

Pulsed Multiple Reaction Monitoring Mode: The Novel Sensitive Approach for Biomolecule Quantitation

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Overview

This work reports on the improved limit of detection of a liquid chromatography (LC)-triple quadrupole instrument operating in the multiple reaction monitoring mode (MRM) by incorporation of an ion funnel trap (IFT) between an ion source and a quadrupole analyzer.

Introduction

Multiple Reaction Monitoring (MRM) offers a highly sensitive analytical platform to quantify trace constituents in complex biological matrices by selectively delivering analyte ions from an ESI source to an MS detector. Further sensitivity improvements with LC-MRM approach are achievable by enabling higher efficiency transport of analyte components from an ion source to the MS analyzer, eliminating dead times in analyses of fragment ions and reducing background ion signals. Electrodynamic ion funnel (IF) or the derivative S-lens² have been shown to drastically improve MS sensitivity. A recently introduced ion funnel trap (IFT) has been demonstrated to further enhance the limit of detection for both the time-of-flight³ and ion mobility time-of-flight mass spectrometers⁴.

The premise for sensitivity improvement with the IFT coupled to a triple quadrupole instrument is due to: i) ion accumulation in the RF-energized trap, which facilitates improved droplet desolvation manifested in the reduced background ion noise at the detector, ii) enhancement in signal amplitude for a given transition because of an order-of-magnitude increase in the ion charge density per unit time compared to the continuous mode of operation, and iii) the unity duty cycle in signal detection, as the use of the trap eliminates dead times between transitions, which are inevitable with continuous ion streams.

This work reports on implementation of LC-IFT-MRM analysis of trace constituents from a complex biological matrix using a commercial triple quadrupole instrument (TSQ, Thermo Fisher Scientific).

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.

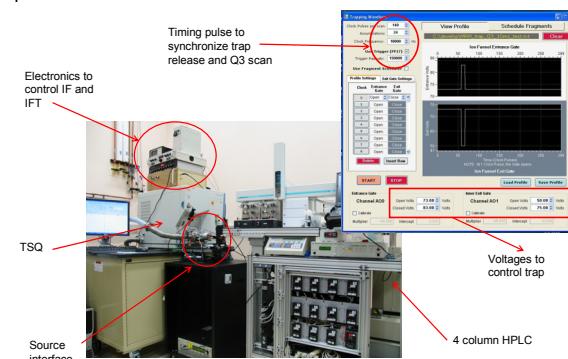


Figure 1. Experimental setup and graphical interface of instrument control software for an ion funnel trap

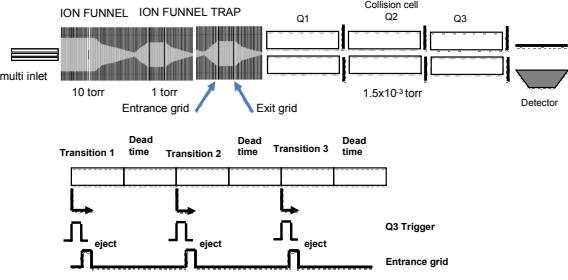


Figure 2. Pulsing sequence of experiment with the ion funnel trap. Ions are accumulated in the trap during dead time and dwell time followed by a 0.5 ms release event. Ion release event is synchronized with the start of Q3 scan.

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Results

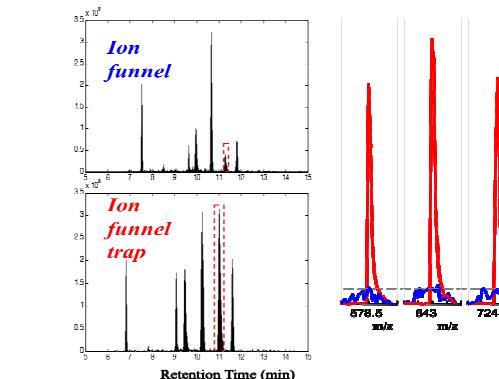


Figure 3. LC-MS/MS experiment with a 0.25 mg/mL *Shewanella oneidensis* digest spiked with nine peptides (see table 1). Inset shows three transitions of neurotensin acquired in continuous (blue) and trapping (red) modes

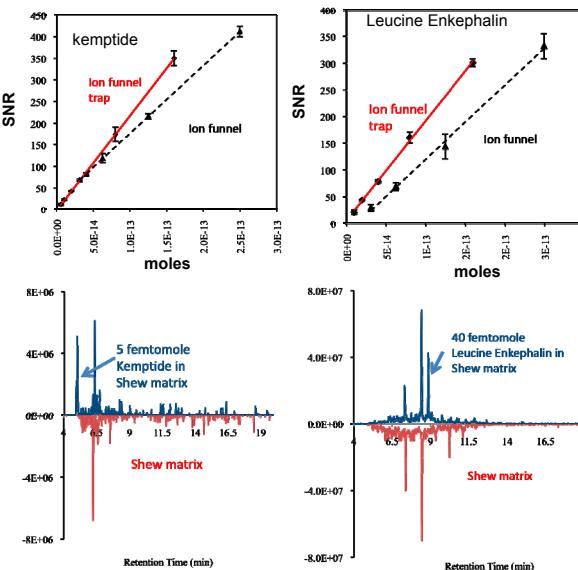


Figure 4. Signal-to-Noise (SNR) of kemptide and leucine enkephalin as a function of the peptide amount added to a 0.25 mg/mL *Shewanella oneidensis* digest. Bottom panels show selected ion chromatograms representing three transitions of the two peptides and the matrix signal in the corresponding m/z ranges.

