

Ion Funnel Interface for a Linear Ion Trap: Enabling Faster, High Resolution LC-MS Analyses

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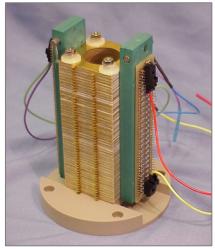
Overview

- An ion funnel interface has been designed and implemented on a linear ion trap mass spectrometer.
- The ion funnel replaced the skimmer and reduced ion loss in the interface by ~90%.
- The greater ion flux afforded by the ion funnel greatly decreased ion accumulation times.
- Increasing the rate of ion accumulation produced higher quality MS/MS spectra.
- Faster high resolution scans enabled better coupling to fast separation techniques for high sample throughput.

Introduction

The linear ion trap mass spectrometer has an increased ion capacity, improved ion trapping efficiency, and faster cycle times compared to the three-dimensional (3D) Paul trap mass spectrometer.¹ However, even with the increased performance, its atmospheric pressure ionization interface still incurs significant ion loss. We have previously developed and reported on an electrodynamical ion funnel that replaces the skimmer interface and greatly improves the ion transmission efficiency.²

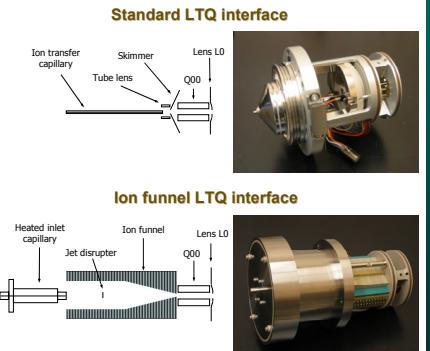
Here, we evaluate an ion funnel interface on a linear ion trap instrument (Thermo Electron, LTQ) and on a hybrid linear ion trap/Fourier transform ion cyclotron resonance instrument (LTQ FT). We show that a large reduction in ion accumulation times enables higher quality tandem mass spectrometry (MS/MS) analyses and fast liquid chromatography (LC) FT ICR MS analyses resulting in increased sample throughput.



Ion Funnel

Methods

An ion funnel interface for a LTQ mass spectrometer has been designed which mimics the original LTQ interface.



Ion funnel parameters:

- 100 electrodes with 0.5 mm thickness and 0.5 mm spacing
- RF = 615 kHz with 60 V_{P-P}
- DC voltage gradient of 20 V/cm
- Pressure of 1.5 Torr

Infusion experiments:

- A range of reserpine solutions (0.001 – 10 μ M) were made in 50:50 MeOH and H₂O with 1% acetic acid.
- Solutions were infused from lowest to highest concentration at 300 nL/min using the same ESI emitter, syringe, voltage, etc. for both the standard and ion funnel interfaces.

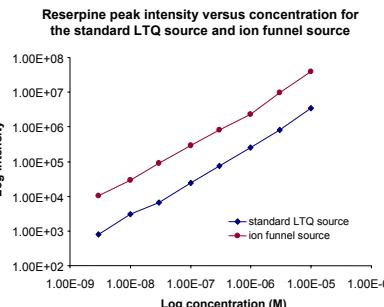
LC MS and LC MS/MS:

- Automated HPLC system based on Isco 100DM syringe pumps using a 150 μ m i.d. x 60 cm capillary column packed with 5 μ m Jupiter C18 stationary phase.³
- Samples made from whole cell lysates of the bacterium, *Shewanella oneidensis* and were digested by trypsin and cleaned using a C18 SPE column.³

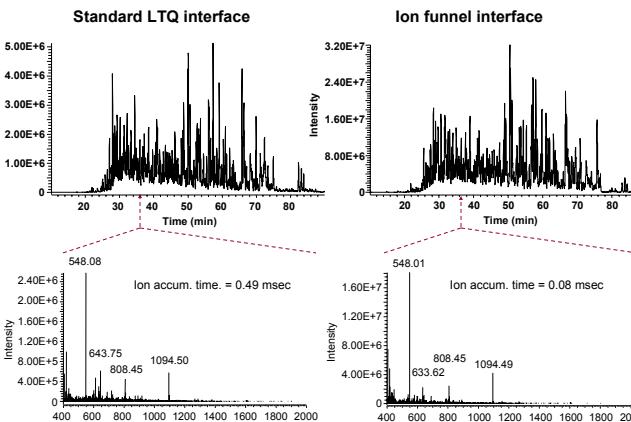
Results

Limit of detection experiment using reserpine solutions to test ion funnel performance with an LTQ ion trap MS

- ~10-fold increase in peak intensities (corresponding to 90% reduction in ion trap "fill" times)
- Experiment was repeated on two different LTQs with similar results
- Minimal change in S/N indicates similar ion population in the ion traps



LC-MS analysis of 10 μ g of *Shewanella oneidensis* digest on LTQ with standard and the ion funnel interfaces



- Peak intensities increased and ion accumulation times decreased when the ion funnel interface was used.
- Similar peak profiles indicate similar ion transmission profiles in this mass range for both interfaces.

