High sensitivity LC-ESI-SRM MS analysis using multi-capillary inlet/tandem electrodynamic ion funnel interface

Errol W. Robinson[1], Keqi Tang[2], Tao Liu[2], Alex Mordehai[3], Anabel Fandino[3], Ed Aisawa[3], and Richard D. Smith[2]
[1]Environmental Molecular Sciences Laboratory and [2]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA; [3]Agilent Technologies, Santa Clara, CA

Pacific Northwest NATIONAL LABORATORY

Overview

- Agilent Technologies triple quadrupole modified to include:
  - Multi-capillary inlet
  - Dual electrodynamic ion funnels
- 2- to10-fold increase in measured ion current compared to the standard source
- 3- to 7-fold improvement in the sensitivity for standard peptides
- 1 to 5 ng/mL limit of quantitation (LOQ) for tryptic peptides from 5 standard proteins spiked into mouse plasma

Introduction

- The achievable sensitivity of electrospray ionization mass spectrometry (ESI-MS) is largely determined by the ionization efficiency in the ESI source and the ion transmission efficiency through the ESI-MS interface [1].
- Selected reaction monitoring (SRM) analysis directly benefits from improvements in the ion source and interface transmission efficiency in terms of limit of detection (LOD), limit of quantitation (LOQ), and reproducibility as determined by the coefficient of variability (CV) [2].
- In many cases, the intensity of the ion beam, or in other words, the ion transmission efficiency determines the SRM MS detection limit.
- The multi-capillary inlet [3] and ion funnel [4] technologies developed at PNNL were designed to increase the transmission efficiency of ions or increase the intensity of the ion beam into the mass spectrometer. In collaboration with Agilent Technologies, these technologies were implemented into an Agilent 6430 Triple quadrupole mass spectrometer.
- We present our latest implementation of a multi-capillary tandem electrodynamic ion funnel interface with triple quadrupole MS and the evaluation of the modified platform's sensitivity for targeted proteomics analysis.

Methods

SRM measurements with multi-capillary inlet and dual ion funnels

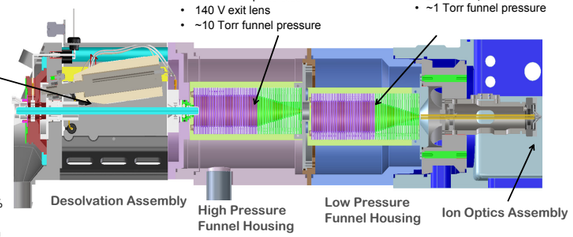
- Sensitivity comparison between standard interface and ion funnel interface
- Precision of peak area
- Precision of LC retention time

High Pressure Funnel compensates for increased gas load from multi-capillary inlet
- RF 3 MHz 250 V_{p-p}
- 340 V first plate bias
- 180 V last plate bias
- 140 V exit lens
- ~10 Torr funnel pressure

Low Pressure Funnel replaces the skimmer
- RF 1.5 MHz 220 V_{p-p}
- 120 V first plate bias
- 20 V last plate bias
- ~1 Torr funnel pressure

Multi-bore Capillary captures more ions from the ESI plume
- 6 capillaries (600 µm i.d.)
- modified end cap

Nano-flow LC and chip cube ESI source
- 300 nL\min
- C18 on-chip column with 3 µm particles 15 µm i.d. × 15 cm length
- Mobile phase A: water with 0.1% formic acid
- Mobile phase B: acetonitrile with 0.1% formic acid



Dynamic SRM conditions

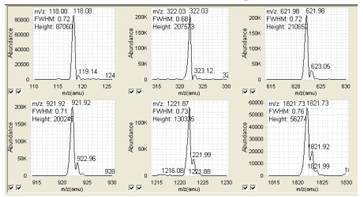
Standard peptides spiked in water at different concentrations

Compound Name	Nominal Mass	Precursor Ion	Product Ion	Collision Energy
Bradykinin	756.39	379.2	527.3	9
Bradykinin	756.39	379.2	614.4	9
Deltorphin II	783.38	784.39	610.29	23
Deltorphin II	783.38	784.39	709.36	23
Kemptide	771.47	386.74	252.2	9
Kemptide	771.47	386.74	409.2	9
Melittin	2845.7	570.2	541.998	16
Melittin	2845.7	570.2	812.494	16
Methionine Enkephalin	573.23	574.23	397.18	16
Methionine Enkephalin	573.23	574.23	425.18	16
Renin Substrate Porcine	1757.93	586.98	696.38	16
Renin Substrate Porcine	1757.93	586.98	745.92	16

