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Evaluation of an Automated “Quick” Optimization Routine to Streamline Low-Flow LC-MS Setup

Joshua A. Silveira, Gary A. Schultz, Matt Tsai, Katherine Walker, Hannah Snyder, Cornelia Boeser, Robert van Ling, Eloy R. Wouters, Thermo Fisher Scientific 355 River Oaks Pkwy, San Jose, CA 95134

Abstract

Purpose: When setting up low flow LC-MS experiments, a user regularly positions an emitter at an arbitrary fixed distance from the inlet based on visual alignment. A prototype ion source featuring an emitter mounted on a 3D motorized stage was used to evaluate a “quick” optimization strategy used to define the active position for subsequent LC-MS measurements.

Methods: The basis of the quick optimization routine was established using m/z- and position-dependent intensity distributions. The routine was evaluated against two methods to manually set the emitter position.

Results: All optimization strategies yielded a statistically equivalent number of protein and peptide identifications. However, the quick optimization routine yielded superior peak area reproducibility that translated into significantly better emitter-to-emitter consistency.

Introduction

Electrospray ionization (ESI) generates gas phase ions when a sufficiently high voltage is applied to a liquid exiting an emitter with micron dimensions. At low flow rates (0.1-5 µL/min), the optimal emitter-to-inlet distance is typically <5 mm. When setting up low flow LC-MS experiments, a user regularly positions an emitter at an arbitrary fixed distance from the inlet based on visual alignment. An automated routine eliminates ambiguity by positioning the emitter based on the mass spectrometer signal detected for solvent or analytical ions, as defined by the user.

Materials and Methods

General

Spatial mapping of ESI plumes was carried out on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer by mounting the emitter on a three-dimensional XYZ stage controlled by stepper motors. In the YZ dimensions, note that 1 step = 48 µm. In all experiments a tapered emitter with 15 µm ID was used. The voltage difference from the liquid junction upstream of the emitter to HCTT inlet was 1.8-2.2 kV. A coaxial sheath gas of nitrogen was used at a flow rate of ~0.5 L/min.

Proteomics Experiments

LC-MS proteomics experiments were carried out using 200 ng loads of HeLa cell protein digest. Samples were analyzed using a prototype µPAC™ Neo HT column at 1500 nL/min. In all cases, a 1 µL injection volume was used. The gradient was ramped from 1% to 45%B (where [A]: 0.1% formic acid in water, [B]: 0.1% formic acid in 80:20 acetonitrile:water) over 5 minutes. Raw files were searched in Thermo Scientific™ Proteome Discoverer™ 3.1 using CHIMERYS™ intelligent search algorithm (MSAID GmbH) with MS1 quantitation.

YZ Heatmaps

Pierce™ Peptide Retention Time Calibration (PRTC) mixture was diluted 1:100 into FlexMix™ calibration solution. The mixture was infused at a flow rate of 1500 nL/min. Heatmaps for select singly and doubly charged ions were acquired as a function of the emitter position in the YZ plane at a predetermined X optimum of 4480 steps (laterally centered with the HCTT). The acquisition time was ~1 hour.

Quick Optimization

Quick optimization routines were performed on six different emitters to establish the active position for proteomics analysis. The routine was compared to two manual alternatives:

(1) Default position: predefined motor coordinates: X, Y, Z = 4440, 100, 118

(2) Constant spatial tip position (±25 µm estimated precision based on camera images)

Once the emitter position was established, the performance was assessed by LC-MS analysis. The solvent composition during the quick optimization routine was 90%B. It was observed that compositions containing higher aqueous content yielded broader distributions (data not shown).

Quick Optimization Description

(1) X-scan to determine lateral center position

(2) At X-optimum, acquire a series of Z-line scans that intersect the YZ diagonal

(3) Determine the optima of each Z-line scans (●) and perform linear regression to determine slope and intercept of the diagonal line

(4) Scan along the diagonal line

(5) Position the emitter at the apex along the diagonal (+)

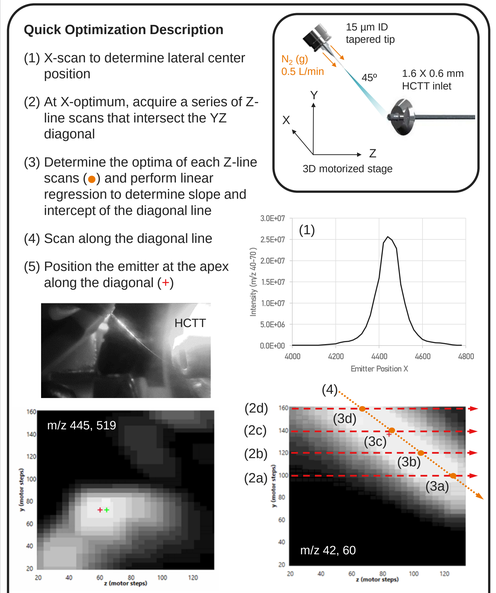
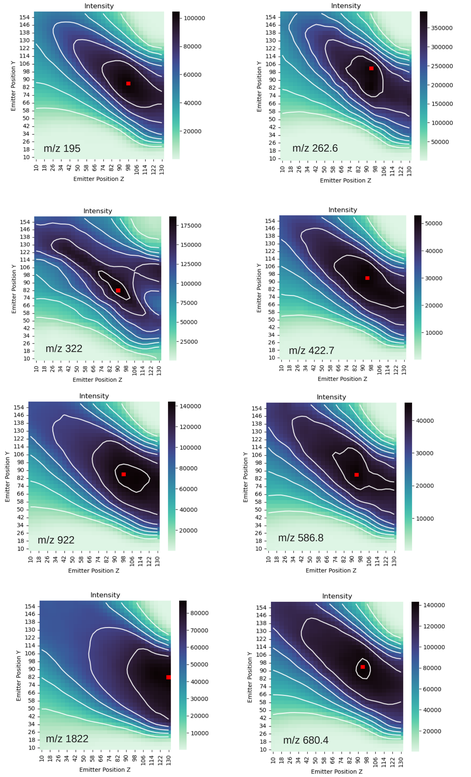


