Interfacing an Ion Funnel Trap (IFT) to a Triple Quadrupole Analyzer for Enhancing Sensitivity in Multiple Reaction Monitoring (MRM) Analyses

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Overview

- Inclusion of an ion funnel trap (IFT) with MRM platforms for improved signal to noise (S/N)
- Increased ion sampling from the ESI source by avoiding “dead times” between transition
- Reduction in chemical noise during ion storage in the IFT

Introduction

Multiple Reaction Monitoring (MRM) offers a sensitive analytical platform to quantify trace constituents in complex biological matrices by selectively delivering analyte ions from the ESI source to the MS detector over ions formed from interferences. This is achieved in multiple stages by using quadrupoles that are tuned to transmit analyte ions over a narrow *m/z* range. Although the S/N is superior with LC-MRM analysis, the approach limits sampling of analyte ions from the source, which translates to limited sensitivity.

Electrodynamic ion funnel[1] and S-lens[2] have been employed to improve delivery of ions from the source to the detector, and delivery can be further improved by using an IFT. During MRM analysis, the continuous flow of ions from the ESI source to the detector is interrupted for short periods owing to re-setting of quadrupole conditions to cycle through multiple transitions. Accumulating ions during this period followed by rapid releases into the quadrupoles can improve sensitivity.

An additional benefit of using an ion funnel trap is anticipated from the conversion of a continuous ion beam into multiple packets of ions where each packet translates into a greater charge density over the continuous ion beam.[3] Signal integration from multiple ion packets can result in lower chemical noise and enhanced analyte signal.

The capillary ESI-MS interface (TSQ, Thermo Scientific) was replaced with an IFT to evaluate improvements in S/N in MRM mode of operation.

Methods

Chemicals and Materials: Lyophilized Kemptide, Angiotensin I, Syntide 2, Bradykinin, Leucine and Enkephalin, Dynorphin A Porcine 1-13, Neurotensin, and Fibrinopeptide A were purchased from Sigma-Aldrich (St. Louis, MO). These were serially diluted to prepare concentrations ranging from 0.25 nM to 500 nM in 0.25 mg/mL of tryptic digest of *Shewanella oneidensis* MR-1 proteins.

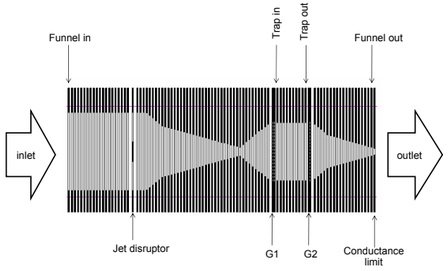

Figure 1. A 2D cross section of a custom built ion funnel trap assembly.

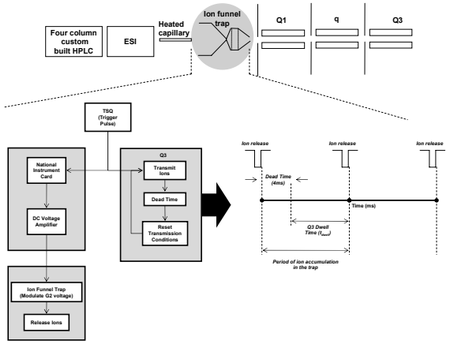

Figure 2. The IFT is situated between the heated capillary and Q1. Ions are accumulated in the trap during dead time and dwell time followed by a 0.5 ms release event. Ion release from the trap is coordinated with the start of Q3 scan through a common 5.0 V pulse that is sourced from Q3.

Results

ESI-IFT-MRM results including MS features owing to IFT

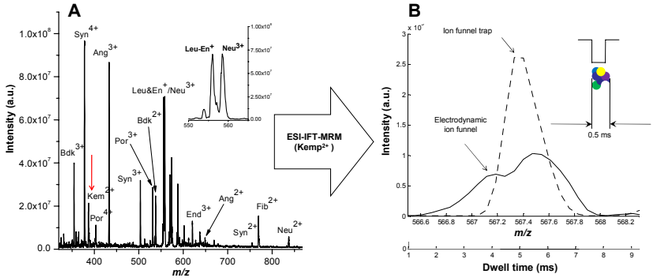

Figure 3. A) A 1 µM eight peptide mix was directly infused into the TSQ where Q1 was scanned from *m/z* = 300 to 900. Analysis conditions included ESI = 2000V and flow rate = 0.3 µL/min. Kemptide (2+, *m/z* = 386.74) was isolated using Q1 (±0.5 Da), fragmented in the collision cell (1.5 mTorr and CE = 25 eV), and the fragments (409.27, 539.34, and 567.33) were monitored using Q3. **B)** MS spectrum of a single transition of Kemptide (*m/z* = 567.33) from ESI-MRM analysis. Spectrum from ESI-IFT-MRM is reduced in spatial spread when displayed as a function of dwell time (ms) and greater in amplitude then the spectrum acquired without trap function.

