Experimental characterization of automated emitter position optimization strategies for a new low-flow ion source and cartridge

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Abstract

Purpose: When setting up low flow LC-MS experiments, a user conventionally positions an emitter at an arbitrary fixed distance from the inlet based on visual alignment. The new Thermo Scientific™ OptiSpray™ Ion Source features a cartridge-based consumable mounted on a 3D motorized stage. Automated routines to position the emitter are evaluated herein.

Methods: The fundamental basis of the nano and capillary flow optimization routines was established using m/z- and position-dependent intensity distributions. The routine for nano flow was evaluated against a 25 cm x 75 µm leading 3rd party column for low to moderate sample input proteomics applications using FAIMS—the most challenging atmosphere-to-vacuum interface owing to the strong dependence of ion transmission on position.

Results: The nano flow automated routine yielded superior proteome coverage and reproducibility compared to a leading 3rd party column. The data also demonstrated that additional sensitivity can be attained by moving the emitter an additional 0.5 mm inward along the diagonal axis—an outcome important when using FAIMS for low sample input applications.

Introduction

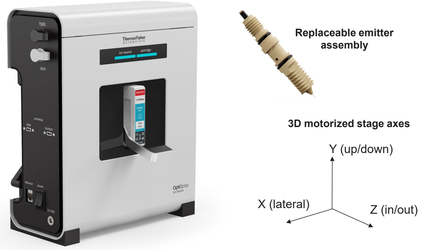
Electrospray ionization (ESI) generates gas phase ions when a sufficiently high voltage is applied to a liquid exiting an emitter with micron dimensions. At low flow rates (0.1-5 µL/min), the optimal emitter-to-inlet distance is typically <5 mm. When setting up low flow LC-MS experiments, a user regularly 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

Experiments were carried out on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer equipped with an OptiSpray ion source and Thermo Scientific™ µPAC™ Neo 50 cm Cartridge with a pulled emitter. The ion source contains a three-dimensional XYZ stage controlled by stepper motors that features automated position optimization software routines run from the Tune UI. In the YZ dimensions, note that 1 step = 48 µm whereas 1 step = 10 µm in the X dimension.

Figure 1. OptiSpray ion source and cartridge, replaceable emitter assembly, and coordinate system used herein.



Proteomics Experiments

LC-MS proteomics experiments were carried out using 1 - 200 ng loads of HeLa cell protein digest. Samples were analyzed using a µPAC Neo column at 300 nL/min delivered from a Thermo Scientific™ Vanquish™ Neo UHPLC System. Raw files were processed in Thermo Scientific™ Proteome Discoverer™ 3.1 using CHIMERYS and Spectronaut® 19.

DDA experiments were carried out using the wide isolation window acquisition program from the method editor template. DIA data was acquired using an isolation window of m/z 40 from m/z 400-800 at a resolving power of 60k. Both experiments used a single FAIMS CV value of -50 V. The total gradient length was kept constant but optimal gradient programs were used for each column type.

Table 1. LC gradients and parameters

uPAC Gradient			3rd party Column (User Guide Gradient)			3rd Party Column (Optimized Gradient)		
Time, min	Flow, µL/min	%B	Time, min	Flow, µL/min	%B	Time, min	Flow, µL/min	%B
0	0.75	4	0	0.3	0	0	0.45	1
0.1	0.75	8	1	0.3	3	0.1	0.45	4
2	0.75	12	12.3	0.3	17	1.9	0.45	12
2.1	0.3	12.1	16.8	0.3	25	2	0.3	12
12.1	0.3	22.5	19.6	0.3	34	12	0.3	22.5
19.6	0.3	40	20	0.3	99	19.5	0.3	40
20	0.3	99	30	0.3	99	22	0.3	99
30	0.3	99				25	0.3	99

Figure 2. X-line scan and representative YZ heatmap showing the line scans that intersect the ions that reside along the diagonal created by the 45-degree angle of the emitter.

Capillary flow optimization (m/z 42, 60)

(1) X scan to determine lateral center position

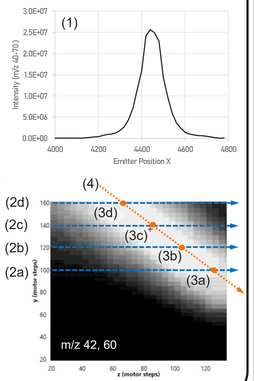
(2) At X optimum, acquire a series of Z line scans that intersect the YZ diagonal

(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) Find the emitter at the optimum position along the diagonal (+)

Note: The routine is intended for use with the 15 µm ID tapered emitter, to be used in conjunction with sheath gas (minimum value 5 psi; not evaluated in this work).



Nano flow optimization (m/z 42)

(1) Acquire an XY heatmap at a fixed Z position (dependent upon the detected configuration of the inlet).

