Extended Mass Range Triple Quadrupole for Routine Analysis of High Mass-to-charge Peptide Ions

Application Note

Targeted Proteomics

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Introduction

Routine, high-throughput protein quantitation by LC/Triple Quadrupole analysis uses peptides as surrogates for the corresponding proteins. A critical step to successful analysis is to select unique peptides and suitable MRM transitions for each targeted peptide. Peptides with high *m/z* precursor or product ions are often not selected for analysis due to instrument mass range limitations. However, to address biological questions these peptides may provide critical information or be the only analytical choice. Some examples where this may arise are membrane proteins with large hydrophobic transmembrane domains, proteins with extensive post-translational modifications, and endogenously produced peptides.

This Application Note demonstrates the performance of the Agilent 6495B Triple Quadrupole for peptides with high *m/z* product ions, showing the benefits of having a mass range up to *m/z* 3,000. Tryptically digested monoclonal antibody (mAb) standard and a HeLa cell membrane-enriched extract were used as simple and complex samples, respectively.

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Experimental Method

Protein/peptide selection

Using an Agilent 6550 Q-TOF Mass Spectrometer, LC/MS data were acquired in a data-dependent mode on anti-IL8 mAb digest and a HeLa membrane-enriched protein digest. Raw data were searched against the appropriate database using Agilent Spectrum Mill (Rev B.05.00.181 SP1) to identify peptides producing high *m/z* product ions for testing the performance of the Agilent 6495B Triple Quadrupole Mass Spectrometer.

LC/MS/MS system

An Agilent 1290 Infinity UHPLC system was interfaced to a 6495B Triple Quadrupole with a mass range of 3,000 u. The LC/MS experiments were performed using an Agilent Jet Stream (AJS) source, and an Agilent Poroshell 120 EC-C18, 2.1 × 100 mm 2.7 µm column (p/n 695775-902). For the mAb digest, a 3.5 minute MRM method was used and for the HeLa membrane digest, a 21 minute MRM method was used.

For glycopeptide quantification, the mAb digest was diluted in series, and the signal response for each injection level was plotted against the total mAb digest amount on-column.

For the HeLa membrane peptide quantification, the signal response for each product ion was plotted against the relative injection amount on-column.

Table 1. Agilent 6495 Triple Quadrupole MS method.

Parameter	Setting
Ion mode	AJS, Positive
Gas temperature	150 °C
Drying gas flow	15 L/min
Nebulizer gas	30 psi
Sheath gas temperature	200 °C
Sheath gas flow	11 L/min
Capillary voltage	3,500 V
Nozzle voltage	0 V
High/Low pressure RF voltage	200/110 V
Delta EMV	100–200 V
Q1 and Q3 resolution	Wide/Unit
Fragmentor	380 V
Cell accelerator voltage	4 V
Cycle time	267–369 ms

Table 2. Selected peptides and ions for LC/MS/MS test.

Protein name	Peptide	Modification	*m/z* Precursor	*m/z* MRM Quantifier	*m/z* MRM Qualifier
Anti-IL8 mAb antibody	EEQYN[+1606.6]STYR	N-glycan G1F	932.7	204.1	366.1 1,392.6 2,065.8 2,227.9
Sterol O-acyltransferase 1	SSTVPIPTVNQYLYFLFAPTLIYR	none	1,402.3	1,215.2	762.5 1,110.1 2,219.2 2,429.3
ATP Synthase subunit d, mitochondrial	NLIPFDQMTIEDLNEAFPETK	none	1,233.1	1,063.0	228.1 341.2 474.3 2,125.0
ADP/ATP Translocase 1	YFAGNLASGGAAGATSLC[+57]FVYPLDFAR	Carbamidomethylation	1,398.7	718.4	175.1 881.5 1,915.9 2,060.0

Results and Discussion

Peptide identification and selection

Anti-IL8 mAb digest and HeLa membrane digest were used for peptide identification and MRM method development with the following results:

One G1F N-glycopeptide from the mAb digest, and three peptides from the HeLa membrane digest with product ions ranging from *m/z* 204.1 to *m/z* 2,429.3 were selected for a LC/MS/MS test (Table 2).

Figure 1 and Figure 2 show the MS/MS spectrum and the optimized MRM chromatography for two of the peptides. The product ions selected for the MRM method are marked with * in the MS/MS plot.

Glycopeptides produce high *m/z* product ions, which have large glycan chains. These product ions are informative for interpreting glycan structure.

