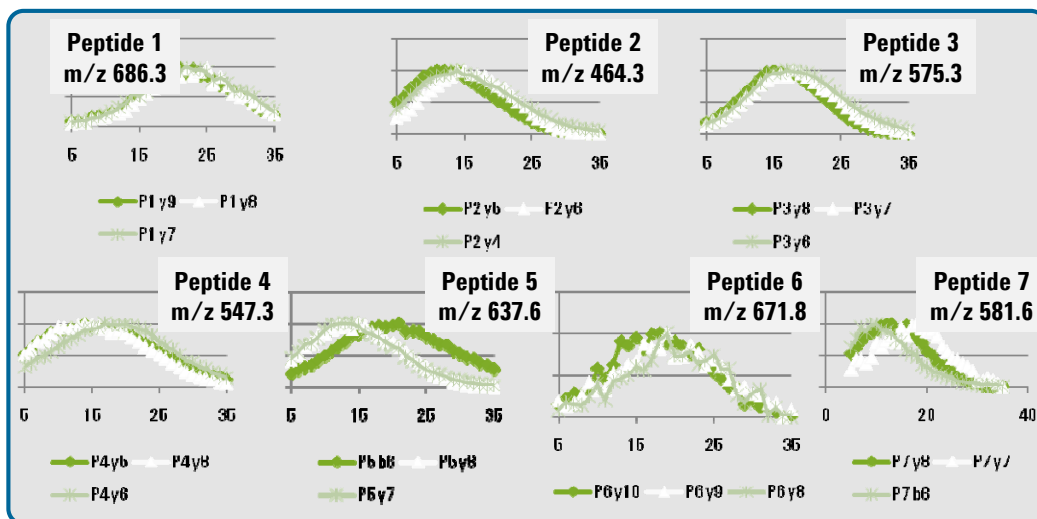


Figure 4: Normalized response versus CE for 3 transitions from 7 peptides.

Comparison of Optimization

With the mix of 16 protein digests, a set of 30 peptides was selected based on Q-TOF discovery data and Spectrum Mill Peptide Selector. The optimal CE for these peptides was determined using both a manual (2 V steps, 88 transitions) and automated (Optimizer, 191 transitions) approach. The results were examined to determine:

- How manual vs. automated optimal CE compare
- How optimal CE compares to the predicted formula (slope = 3.6 and intercept = -4.8).

Over the 88 transitions optimized manually, the average delta of the determined CE from the predicted (formula) CE was -3 V. There was no clear deviation from the predicted CE based on charge state (precursor or product ion), or m/z (precursor or product ion).

Similarly, for the 191 transitions optimized automatically, the average delta of the optimized CE from the predicted was 4 V.

A limited comparison of the automatically and manually optimized values (table to the right) shows good agreement between for the optimized CE values.

Compound Name	Precursor Ion	Product Ion	Auto CE	Manual CE	Formula CE
AAGLATGNVSTAELODQATPAALVA HVTSR (+3)	931.5	1019.5	32	29	28
	931.5	1205.1	32	29	28
AISNNEADAVTLDDGLVYEAGLKPN NLKPVVAEFHGTK (+5)	791.2	1084.6	33	29	23
	791.2	1156.6	25	27	23
GYQLVSDAASLNSVTEANQQKPLL GLFADGNMPVR (+4)	926.7	1006.5	31	23	28
	926.7	1300.2	31	29	28
IGEEYISLDLQLRK (+3)	560.3	783.4	15	15	15
	560.3	974.5	23	23	15
KPVTD AENCHLAR (+3)	504.3	691.8	13	13	13
	504.3	1186.5	21	21	13
LVNELTEFAK (+2)	582.3	951.5	16	15	16
	582.3	708.4	16	17	16
	582.3	595.3	20	17	16
WSGFSGGAIECETAENTECCIAK (+3)	849.4	964.4	32	23	25
	849.4	1093.5	32	23	25
	722.3	1167.5	22	23	21
YICDNQDTISSK (+2)	722.3	1007.5	22	23	21
	722.3	892.4	26	27	21

Collision Energy on QQQ and Q-TOF

Because the Agilent 6410 Series QQQ and 6500 Series Accurate Mass Q-TOFs use the same collision cell and ion optics (Figure 5), the observed fragmentation is the same between the two mass analyzers when the same CE is used. This facilitates the transfer of information between the platforms. Utilizing the new CE formula for data-dependent MS/MS on the Q-TOF for the protein digest mix yielded a 31% increase in the number of assigned MS/MS spectra, a 24% increase in protein score and a 16% increase in number of unique peptides.