Comparison of standard and ion funnel interfaces in LC-MS/MS analyses of *S. oneidensis* samples

- Ion funnel increased the number of identifications, especially for the lower concentration samples
- Largest factor for the increased identifications is higher quality MS/MS spectra
- The ion funnel increased the number of ions in the ion trap for MS/MS analyses

Peptide and protein identifications from the LC MS/MS analysis of a range of *S. oneidensis* tryptic digest concentrations and 10 μ L injections on the same LC column using the standard and ion funnel interfaces

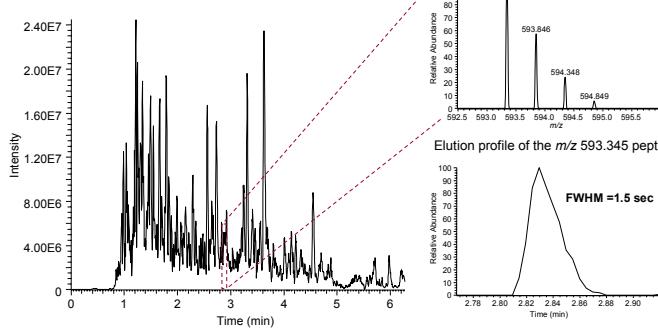
	Conventional interface			Ion funnel interface				
Concentration (μ g/ μ L)	1.0	0.3	0.1	0.03	1.0	0.3	0.1	0.03
Peptide identifications	4770	3681	2548	1415	5187	4712	4106	3851
Unique peptide identifications	3107	2405	1726	1012	3126	2835	2302	1992
Unique protein identifications	940	780	587	357	953	895	757	699

* Peptide hits filtered for > 95% confidence (Qian WJ et al. *J. Proteome Res.* 2005, 4, 53-62).

Proteomics analyses using fast HPLC separations on LTQ FT MS with ion funnel interface

- ~10 cm capillary column packed with 0.8- μ m porous C18-bonded silica particles at 8000 psi⁴
- 5 μ L injected of a 0.2 μ g/ μ L S. *oneidensis* tryptic digest
- Mass range of m/z 400-1800
- Target ion population of 10^6 for the ICR mass analyzer
- Electrospray ionization directly from the pulled exit of the LC column

HPLC chromatogram of the analysis of 1 μ g of a global digest from *S. oneidensis* using a LTQ FT with an ion funnel interface



Results from the analysis of a *S. oneidensis* sample using ~5 min HPLC separations and a LTQ FT with an ion funnel interface

Transient size (kB)	Total scan time (msec)	Peptide identifications	Protein identifications	Mass accuracy (ppm)
128	150	854	334	2.4
256	240	1047	371	1.3
512	440	1109	394	1.2

* Peptides and proteins identified and filtered using an accurate mass and elution time database

Conclusions

- Replacing the standard LTQ-ESI interface with an ion funnel increases transmitted ion currents ~9-fold.
- The increased ion beam intensity from the ion funnel greatly reduced the linear ion trap "fill" times.
- Faster ion trap "fill" times increased the duty cycle and provided higher quality data in MS/MS spectra.
- Increased scan speed by shorter ion trap "fill" times improved the use of high resolution MS with fast separation techniques.
- An LTQ FT with ion funnel interface was coupled to fast HPLC separations (~5 min) which produced several hundred protein identifications from a 1 μ g proteomic sample

Acknowledgements

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References

- Schwartz, J.C.; Senko, M. W.; Syka, J. E. P. *J. Am. Soc. Mass Spectrom.* 2002, 13, 659.
- Taeman, K.; Tolmachev, A.V.; Harkewicz, R.; Prior, D.C.; Anderson, G.; Udseth, H.R.; Smith, R.D.; Bailey, T.H.; Rakov, S.; Futrell, J.H. *Anal. Chem.* 2000, 72, 2247.
- Lipton MS, Pasa-Tolic L, Anderson GA, Anderson DJ, Auberry DL, Battista JR, Daly MJ, Fredrickson J, Hixson KK, Kostandarithes H, Masselon C, Markillie LM, Moore RJ, Romine MF, Shen Y, Stritmatter E, Tolic N, Udseth HR, Venkateswaran A, Wong K-K, Zhao R, Smith RD. *Proc. Natl. Acad. Sci.* 2002, 99, 11049.
- Shen, Y.; Stritmatter, E. F.; Zhang, R.; Metz, T. O.; Moore, R. J.; Li, F.; Udseth, H. R.; Smith, R. D. *Anal. Chem.* 2005, 77, 7763.

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