Product ions with higher *m/z* tend to have less background noise or higher signal-to-noise ratios in complex samples.

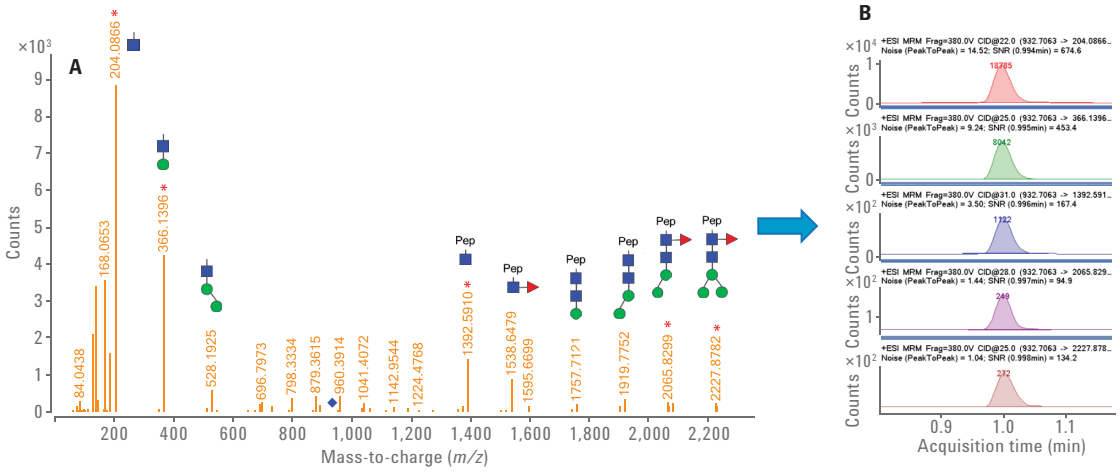


Figure 1. MS/MS spectrum and the optimized MRM chromatography for G1F glycopeptide EEQYN[+1606.6]STYR.

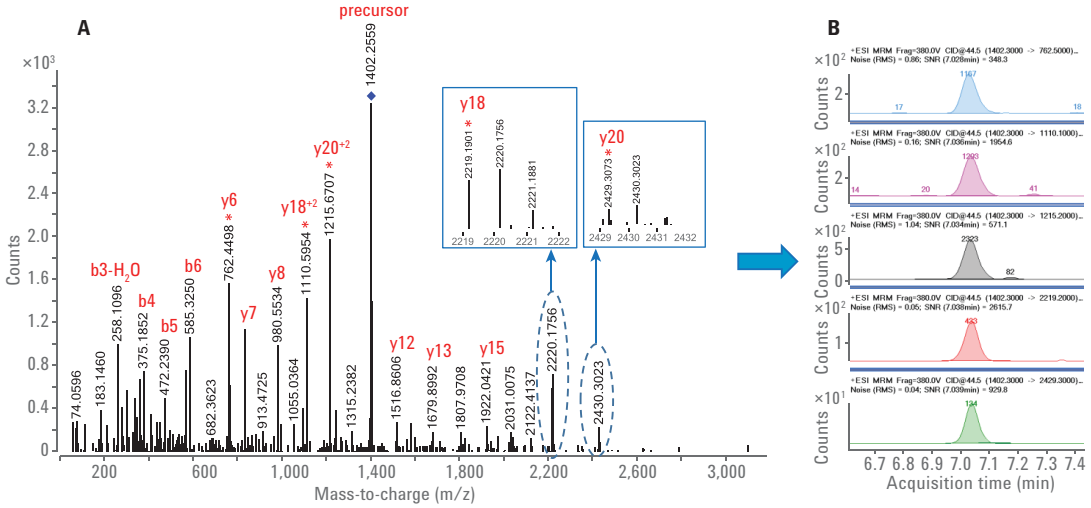
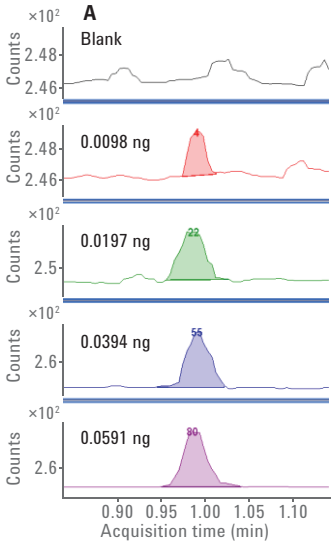


Figure 2. MS/MS spectrum and the optimized MRM chromatography for peptide SSTVPIPTVNQYLYFLFAPTLIYR.