(2) Find the optimum position in the 2D heatmap (+)

Note: the routine may be used with either pulled or tapered emitters. Sheath gas is not used with pulled emitters but may be used in conjunction with tapered emitters.

Figure 3. Outcome of optimization routine: spatial position (left) and heatmap plot (right).

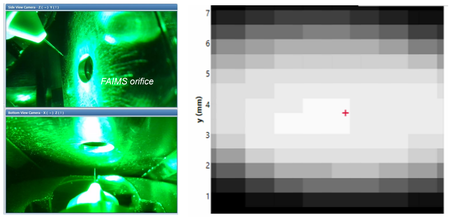


Figure 4. Comparison of proteome coverage at various low input sample loads acquired by DIA. Each bar represents the total number of identifications from n=3 replicates searched together in Spectronaut 19. Note that the combined "Automated+Manual" position was first determined by the nano flow routine, then manually adjusted to be closer to the inlet by moving 0.5 mm along the diagonal axis.

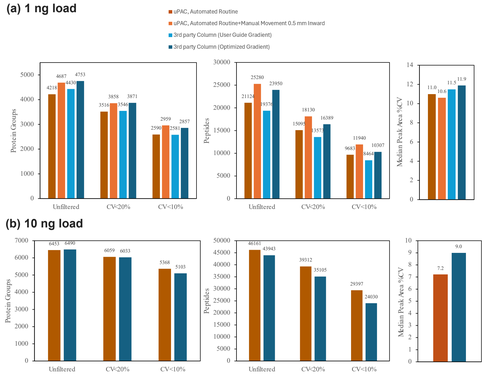


Figure 5. Comparison of proteome coverage at moderate input sample loads acquired by DDA. Each bar represents the average identifications per run (searched individually in Proteome Discoverer).

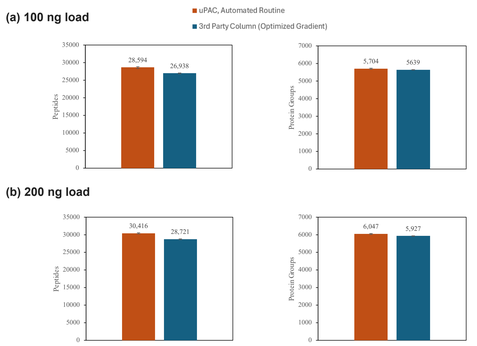


Figure 6. Overlay of three replicate 200 ng injections using a 3rd party column (a) and µPAC cartridge with pulled emitter (b).

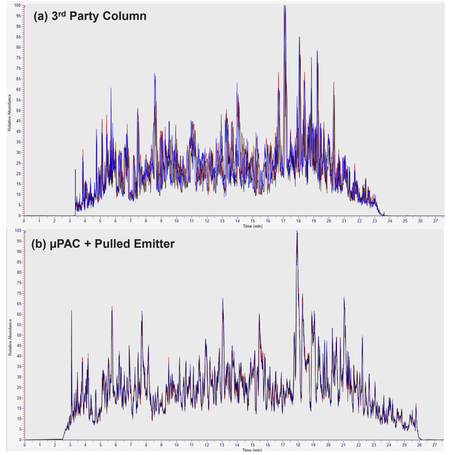
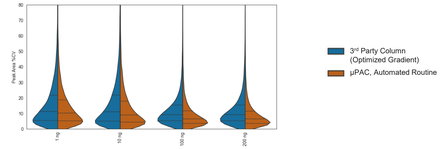


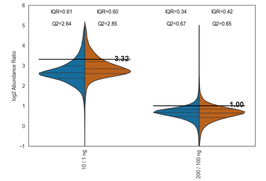
Figure 7. Images of the emitters in functional operation with measured tip dimensions. Note the 3rd party column column features a 24 µm OD emitter and 9 µm rim thickness whereas the OD of the replaceable µPAC pulled emitter is 8 µm with a rim thickness of ~ 1 µm.

Figure 8. Comparison of figures-of-merit

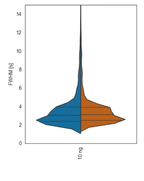
(a) Run-to-run reproducibility for n=3 replicates



(b) Quantitative accuracy



(c) Peak width



Conclusions

- The automated emitter position optimization routine offers a sensitive and reproducible strategy to set the emitter position based on MS-signal, eliminating the ambiguity of human judgement.
- Compared to a 3rd party column, the µPAC Neo 50 cm cartridge with pulled emitter positioned with the automated routine yielded superior proteome coverage with a larger fraction of identifications having superior reproducibility.
- Importantly, while a majority of the comparisons were made using the automated routine, the DIA dataset demonstrated that additional sensitivity can be attained by moving the emitter an additional 0.5 mm inward along the diagonal axis. At a 1 ng load, the seemingly inconsequential adjustment translated to a 20% gain in peptide and 3% gain in protein identifications illustrating the importance of proper emitter alignment—especially when using FAIMS.

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