As shown in Figure 6, the MRM Selector in Spectrum Mill can now directly create an MRM or dynamic MRM (retention-time based MRM) method for subsequent validation studies.

With the yeast digest, Q-TOF data was searched using Spectrum Mill. A Dynamic MRM list were generated using MRM Selector (Figure 7) based on the validated peptide hits, which contains protein accession number and peptide sequence, MRM transition values, retention time (RT), peak width, collision energy and fragmentor value.

MRM Selector parameters:

- number of peptides per protein,
- number of product ions per peptide, choice of above precursor and y-ions
- Peptide score and %SPI
- Required AA and disallowed AA
- Peptide pl
- Protein accession number

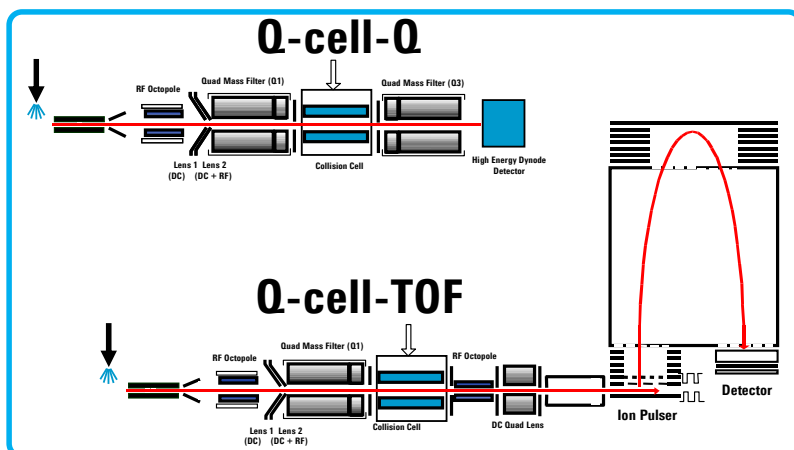


Figure 5: QQQ and Q-TOF design implementations.

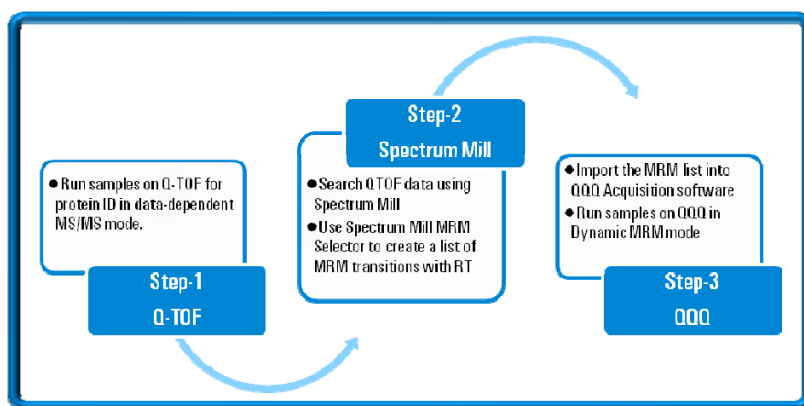


Figure 6: Biomarker validation workflow benefits from sharing of optimized parameters, retention times etc. between Q-TOF discovery data and QQQ

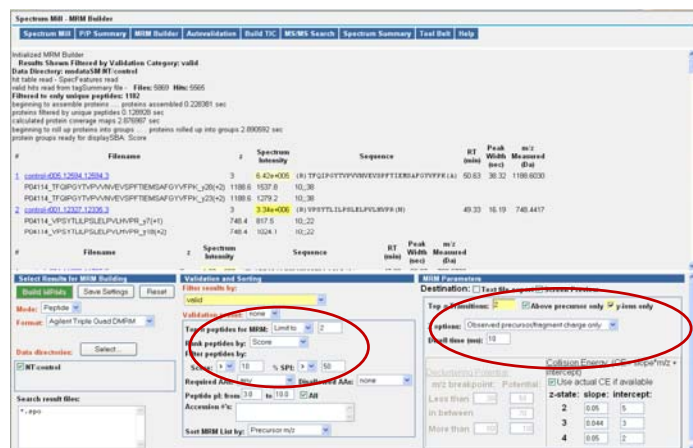


Figure 7: MRM Selector generates dynamic MRM methods from discovery Q-TOF data.

Conclusions

- An automated optimization software has been developed specifically for peptides.
- The manual and automated optimal CE values were generally in very good agreement.
- MRM Selector allows the user to create dynamic MRM methods based on experimental discovery data.
- The transfer of hundreds of transitions from discovery data to a dynamic MRM method has been demonstrated and is shown in poster MPT 456.