Glycopeptide quantification

An 1290 Infinity UHPLC system coupled with Agilent Jet Stream Technology, and a 6495B Triple Quadrupole LC/MS system with a mass range of 3,000 u were used for glycopeptide quantitation with the following results (Figure 3):

- Excellent precision and accuracy at all levels including injection amount as low as 0.0197 ng (130 amol) of total mAb digest on-column (18.5 % RSD, 97.5 % accuracy). This target peptide is estimated to represent about 10 % of the total protein so LOQ for this glycopeptide is estimated at about 13 amol on-column
- Reproducible responses for product ions with extended mass range (*m/z* 204.1 ~ *m/z* 2,227.9)
- Excellent retention time (RT) reproducibility (RSD% = 0.47 % for n = 90)
- Excellent linearity at low injection levels with small fold changes (0.0197 ng ~ 19.7 ng, R^2 = 0.998)



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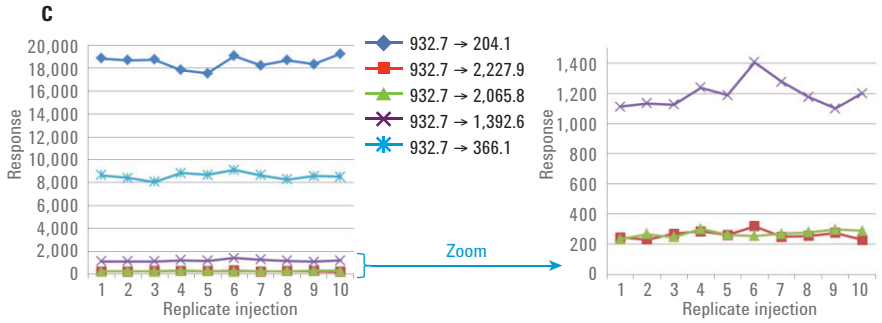
Amount of total mAb digest on-column (ng)	%RSD (n = 10)	% Accuracy
0.0197	18.5	97.5
0.0394	18.3	104.2
0.0591	12.5	108.3
0.0985	9.3	102.4
0.197	13.0	95.6
0.394	5.9	84.8
0.985	5.7	102.1
1.97	1.6	105.5
19.7	2.9	99.7



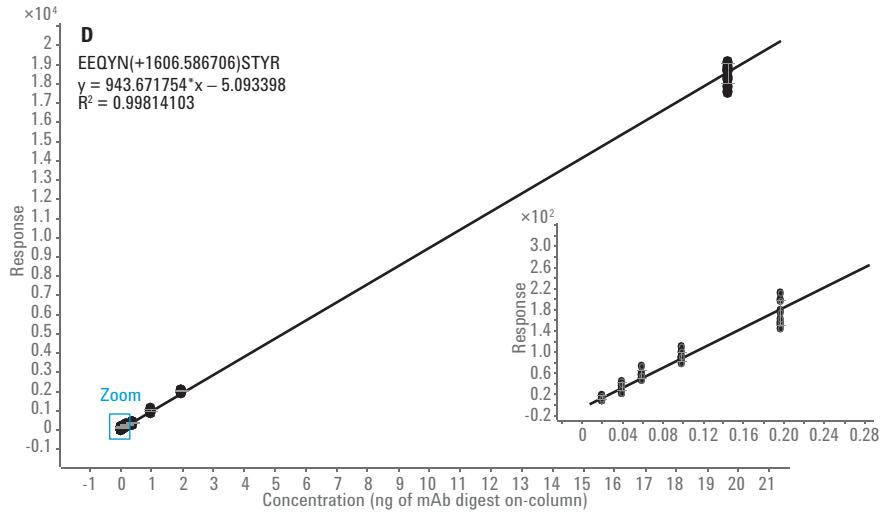


Figure 3. Quantification for G1F glycopeptide EEQYN[+1606.6]STYR.

HeLa membrane peptide quantification

Three peptides from the HeLa membrane-enriched sample digest were quantified following the same protocol as the glycopeptide to test high *m/z* product ion behavior (Figure 4).

- The quantifier response for each of these three peptides were plotted against the relative injection amount on-column (left plots) as the stoichiometry of these peptides were unknown in the sample.
- Good linearity was observed (R^2 ranges from 0.989 to 0.997).
- Reproducible responses for product ions with extended mass range (up to *m/z* 2429.3) were observed (right plots).

