Quantification of Peptides with Poor MS/MS Fragmentation using Novel Jet Injector SelexION® + MIM Workflow

Enhanced signal intensities with novel differential mobility cell design

Key Challenges of Peptide Quantification

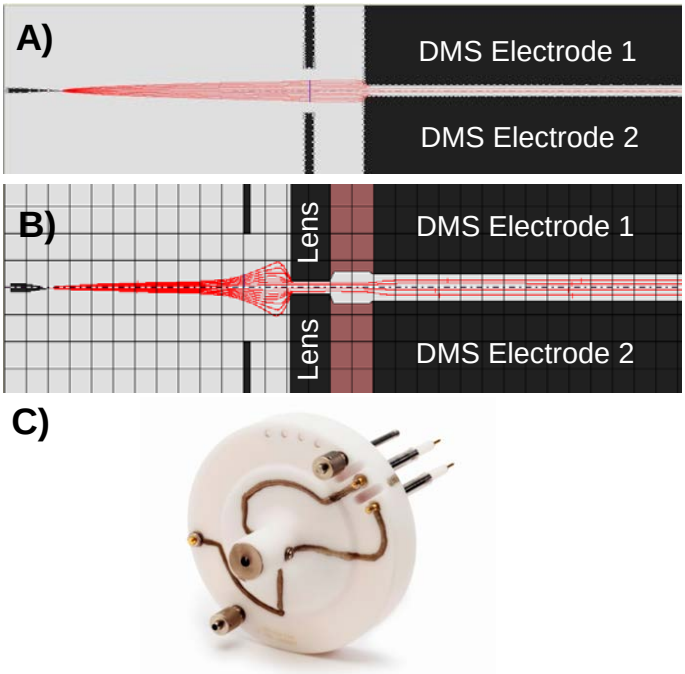
- **Poor MS/MS fragmentation**-Cyclic peptides or peptides with disulphide bond have either poor fragmentation or too much fragmentation
- **Lack of sensitivity**-Regular MRM which relies on daughter ion fragment has challenges in achieving highly sensitive assay specially in complex biological matrices
- **Selectivity challenges**-Parent ion to parent ion quantification, otherwise called Multiple Ion Monitoring (MIM) is the best choice but will encounter very high noise background and matrix interference

Key Benefits of SelexION®+ Multiple Ion Monitoring workflows

- **Addressing isobaric interferences** from complex matrix – SelexION+ reduces matrix interference and background noise originating from isobaric compounds present in complex plasma matrix.
- **Enhanced Selectivity & Sensitivity**- With new cell design, SelexION+ provides enhanced sensitivity and specificity without any compromise.
- **Improving Signal-to-Noise Ratio**– Reduces high background noise in MRM or MIM in complex matrices such as plasma or tissues.
- **Ensuring better Data quality**- SelexION+ improves precision and accuracy statistics at lowest level of quantitation.

Key Features of New Jet Injector SelexION®+ Technology

- **Novel Cell Design**- Addition of lens increases ion velocities into cell, reducing transit times through detrimental fringing fields.

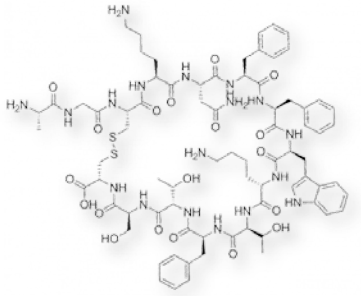


New SelexION®+ Jet Injector: A) Schematic showing old cell design and ion transmission through 2 electrodes in DMS cell B) Novel jet injector cell design with 2 additional lens increases ion velocities into the cell and reduces transit times through detrimental infringing fields C) New SelexION cell showing design changes compared to old cell. Approximate dimensions are cited (not drawn to scale).

- **Increased Ion Transmission Efficiency** – Potential interferences originating from isobaric compounds present in complex plasma matrix.
- **Shorter Ion Residence Time–** High background noise in MRM mode in processed plasma sample.
- **Robustness**- In certain circumstances, proven to meet guidelines for inter day and intraday precision and accuracy statistics.

