INTELLIGENT DESIGN OF MRM ASSAYS FROM LC-MS^E INVENTORIES

Waters

Michael J. Nold, Gordon Fujimoto, Asish B. Chakraborty, Scott J. Berger, Scott J. Geromanos, Jeffrey C. Silva
Waters Corporation, Milford, MA

OVERVIEW

- Multiple reaction monitoring (MRM) by tandem quadrupole mass spectrometry is a powerful method for quantitatively screening protein biomarkers in biological samples. However, prediction of optimized MRM peptide transitions is challenging due to sample complexity and dynamic range.
- Empirical determinations of optimum transitions for MRM experiments are facilitated by alternate scanning (LC-MS^E) data acquisition which records a comprehensive inventory of peptide precursor and product ion accurate mass, retention time, and intensity information for a complex protein digest (Figure 1).
- The LC-MS^E discovery method was used to generate a targeted MRM assay for monitoring a panel of twenty serum proteins (Table 1).

INTRODUCTION

There is consensus that high and medium abundance serum proteins hold promise as potential clinical biomarkers. Broadband monitoring of these proteins is needed to improve clinical diagnostic capabilities across a diverse spectrum of human diseases. Mass spectrometry (MS) provides a means for such high throughput monitoring.

In this study, a comprehensive MS analysis of whole and depleted serum samples has been carried out via a label-free, alternate scanning LC-MS methodology (LC-MS^E).[1-4] The inventories of precursor and fragment ion information produced in this discovery method are a qualitative and quantitative record of the proteomic condition of the sample. The LC-MS^E data allows the empirical selection of optimum MRM transitions for any targeted peptide (Figures 2-3).

Optimum MRM transitions for peptides representing 20 serum proteins have been extracted for this work resulting in four monitored transitions per protein. In some cases, multiple charge states for a peptide were monitored. Limited cone voltage and collision energy profiling was conducted to crudely estimate preferred operating conditions for a given transition.

GOALS

- Demonstrate the utility of LC-MS^E for the development of optimized MRM quantitation of peptides associated with targeted proteins.
- Illustrate the sensitivity improvements associated with MRM for targeted peptide analysis.
- Compile retention time, peptide precursor and fragment ion information for the most sensitive and selective MRM transitions for serum proteins to be posted in the future at the Waters website.

REFERENCES

1. Bateman, RH. et al., *J Am Soc Mass Spectrom* **2002**, 13, 792-803
2. Purvine, S. et al., *Proteomics* **2003**, 3, 847-997
3. J. Silva et al., *Anal. Chem.* **2005**, 77, 2187-2200
4. J. Silva et al., *Mol. Cell. Proteomics* **2006**, 5, 144-156
5. J. Silva et al., *Mol. Cell. Proteomics* **2006**, 5, 589-607
6. M. Hughes et al., *J. Proteome Res.* **2006**, 5, 54-63

METHODS

Depletion

Depletion of serum was carried out using a ProteoPrep® 20 Plasma Immunodepletion Kit (Sigma Product No. PROT20, Sigma-Aldrich, St. Louis, MO)

Samples

Whole and depleted serum protein samples were taken up in 50 mM NH_4HCO_3, 0.1% *Rapi*Gest™ SF (Waters Corp., Milford, MA), pH 8.5 to a final concentration of ~ 1 µg/µL. Reduction and alkylation was carried out with DTT and IAA, respectively. The proteins were digested with 1:25 (w/w) proteomics grade, dimethylated trypsin (Sigma Product No. T6567, Sigma-Aldrich, St. Louis, MO) overnight (16 hr). Tryptic activity was degraded by the addition of 2 µL concentrated HCl, followed by centrifugation and the supernatant collected. Samples were spiked with known quantities of rabbit phosphorylase B and yeast enolase digest and diluted with 50 mM NH_4HCO_3 to a final concentration of 200 ng/µL of serum peptides for 600 ng loads.

LCMS^E Data Collection

The peptides were separated and analyzed by LC-MS^E using a nanoACQUITY™-Q-Tof Premier™ (Waters Corp., Milford, MA), and then processed to produce a ***COMPREHENSIVE INVENTORY*** of identified peptides with their corresponding precursor and product intensities, their accurate mass and retention time information (AMRT), that was then used to select optimum MRM transitions.

