

## LCQ Advantage Instrument Methods for Typical Proteomics Problems

**Problem 1: Detect whether the m/z of a certain peptide analyte is present and perform an MS/MS scan to verify its structure.**

Example: Angiotensin II (DRVHHPF). Monoisotopic mass: 1045.52

If it is doubly charged,  $m/z = (1045.52 + 2) / 2 = 523.8$  m/z

Scan 1: Full scan, with a low mass range. (The narrower the mass range, the faster the scan time.)

Mass range: 500-550 m/z

Positive polarity, centroid

Scan 2: Full, MS/MS

Set up the following under “parent masses”:

Parent mass: 523.8 m/z

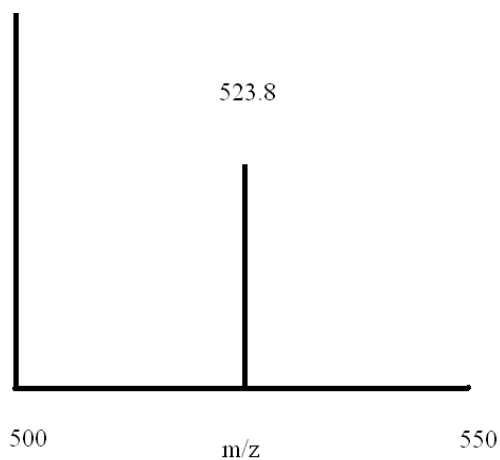
Isolation width: 3.0 m/z

Normalized collision energy (CE): 35%

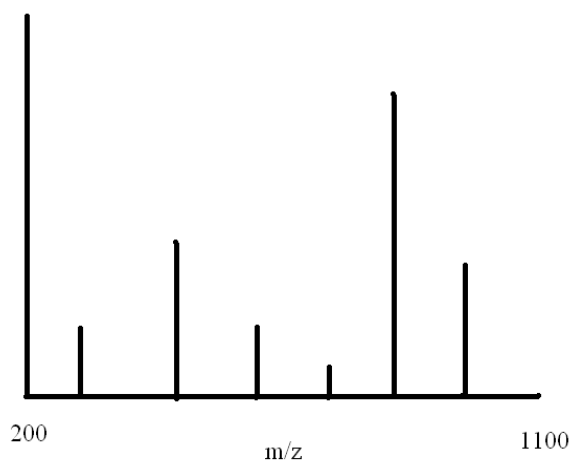
Positive polarity, centroid

SRM range: 200-1100 m/z (This depends on the MS/MS spectrum of the particular analyte. Since the mass of the peptide is 1045.52, no product ions can exceed that mass so I set the top of the mass range to 1100 m/z.)

Hypothetical output:



Full scan



MS/MS scan

Method: test1\_080403.meth

**Problem 2: From a complex peptide mixture, perform sequential MS/MS scans on the top three most intense ions of a full scan.**

This is a typical proteomics experiment in which an attempt is made to identify as many peptides as possible from the digest of a protein or protein mixture. Here, dynamic exclusion may be performed so that after an ion is sequenced several times it will be put onto an exclusion list so that less intense ions may be sequenced.

Scan 1: Full scan (MS), 400-1800 m/z, positive, centroid

400-1800 m/z is a typical scan range for peptides. If the scan range begins at a value less than 400 m/z, solvent and noise ions will often be more intense than the peptide ions of interest. It is rare for peptides of greater than 1800 m/z to be successfully sequenced and identified.

Scan 2: MS/MS of most intense ion of scan 1

Scan 3: MS/MS of 2<sup>nd</sup> most intense ion of scan 1

Scan 4: MS/MS of 3<sup>rd</sup> most intense ion of scan 1

For scans 2-4 set the following:

On the main page: MS/MS, full, positive, centroid.

Set each scan event to “dependent scan” and then click “advanced” to set the following:

Global/Dynamic Exclusion:

Repeat count: 3 Repeat duration 0.5 minute

Exclusion list: 50 Exclusion duration: 1.0 minute

Exclusion mass width: 1.5 m/z

(The duration times should be set depending on the peak width and the scan rate of the instrument. The above values are assuming a peak width of 2.0 minutes.)