Introduction

Differential Mobility Spectrometry[1,2] (DMS) can be used as an orthogonal separation method coupled to a mass spectrometer. Separation occurs based on the chemical properties of the analyte and can be used to separate isobaric and isomeric compounds. MRM (multiple reaction monitoring) is widely used for the quantitation of peptides. However, frequently one encounters a peptide that is not readily amenable to MRM analysis. Poor fragmentation or fragmentation that produces common low m/z ions can result in high background and a poor lower limit of detection (LOD). Peptides that don't fragment well include cyclic peptides. Low fragmentation efficiency typically translates to poor sensitivity. An alternative to MRM is that has the potential for higher signal is SRM (Selected Ion Monitoring). However, the increased in signal response tracks with the increase in the noise thus giving poor selectivity. Here we use MIM (Multiple Ion Monitoring) coupled to SelexION®+ to achieve low level selective quantitation of Somatostatin (Figure 1).



C76H104N18O19S2

1636.7Da

+2 charge- m/z 819.7

Figure 1 Somatostatin structure

Typically, the preferred MS method for quantitation is MRM; however, as can be seen in Figure 2, Somatostatin generates many fragments. While the number of fragments observed makes picking the best daughter difficult the main issue is lack of sensitivity due to the detector counts being spread across so many fragments. In complex fragmentation situations a SIM (Selected Ion Monitor) or a MIM can be used if the background isn't significant. However, this is rarely the case when running samples in biological matrices. While the selectivity of the assay can be increased approximately 2-fold by using MIM (vs SIM) the background still makes quantitation difficult (Figure 3). In this assay, the addition of SelexION®+ makes the use of MIM an effective methodology for quantitation by dramatically dropping the background by 10-fold (Figure 4).

Using the QTRAP® 6500+ system with the SelexION®+ we are able to achieve increases in sensitivity and lowered limits of quantitation for the detection down to 50 pg/mL in plasma (Figure 5).

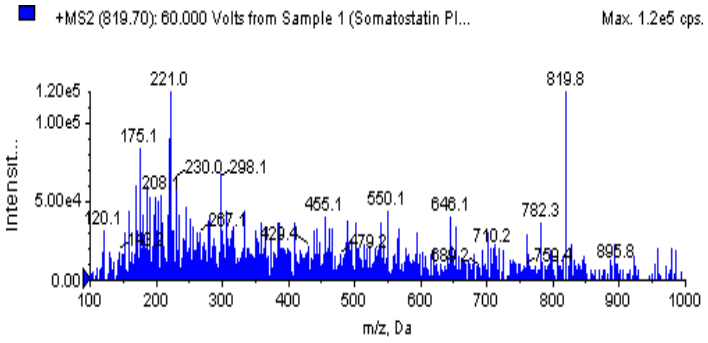


Figure 2 MS^2 spectrum of Somatostatin displays poor fragmentation
Poor fragmentation of Somatostatin.

Blank Plasma-*Without* SelexION®+ in MIM Mode

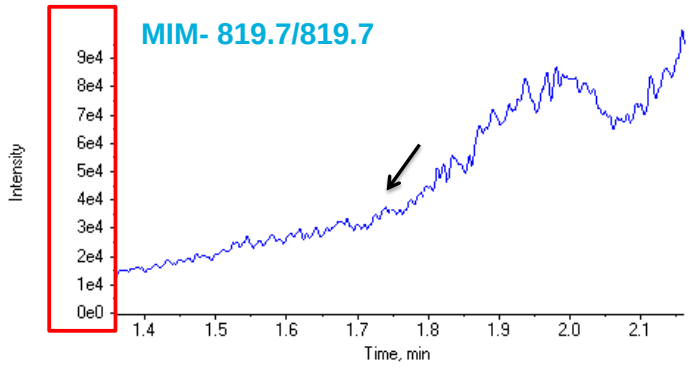


Figure 3 High background in plasma blank without SelexION$^+$

Blank Plasma-*With* SelexION®+ in MIM Mode

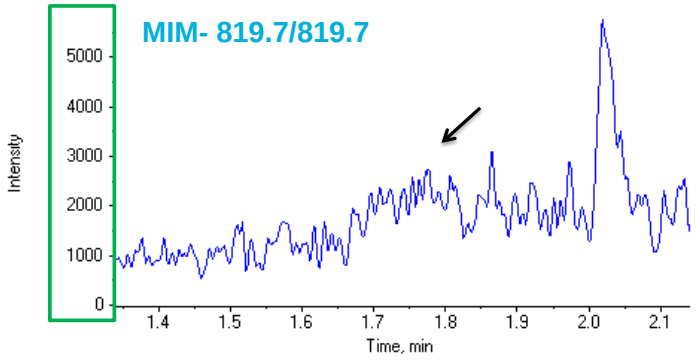


Figure 4 Dramatic decrease in background observed.