MRM Data Collection

MRM analyses were performed on a Quattro Premier™ XE tandem quadrupole mass spectrometer (Waters Corp., Milford, MA), monitoring four transitions per protein. They were selected from the AMRT inventories of each protein based upon product ion intensity and replication in at least two out of three experiments.

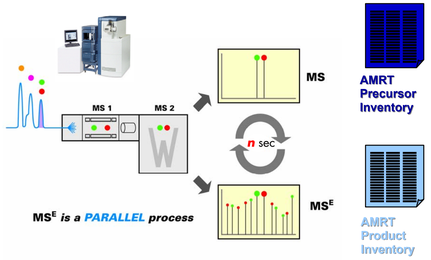

Figure 1. Alternate Scanning LC-MS (LC-MS^E).
LC-MS^E data acquisition produces an inventory of all precursors and fragment ions detected throughout the entire experiment, with their corresponding accurate mass, retention time, and intensity.

RESULTS & DISCUSSION

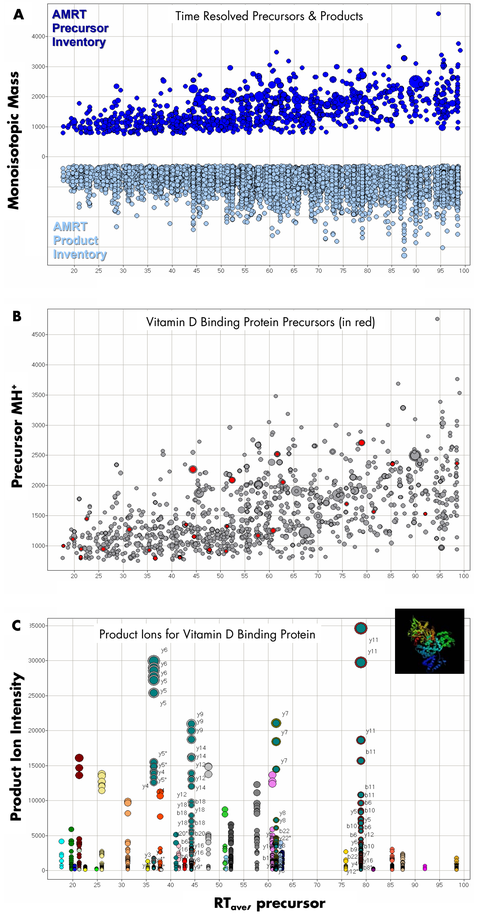

Figure 2. Extraction of MRM Transitions from AMRT Inventories.
A. Total AMRTs from 3 replicates of whole and depleted serum tryptic digests
B. Filtered AMRT precursor inventory - Found in at least 2 out of 3 experiments
C. Vitamin D binding protein product inventory

Number	Protein.Name	T-1	T-2	T-3	T-4
1	beta 2 glycoprotein I precursor	795.0 → 1842.8	638.7 → 927.4	638.7 → 987.6	511.8 → 652.3
2	complement component 3 precursor	595.8 → 567.4	895.4 → 602.3	595.8 → 666.4	595.8 → 624.3
3	complement component 4 binding protein	569.6 → 1550.8	564.8 → 591.3	569.6 → 1083.6	625.3 → 644.4
4	complement component 4B preproprotein	828.4 → 869.5	828.4 → 956.5	771.4 → 1129.6	771.4 → 574.3
5	complement component 5	626.9 → 925.5	728.3 → 843.4	629.3 → 1739.8	626.9 → 1038.6
6	complement component 6 precursor	623.8 → 747.4	1005.5 → 811.5	621.0 → 538.3	1000.8 → 701.4
7	complement component 7 precursor	409.2 → 799.4	550.3 → 887.4	605.3 → 677.4	753.0 → 581.3
8	hemopexin	610.8 → 959.5	571.7 → 586.3	833.4 → 663.3	610.8 → 775.4
9	heparin cofactor II	514.8 → 814.4	465.7 → 712.4	540.3 → 893.5	410.3 → 706.4
10	histidine rich glycoprotein precursor	912.9 → 300.2	841.9 → 1058.4	841.9 → 1171.5	1008.5 → 1106.6
11	I factor complement	728.9 → 300.2	579.8 → 924.4	728.9 → 856.4	719.9 → 841.5
12	inter alpha globulin inhibitor H1	579.3 → 902.5	669.3 → 775.4	669.3 → 874.4	437.3 → 631.4
13	inter alpha globulin inhibitor H2	791.9 → 1341.7	669.4 → 686.4	514.3 → 797.5	423.7 → 570.3
14	inter alpha globulin inhibitor H4	467.3 → 720.4	619.3 → 894.5	464.8 → 702.3	572.8 → 663.4
15	PREDICTED similar to Apolipoprotein A I precursor	626.8 → 1025.5	626.8 → 1025.5	516.3 → 831.4	693.9 → 940.5
16	serine or cysteine proteinase inhibitor clade A	888.5 → 718.4	508.3 → 829.5	888.5 → 605.3	624.3 → 1134.5
17	serine or cysteine proteinase inhibitor clade C	695.4 → 1161.6	625.6 → 687.3	454.7 → 666.3	454.7 → 795.4
18	serine or cysteine proteinase inhibitor clade F	528.3 → 855.5	652.7 → 1771.9	489.3 → 656.4	780.4 → 1134.5
19	vitamin D binding protein precursor	903.1 → 1351.6	400.3 → 700.4	400.3 → 587.3	1259.5 → 926.4
20	vitronectin precursor	657.8 → 686.3	657.8 → 629.3	556.3 → 691.3	835.4 → 591.3

