In-Funnel Orthogonal Ion Injection for Highly Sensitive Pulsed SRM Analysis of Biological Samples

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Overview

- **Ion funnels are well suited for transferring ions from an atmospheric pressure ion source into a mass spectrometer.**
- **However, ion funnels utilizing an inline ion injection method where the inlet capillary is positioned directly inline with the ion funnel axis can lead to contamination of mass spectrometer elements.**
- **Here we describe the implementation of a new ion injection method incorporating a dual ion funnel that considerably reduced the contamination of the ion funnel elements as well as down stream mass spectrometer components and its use in pulsed-SRM experiments.**
- **The pulsed SRM approach with this source is shown to improve the signal intensity by factor of 4-8 compared to the single ion funnel configuration.**

Introduction

The success of selected reaction monitoring (SRM), a widely used method in proteomics and metabolomics applications, is highly dependent on the sensitivity of the instrument. We recently introduced a pulsed-SRM method that significantly improves the sensitivity of current instrumentation.

In this study, we combined a novel in-funnel orthogonal ion injection method with pulsed-SRM method to further improve the sensitivity and reliability of the technique. The inlet capillary is partially inserted into the high pressure ion funnel through an opening on the side of the ion funnel, and a multicapillary or large bore capillary inlet affords efficient ion introduction into the ion funnel. We utilized the tandem ion funnel design with a low pressure ion funnel trap (IFT) for pulsed-SRM experiments [1].

Methods

Samples were prepared in water : methanol : acetic acid (49.5 : 49.5 : 1% by volume) solution. A nine peptide mixture with concentrations ranging from 0.1 nM - 10 nM was used for limit of detection (LOD) study. Agilent tune mix was used for comparing different instrument configurations. Both inline and orthogonal injection experiments were carried out at a flow rate of 300 nL/min.

A nine peptide mixture at 1 µM concentration was used for comparing pulsed-SRM mode and conventional SRM mode. Also, the nine peptide mixture was spiked into a 0.25 mg/mL tryptic digest of *Shewanella oneidensis* strain MR-1 protein at 0.1 nM – 10 nM concentrations.

Instrumentation

- Experiments comparing inline and orthogonal injection methods and experiments to evaluate the performance of the orthogonal ion injection method were carried out using an Agilent TOF instrument.
- SRM experiments were performed using a TSQ Quantum Ultra (Thermo Fisher Scientific) mass analyzer.
- Both instruments were modified with a tandem funnel source that can be converted between inline and orthogonal injection configurations. The high pressure funnel was operated at 9 Torr and the low pressure funnel was operated at 1 Torr.
- For pulsed-SRM experiments, the ion funnel trap exit gate pulse was synchronized with the third quadrupole (Q3) scan. Reversed-phase LC separations were carried out using an Eksigent constant flow HPLC system.

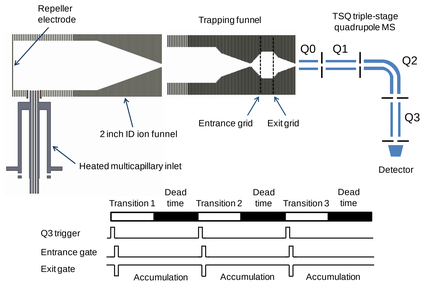

Figure 1. Schematic diagram of the TSQ instrument with orthogonal ion injection funnel and trapping funnel, and the instrument triggering sequence for pulsed-SRM experiments.

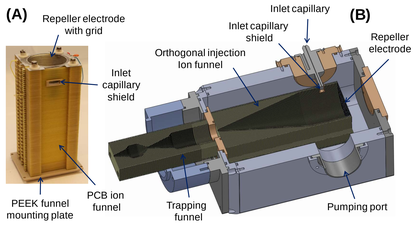

Figure 2. Printed circuit board (PCB) orthogonal injection ion funnel (A) and 3D cross section drawing of the tandem funnel design (B). A 750 µm I.D. inlet capillary was used for pulsed-SRM experiments.

Results

- Orthogonal injection produced signals comparable or slightly higher than the inline method and showed 1.5-2 times less noise making overall factor of 2 signal to noise improvement for most of the low intensity peptide ions studied.
- For these experiments, 1 inch maximum I.D. funnels were used. For inline method, a jet-disruptor [2] was used in the funnel.
- LOD experiment was carried out using a multicapillary inlet with three 500 µm I.D. channels

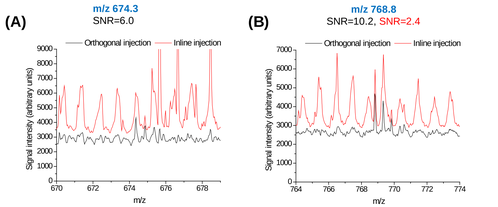

Figure 3. Signal intensity versus m/z plots for [Substance P + 2H]$^{2+}$ ion (A) and [Fibrinopeptide A + 2H]$^{2+}$ ion (B) at 0.5 nM concentration using inline injection and orthogonal injection methods.

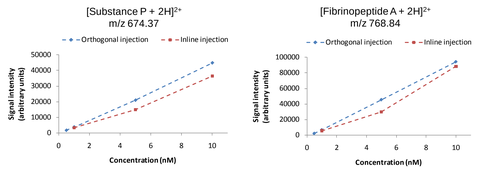

Figure 4. Signal intensity versus concentration plots for [Substance P + 2H]$^{2+}$ and [Fibrinopeptide A + 2H]$^{2+}$ ions. For orthogonal injection method, peptide signal was detected for both substance P and fibrinopeptide A ions at 0.5 nM while for inline injection signal was buried in the noise for both ions.