Figure 1. The quick optimization procedure uses a SIM scan of m/z 40-70 where the dominant signals come from solvent and cluster ions m/z 42 and 60. Isolating these ions from background signals (m/z 445 and 519, for example) is critical because the different chemical classes optimize in different spatial positions. Note the position of the HCTT is in the bottom right corner of the heatmap.

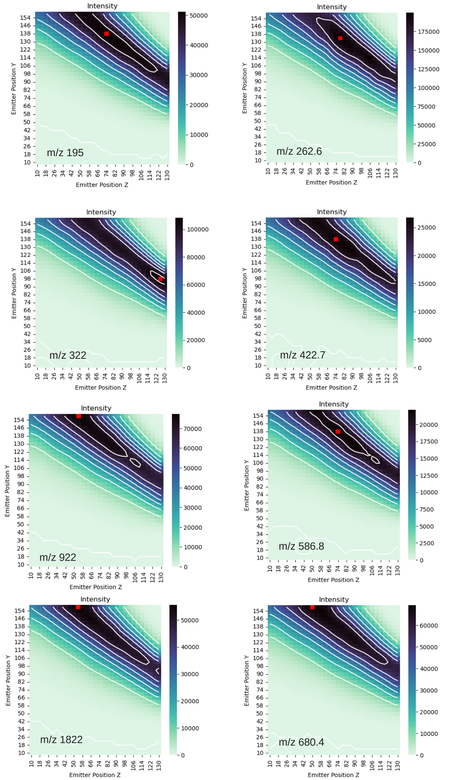
Results – YZ Heatmaps without Sheath Gas

Figure 2. YZ heatmaps of 1+ (left) and 2+ (right) ions without sheath gas. Singly charged ions exhibit more spatial variation compared to doubly charged peptides. Interestingly, the heatmap for m/z 322 splits into two discrete regions as the emitter approaches the inlet, corresponding to local maxima near the top and the bottom of the HCTT. Additionally, the phosphazine series optima shifts toward the inlet as m/z increases. Comparatively, the spatial patterns of doubly charged peptides are more consistent with one another.



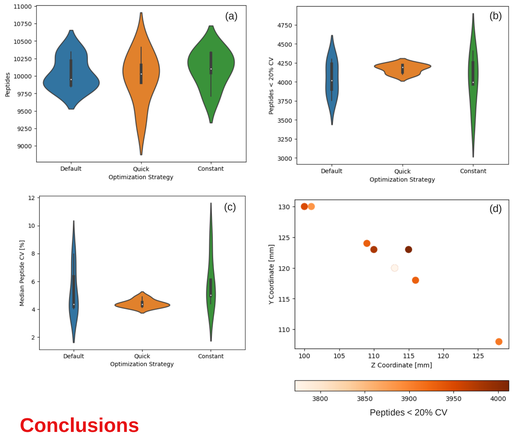
Results – YZ Heatmaps with Sheath Gas

Figure 3. YZ heatmaps of 1+ (left) and 2+ (right) ions with 0.5 L/min sheath gas. Spatial patterns compress along a diagonal as defined by the 45° emitter angle. As compared to the conditions without sheath gas, the optima for most analytes shifted away from the HCTT – the exception being m/z 322 which shifted closer. Though the optima for analytes varies, the profile along the diagonal is substantially flat.



Results – Quick Optimization with Sheath Gas

Figure 4. (a) Distribution of total number of peptides identified across 3 technical replicates shown as a function of the optimization strategy. Distributions are comprised of six different emitters. Note that the same physical emitters were used in each of the datasets. (b) Peptides identified with < 20% CVs. (c) Median peptide CVs. (d) Scatter plot showing the YZ coordinates derived from the quick optimization performed nine different times on the same emitter. The color hue indicates the number of peptides identified with < 20% CVs. Note that the outcome from subsequent executions of the routine changes because the profile along the diagonal line is substantially flat.



Conclusions

- The YZ heatmaps reveal interesting details about the optimal placement of the emitter for maximal sensitivity.
- Sheath gas confines ions to a diagonal following the angle of the emitter. Knowing that a diagonal line is expected in the YZ plane allows for use of a quick optimization routine that provides a suitable strategy for setting up low flow LC-MS experiments in a reasonable timeframe (~3 mins).
- As compared to manual optimization strategies, the quick optimization routine was found to yield statistically equivalent proteome coverage and significantly narrower CV distributions.
- Successive quick optimization routines performed on the same emitter yielded a diagonal pattern analogous to that which was observed in the heatmaps. Different outcomes were observed owing to the lack of a clear apex along the diagonal line.

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