Table 1. Summary of 20 representative serum proteins and transitions derived from an LC-MS^E inventory of whole and depleted serum digests.
This is a subset of LC-MS^E identified proteins from whole and depleted human serum which were then monitored by MRM. The MRM experiments were configured to monitor at least 4 transitions per protein (designated T-1 through T-4), with many peptides having more than 1 transition monitored. The first number of each transition represents the precursor m/z, and the second number represents the fragment m/z.

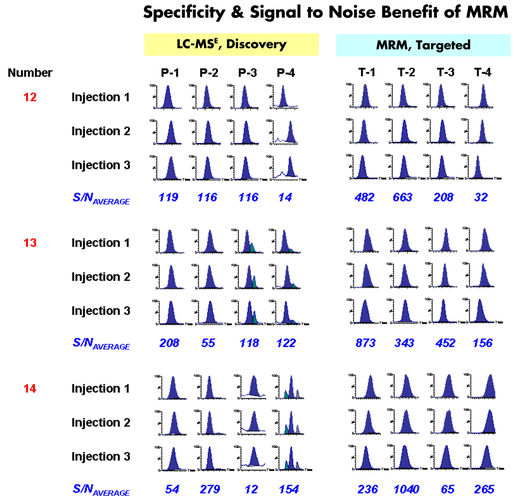

Figure 3. Signal to Noise for inter alpha globulin inhibitors from Whole Serum
Numbers 12-14 refer to the inter alpha globulins listed in Table 1. P-1 through P-4 are selected ion chromatograms, with 50 mDa tolerance, of the precursors from the low energy LC-MS^E data that were selected for each inter alpha globulin inhibitor, respectively. T-1 through T-4 represent the corresponding MRM traces from the targeted assay. These data were collected with dedicated collision energies, and illustrate the sensitivity benefit (S/N) of MRM even before collision energy optimization.

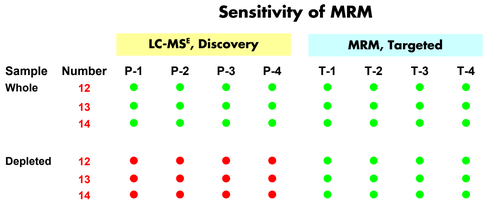

Figure 4. Detection Array for inter alpha globulin inhibitors.
Numbers 12-14 refer to the inter alpha globulins listed in Table 1. LC-MS^E precursor ion detections or MRM transition detections made in at least 2 out of 3 of the respective experiments are colored green, otherwise they are colored red. The sensitivity and specificity of the MRM method allowed detection of all 12 transitions from the depleted serum sample that were not detected by LC-MS^E.

CONCLUSIONS

- **LC-MS^E data is a comprehensive, empirical guide to the best MRM transitions for quantitative monitoring of targeted proteins.**
- **MRM provides additional sensitivity to take advantage of the specificity revealed by LC-MS^E.**
- **In controlled experiments, a 50x or greater improvement in detection limit was demonstrated in MRM acquisition mode relative to LC-MS^E acquisition mode.**

FUTURE WORK

- **Increase the panel of proteins within a single experiment.**
- **Optimize the LC-MS^E inventories through detailed evaluation of precursor and fragment ion energetics.**

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS

720001923EN