Characterizing ESI-IFT-MRM for optimum ion accumulation amidst interferences from BSA digest

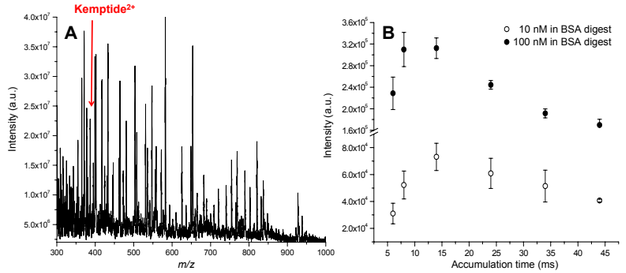

Figure 4. A) MS spectrum acquired from direct infusion of 100 nM of eight peptide mixture spiked into ~160 nM BSA digest. Kemptide (2+, *m/z* = 386.74) was isolated (Q1) for MRM analysis where accumulation times were varied from 8 ms to 44 ms. **B)** Optimum accumulation time was near 14 ms for 10 nM and 100 nM peptide concentration in BSA digest.

A list of transitions and collision energy (CE) of the eight target peptides monitored during LC-IFT-MRM

Parent Ions	Ion	Trans.	Frag.	Trans.	Frag.	Trans.	Frag.	CE
Kemptide	386.74 (2+)	409.27	b_2-NH_3	539.34	a_5-NH_3	567.33	b_5-NH_3	25
Angiotensin I	432.90 (3+)	534.27	b_4	619.36	a_5	647.35	b_5	21
Syntide 2	503.32 (3+)	283.18	b_3	429.28	y_4	705.94	y_{14}^{2+}	23
Bradykinin	530.79 (2+)	522.27	y_9^{2+}-NH_3	710.36	y_9	807.42	y_7	30
Dynorphin A Porcine 1-13	535.34 (3+)	455.21	y_{11}^{3+} - NH_3	529.70	y_{13}^{3+}-NH_3	712.68	y_{12}^{3+}-NH3	28
Leucine Enkephalin	556.28 (+)	278.11	b_3	397.19	a_4	425.18	b_4	22
Neurotensin	558.31 (3+)	578.85	y_9^{2+}	643.73	y_{10}^{2+}	725.90	y_{11}^{2+}	26
Fibrinopeptide A	768.85 (2+)	445.25	y_8	645.33	y_7	1077.5 3	y_{11}	29

Enhanced S/N in LC-MRM analysis with the inclusion of IFT

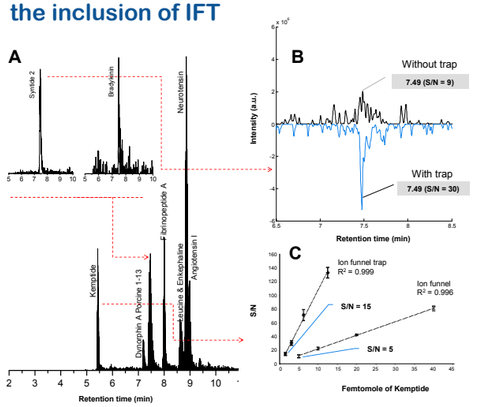

Figure 5. A) Chromatographic trace from LC-IFT-MRM of 100 nM of eight peptides spiked into 0.25 mg/mL tryptic digest of *Shewanella oneidensis* MR-1 proteins where separation was achieved on a 15 cm x 75-µm i.d fused-silica capillary in 30 min. **B)** Selected ion chromatogram of Syntide from LC-IF-MRM (black trace) and LC-IFT-MRM (green trace) analysis of 8 nM peptide spike where the latter shows ~3X enhanced S/N. Comparable improvement in S/N was observed for Kemptide which translated in 2X improvement in level of detection (Figure 5C) over the electrodynamic ion funnel with excellent linearity between 0.5 nM to 40 nM.

Conclusions

- An IFT can be synchronized with Q3 to deliver ion packets prior to a transition event with high duty cycle
- Ion accumulation between transitions add to ion signal when compared to electrodynamic ion funnel or standard interface
- Ion storage in the IFT results in reduced chemical noise and yields chromatographic trace superior in S/N
- Lower level of detection is attained with IFT with improved linearity between signal versus peptide concentration compared to standard or electrodynamic ion funnel

Acknowledgements

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References

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