50 pg/mL LOQ in Rat Plasma-Somatostatin DMS MIM

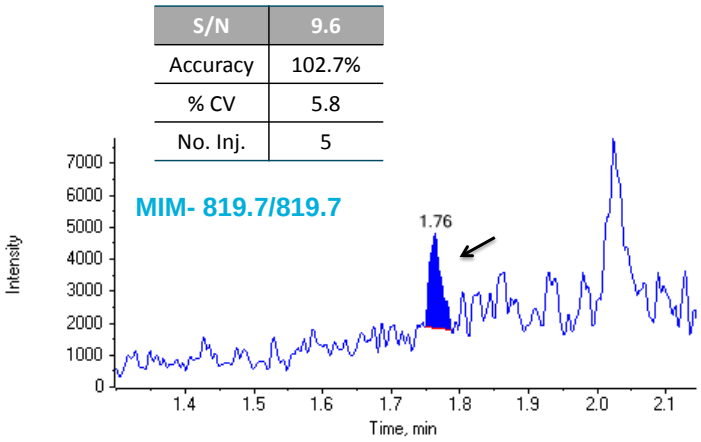
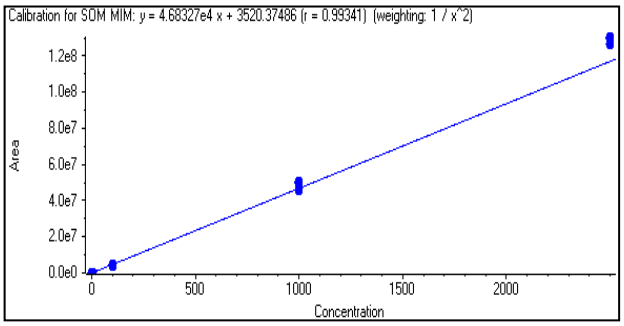


Figure 5 At low level quantitation without SelexION®+ the high background presents a serious challenge. With the SelexION®+ a **0.0**5 ng/mL sample can be quantitated.

Somatostatin SelexION®+ MIM Assay in Rat Plasma



Actual Conc.	Percent CV	Accuracy
0.05	5.83	102.77
0.1	9.39	96.64
0.5	3.93	88.21
5	3.75	109.87
100	6.41	88.95
1000	4.70	103.29
2500	1.17	110.27

Figure 6 Calibration Curve: 0.05 ng/mL-2500 ng/mL;**(**Top)

Conclusions

Quantitation of cyclic peptides or peptides with disulphide bonds can be difficult due to poor fragmentation.

- SelexION®+ (Differential Mobility Spectrometry) provides an orthogonal level of selectivity by separating components based on their chemical properties and mobility.
- The increased ion transmission of the new SelexION®+ cell increases sensitivity while maintaining selectivity.
- The use of SelexION®+ as an orthogonal separation method allows isobaric interference to be reduced so that a MIM can be used for high background assays.
- SelexION®+ improves Signal-to-Noise (S/N) in certain biological assays which improve precision and accuracy even at the lowest levels of quantitation.

References

1. SCIEX SelexION Technology: A New Solution to Selectivity Challenges in Quantitative Bioanalysis – Differential Mobility Separations Enhanced with Chemical Modifiers: A New Dimension in Selectivity. SCIEX Technical Note, Publication 296011-01

2. Schneider et al, (2010) Planar Differential Mobility Spectrometer as a Pre-Filter for Atmospheric Pressure Ionization mass Spectrometry, Int. *J. Mass Spectrometry*, 298 (1-3), 45-54.

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