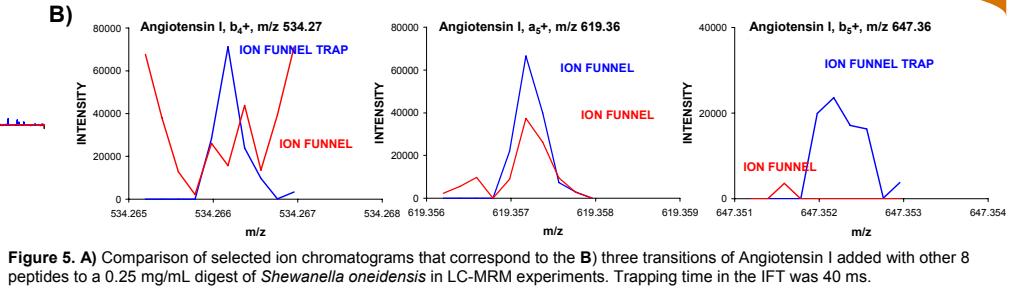


Figure 5. A) Comparison of selected ion chromatograms that correspond to the B) three transitions of Angiotensin I added with other 8 peptides to a 0.25 mg/mL digest of *Shewanella oneidensis* in LC-MRM experiments. Trapping time in the IFT was 40 ms.

Table 1. List of peptides and the corresponding fragments monitored in LC-MRM experiments with a 0.25 mg/mL *Shewanella oneidensis* digest

Parent Ions	Ion	Trans.	Frag.	Trans.	Frag.	Trans.	Frag.	CE
Kemptide	386.74 (2+)	409.27	$b_2\text{-NH}_3$	539.34	$a_5\text{-NH}_3$	567.33	$b_2\text{-NH}_3$	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	$y_9^{2+}\text{-NH}_3$	710.36	Y_6	807.42	Y_7	30
Dynorphin A Porcine 1-13	535.34 (3+)	455.21	$y_{11}^{2+}\text{-NH}_3$	529.70	$Y_{13}^{2+}\text{-NH}_3$	712.68	$Y_{12}^{2+}\text{-NH}_3$	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_5	645.33	Y_7	1077.53	Y_{11}	29

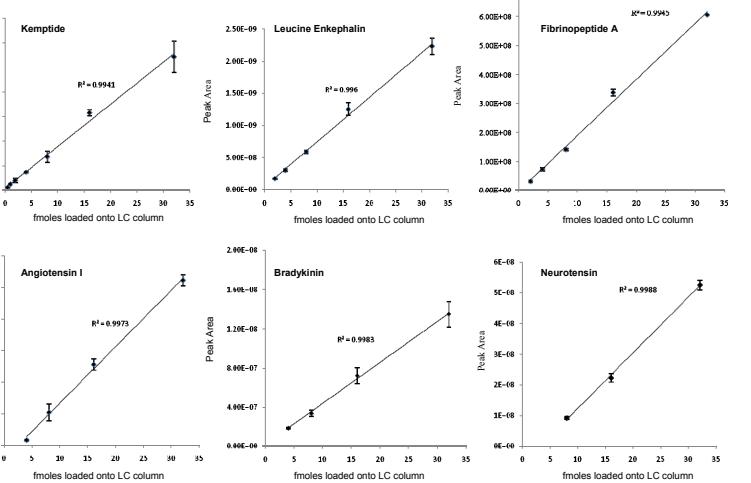


Figure 6. LC-IFT-MRM peak areas as a function of the peptide amount for several peptides added to a 0.25 mg/mL *Shewanella oneidensis* digest.

Conclusions

- Incorporation of an ion funnel trap (IFT) into a triple quadrupole analyzer operating in the multiple reaction monitoring mode (MRM) resulted in 3 to 10 fold improvement in the limit of detection (LOD) as compared to the ion funnel interface and 20 to 50 fold LOD improvement in comparison to that of the commercial instrument.
- Rigorous studies of signal intensities of peptides added to a highly complex biological matrix at concentrations ranging from 0.5 nM to 1000 nM demonstrated a linear response of the LC-IFT-MRM instrument with respect to the concentrations of low abundance peptides.

Acknowledgements

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