Segment/Current Segment/Advanced:

Normalized CE: 35%

Default charge state: 2

Min. MS signal count  $0.5 \times 10^4$

Isolation width: 3.0 m/z

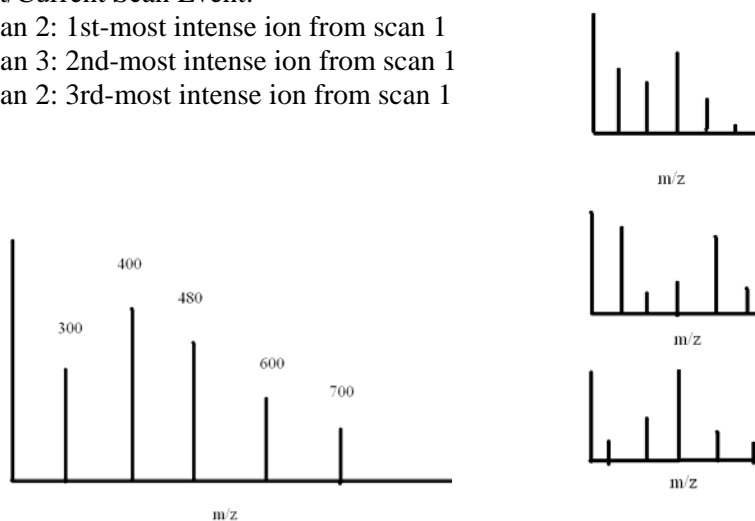
(A CE of 35% is typical for a peptide experiment. The minimum MS signal count (signal intensity required to trigger a MS/MS scan) should be determined from experimental results so that the maximum results can be achieved. It is very important to use an isolation width of about 3.0 m/z, since with a lower value (the default is 1.0 m/z) there is a great loss in sensitivity.)

Scan Event/Current Scan Event:

Scan 2: 1st-most intense ion from scan 1

Scan 3: 2nd-most intense ion from scan 1

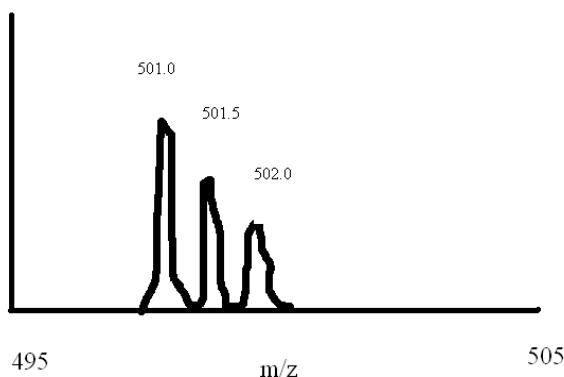
Scan 4: 3rd-most intense ion from scan 1



Method: test2\_080403.meth

**Problem 3: Use Zoom Scan to determine the charge state of peptides, followed by MS/MS scan to identify their sequence.**

Imagine a hypothetical peptide with a monoisotopic mass of 1000 amu. When it is doubly charged, the addition of two positively-charged protons also adds a mass of 2 amu, so the  $m/z$  of this will be:  $(1000 + 2)/2 = 501.0$   $m/z$ . For that portion of the peptides with one carbon-13, the  $m/z$  will be:  $(1001+2)/2 = 501.5$   $m/z$ . With two carbon-13's on the peptide, the  $m/z$  will be:  $(1002 + 2)/2 = 502.0$   $m/z$ . This example illustrates that when the peptide is doubly-charged, if you “zoom in” on the ion there will be a mass envelope of ions separated by 0.5  $m/z$ :



Zoom scan of mass envelope

Scan 1: Full scan, 400-1800  $m/z$ , positive, centroid

Scan 2: Zoom scan of most intense ion of scan 1, positive, profile (zoom scan must be profile)

Scan 3: MS/MS scan of most intense ion of scan 1, MS/MS, full, positive, centroid

Select “dependent scan” for scans 2 and 3 and enter as follows under “Advanced”:

Global/Dynamic Exclusion: enter as in Problem 2 (if desired)

Segment/Current Segment/Advanced: as in problem 2

Scan event/current scan event:

Scan 2: 1<sup>st</sup>-most intense ion from Scan 1

Scan 3: 1<sup>st</sup> most intense ion from scan 1

The LCQ advantage does not seem to be able to do triple-play except with the most intense ion from scan 1, so dynamic exclusion may be the only way to perform data-dependent analysis on multiple ions eluting in the same peak.

Also, with some instruments you can set the size of the mass range around the parent ion in the zoom scan, but the LCQ Advantage automatically sets this to 10 amu.

Method: test3\_080403.meth