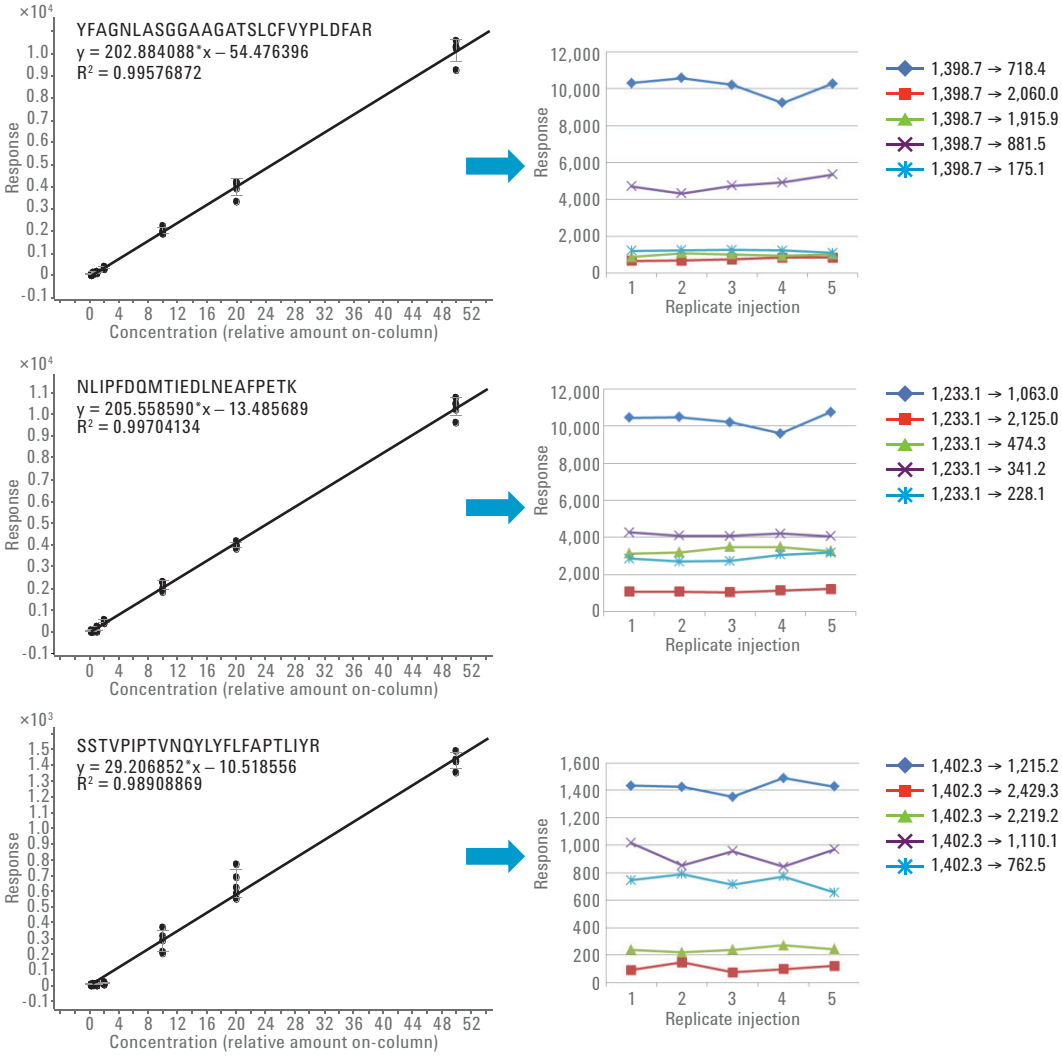


Figure 4. Quantification for peptides from a HeLa membrane digest.

Conclusions

The Agilent 6495B Triple Quadrupole LC/MS system has a mass range of 3,000 u, which is useful for detecting high *m/z* peptide ions. One benefit of using high *m/z* precursor or product ions, particularly in a complex digest, is these ions have less background noise compared to those with low *m/z*. Some high *m/z* product ions may provide important biological information such as location and size of post-translational modifications, for example glycosylation.

This application notes demonstrates the excellent reproducibility for MRM transitions with high *m/z* product ions achieved on the Agilent 6495B Triple Quadrupole LC/MS system. The work shown here uses Agilent Jet Stream Technology at standard flow rates and resulted in outstanding precision and accuracy for both glycopeptide and HeLa membrane peptide quantification.

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Published in the USA, March 16, 2018
5991-7298EN

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