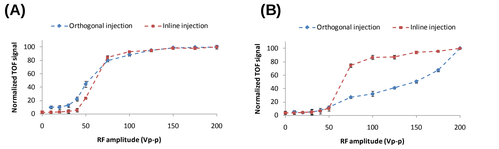

Figure 5. Normalized signal intensity versus RF amplitude for m/z 622 (A) and m/z 1822 (B) ions from Agilent tune mix sample using orthogonal injection and inline injection methods. For m/z 622 ion, both injection methods showed similar curves indicating that DC field applied to orthogonal injection funnel is strong enough to divert the ions into the funnel. However, for m/z 1822 ion, the DC field was not strong enough to divert the ions into the funnel before they reach the opposite side of the funnel electrode requiring stronger RF fields to contain ions inside the funnel. DC field was maintained at 23 V/cm for orthogonal injection experiment. This observation indicates that to obtain the optimum transmission for higher m/z ions, either stronger DC and RF fields must be employed or the funnel I.D. must be increased.

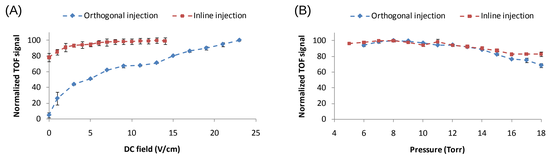

Figure 6. Normalized signal intensity versus DC field for the funnel (A) and normalized signal intensity versus pressure in the funnel chamber (B). For inline injection method, about 80% of the signal is transmitted through the funnel even at zero DC field. This indicates that there is a strong directional gas flow that carries ions, neutrals, ion clusters and large liquid droplets through the funnel into the next pumping stage causing contamination of the mass spectrometer elements. For orthogonal injection method less than 5% of the signal is transmitted through the funnel at zero DC field, indicating the significantly reduced contamination with this new technique. Figure (B) shows that this funnel can be operated over a wide range of pressure. The signal fall off at higher pressures is due to insufficient RF frequency and amplitude to confine ions in the funnel.

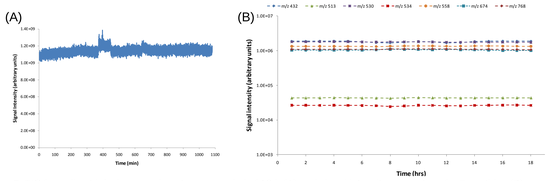

Figure 7. TIC for a direct infusion experiment over a period of 18 h (A) and signal intensity versus time for 7 peptides (B).

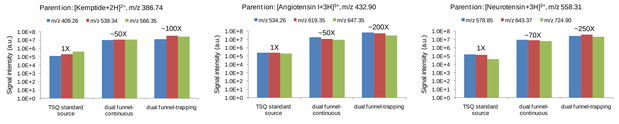

Figure 8. Comparison of integrated signal intensity for Kemptide, Angiotensin I and Neurotensin using TSQ standard source, dual ion funnel source operated in continuous mode and dual ion funnel source operated in trapping mode. 13.64

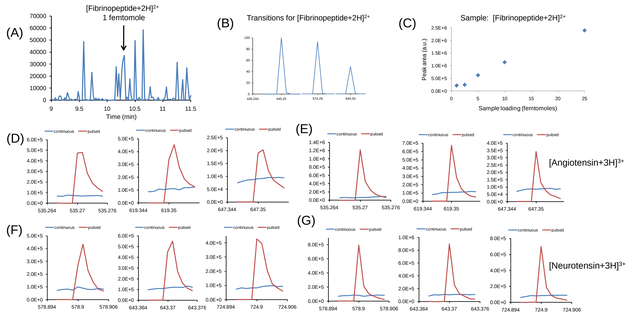

Figure 9. BPC for [Fibrinopeptide+3H]$^{3+}$ ion in pulsed-SRM mode using dual ion funnel source with orthogonal ion injection method (A), transitions for [Fibrinopeptide+3H]$^{3+}$ ion (B) and concentration curve for [Fibrinopeptide+3H]$^{3+}$ ion (C). Here, 9 peptides were spiked at 0.1 nM to 10 nM concentrations into a 0.25 mg/mL *Shewanella oneidensis* tryptic digest and eluted through a reversed-phase LC column. Figure 9 D, E, F and G correspond to direct infusion experiments for [Angiotensin+3H]$^{3+}$ ion (10 nM) at 4ms and 10 ms trapping times and [Neurotensin+3H]$^{3+}$ ion (10 nM) at 4 ms and 10 ms trapping times respectively.

Conclusions

- **We have developed a new orthogonal ion injection method that can be coupled to high pressure or low pressure ion funnels. With this new method, multicapillary inlets can be employed to obtain higher sensitivity and improved signal-to-noise ratio.**
- **Ions are injected into the DC and RF fields in the funnel through an opening on the side of the funnel. Strong DC fields divert the ions into the funnel while RF field confine ions inside the funnel. Particles and neutral species are removed through a pumping port positioned directly opposite to the capillary inlet.**
- **This method produced ion signal intensities similar to conventional inline ion injection while considerably reducing the contamination of mass spectrometer components.**
- **The tandem ion funnel configuration with the in-funnel orthogonal ion injection improved the signal intensity by a factor of 4-8 compared to the single ion funnel design while providing excellent stability to the Thermo TSQ instrument. This instrument was configured with a large 750 µm I.D. inlet capillary and the instrument operated continuously over a month without any signal decay.**

Acknowledgements

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References

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