Methods

Chemicals and Materials: Lyophilized Kemptide, Argangistran I, Synthet 2, Bradykinin, Leucine and Enkephalin, Dynorphin A Peron 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 Haliotis oenoides MR-1 protease.

Results

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

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

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

Conclusions

• An IFT can be synchronized with Q3 to deliver ion packets at 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

Throughout this work, Pacific Northwest National Laboratory (PNNL) researchers utilized National Research Resources (RR18522) and the U.S. Department of Energy (DOE/BER) national scientific user facility at Pacific Northwest National Laboratory operated by Battelle under contract DE-AC05-76RL01830.

References

1. Belov ME, Sandhu MM, Upholt HK, Arakelian GA, Tolstikhin AV, Prasad SD, Harkewicz R, Smith RD. 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 = 368.74) was isolated using Q1 (0.5 ms). Fragmented in the collision cell (1.5 ms from MS; CE = 25 eV), and the fragments (409.27, 539.34, and 567.33) were monitored using Q3. MS/MS spectrum of a single transition of Kemptide (m/z = 567.33) from ESI-IFT-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 than the spectrum acquired without trap function.

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 0.5 V pulse that is sourced from Q3.

Figure 3. 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 = 368.74) was isolated using Q1 (0.5 ms). Fragmented in the collision cell (1.5 ms from MS; CE = 25 eV), and the fragments (409.27, 539.34, and 567.33) were monitored using Q3. MS/MS spectrum of a single transition of Kemptide (m/z = 567.33) from ESI-IFT-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 than the spectrum acquired without trap function.

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 = 368.74) 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.

Figure 5. A) Chromatographic trace from LC-IFT-MRM of 100 nM of eight peptides spiked into 0.25 mg/mL tryptic digest of Haliotis oenoides MR-1 protease where separation was achieved on a 15 cm x 7.5 mm, 5 µm, fused silica capillary in 30 min. B) Selected ion chromatogram of Synthet from LC-IFT-MRM (black trace) and LC-IFT-MRM (green trace) analysis of 5 µM peptide spike where the latter shows ~5X enhanced S/N. Comparable improvement in S/N was observed for Kemptide which translates to 5X improvement in level of detection (Figure 5C) over the electrodynamic ion funnel with excellent linearity between 0.5 nM to 40 nM.

CONCLUSION: Satendra Prasad, Ph.D. Biological Sciences Division 98 Pacific Northwest National Laboratory P.O. Box 999 Richland, WA 99352 E-mail: satendra.prasad@pnl.gov