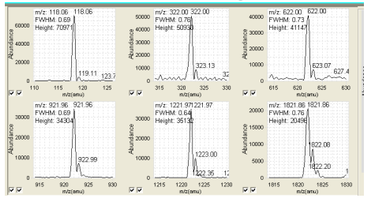
Standard proteins spiked in non-depleted mouse blood plasma matrix at different concentrations

Compound Name	Precursor Ion	Product Ion	Collision Energy
Bovine carbonic anhydrase VLDALDSIK	487.28 (2+)	761.40 (+1, y^7)	11
E. coli beta-galactosidase LWSAEIPNLYR	681.36 (2+)	1062.56 (1+, y^9)	20
Equine myoglobin LFTGHPETLEK	424.56 (3+)	506.26 (2+, y^9)	8
Chicken ovalbumin GGLEPINFQTAADQAR	844.42 (3+)	666.34 (2+, y^{12})	24
Bovine cytochrome *c* EDLIAYLK	482.77 (2+)	494.30 (1+, y^4)	11

Ion funnel source tuning results



Standard source tuning results

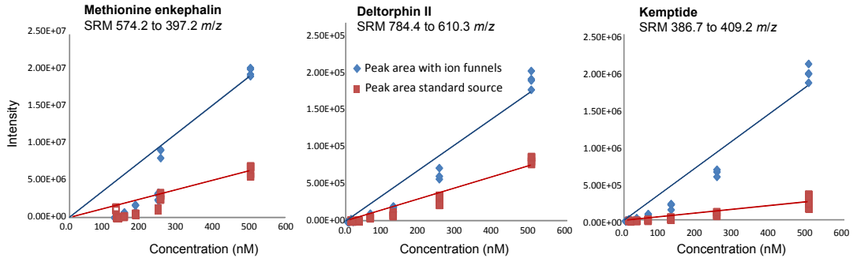


Samples for LC-SRM-MS

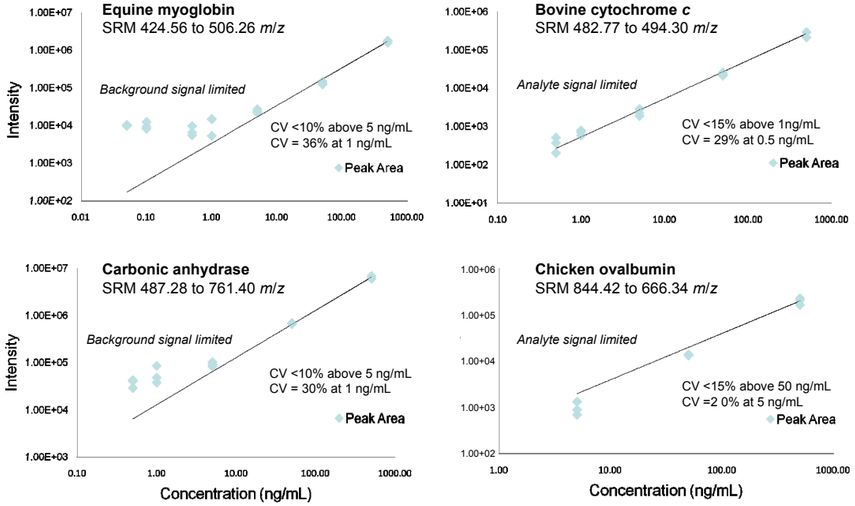
Protein standards were digested and then spiked into non-depleted mouse plasma digest. 5 µL of the sample mixture was injected on column. 2 µL of the peptide standard mixture in water was injected on column. Three replicate analyses were performed for all samples.

Results

Sensitivity improves ~3-7 fold using ion funnel multi-capillary inlet and tandem ion funnel interface for LC-SRM-MS of peptides in water



Limit of quantitation (LOQ) with the multi-capillary inlet and tandem ion funnel interface for digested protein standards spiked into digested mouse plasma



Conclusions

- The present design achieved a 1-5 ng/mL instrument limit of quantitation (LOQ)
- The LOQ for some protein standards is limited by the instrument sensitivity, while for others, the LOQ is limited by the instrument specificity
- Significant room for further gains

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References

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CONTACT: Robby Robinson, Ph.D.
Environmental Molecular Sciences Laboratory, K8-98
Pacific Northwest National Laboratory
P.O. Box 999, Richland, WA 99352
E-mail: errol.robinson@pnl.gov