Electrospray Ionization Stability: The Importance of Nano-Electrospray Quality for Relative, Quantitative Proteomics

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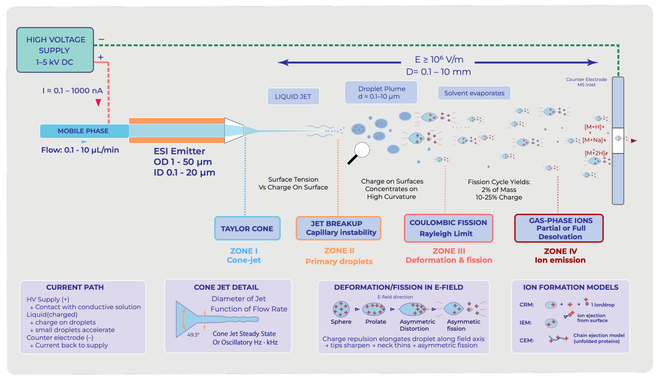
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Introduction

Electrospray ionization (ESI), introduced by John Fenn in 1989 and recognized with the Nobel Prize in 2002, has been central to the evolution of mass spectrometry-based proteomics. Despite decades of refinement, the fundamental spray physics governing low-flow (nano/micro) LC-ESI remain underappreciated in practical application. Modern high-definition mass spectrometers now acquire data at speeds and sensitivities that can reveal previously masked instabilities in the electrospray process. Specifically, the oscillatory nature of common ESI spray modes — spindle, pulsed cone-jet, and multi-jet — can introduce signal dropout and compromise LC peak shape for relative quantification, particularly in complex proteomics experiments and emerging clinical proteomics workflows. This work revisits the foundational 2004 manuscript "Automated Orthogonal Control System for Electrospray Ionization" (JASMS, 2004) in the context of modern instrumentation and proteomics demands.

Methods

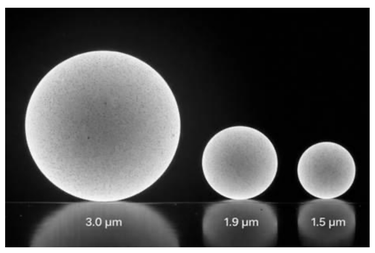
Thermo Pierce™ HeLa cell tryptic digest (100 ng on-column) was separated using a Dionex™ 3000 nanoHPLC (300 nL/min). Two columns were compared: the New Objective Integrity™ PicoFrit® Resolve™ (75 µm × 250 mm, sub 2 µm C18, integrated PicoFrit emitter) and a commercially available blunt ended emitter column (75 µm × 250 mm, sub 2 µm C18, integrated emitter). A 30-minute acetonitrile gradient (2–35% B, 0.1% formic acid) was employed under identical conditions for both columns, with emitter voltage fixed at 1800 V. Data were acquired on a Thermo Orbitrap Exploris™ 480 in two modes: (1) DDA mode (120,000 resolution, 3-second cycle time, 2 m/z isolation, 350–1600 m/z) for peptide and protein identification, and (2) MS1-only mode at ~18 Hz (30,000 resolution, 375–1200 m/z) to assess chromatographic peak shape and spray stability. Data analysis employed Thermo FreeStyle™ 1.8, Skyline, the Trans Proteomic Pipeline, MSconvert, and custom Python 3.12/pyOpenMS software for selected ion chromatogram (SIC) extraction. Triplicate injections were performed for each column.



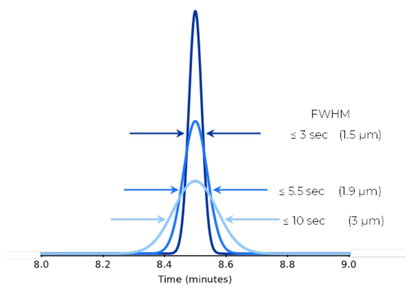
Schematic of the electrospray ionization process.

Peak Width (w)	# of Points Across Peak	Needed Chromatographic Sampling Rate to reach x points across the peak	Scan Cycle (seconds)
3.0 s	7	2.33 Hz	0.43
5.0 s	7	1.40 Hz	0.71
10.0 s	7	0.70 Hz	1.43
20.0 s	7	0.35 Hz	2.86
3.0 s	12	4.00 Hz	0.25
5.0 s	12	2.40 Hz	0.42
10.0 s	12	1.20 Hz	0.83
20.0 s	12	0.60 Hz	1.67
3.0 s	15	5.00 Hz	0.2
5.0 s	15	3.00 Hz	0.33
10.0 s	15	1.50 Hz	0.67
20.0 s	15	0.75 Hz	1.33

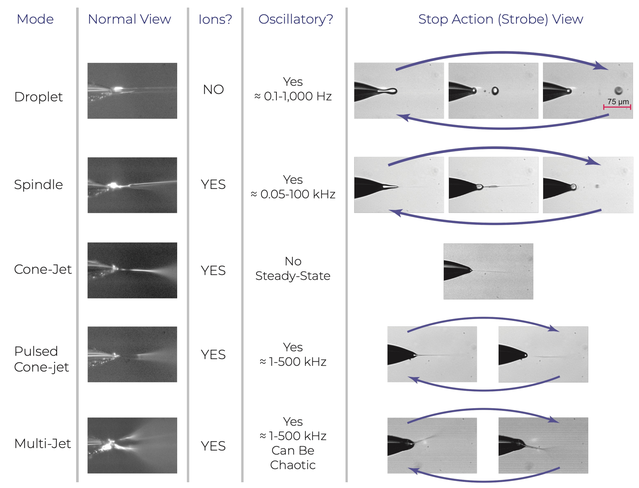
Theoretical points across the peak, matched to chromatographic speed.



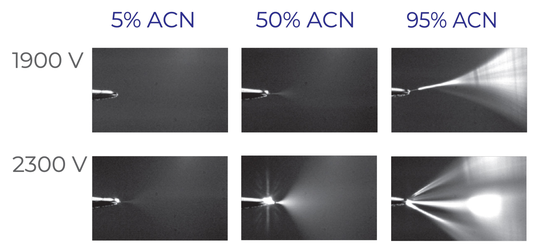
Particle size — smaller size enables faster gradients: 3.0 µm, 1.9 µm, and 1.5 µm shown.



Idealized single peptide analyte FWHM using a 30-minute gradient with different size porous packing in the same column format. Resolving power for the 1.5 µm packed column can now be utilized with fast acquisition, modern mass spectrometry instruments.

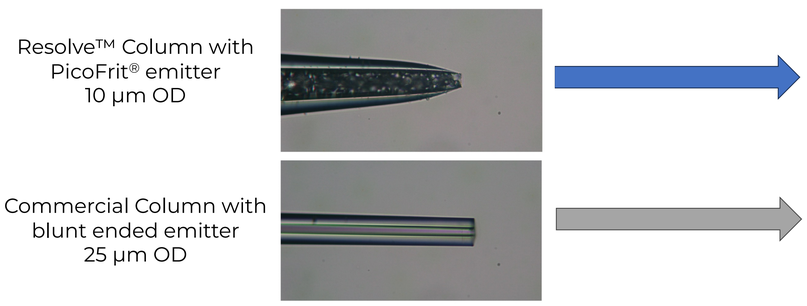


Visualization of common spray modes observed during low-flow ESI. Note the only spray mode that is non-oscillatory is the pseudo steady state cone-jet mode. All other modes have time-dependent (oscillatory) aspects. These are the most common modes that are seen in practice with nanospray LC–MS. There are additional electrospray modes not shown.

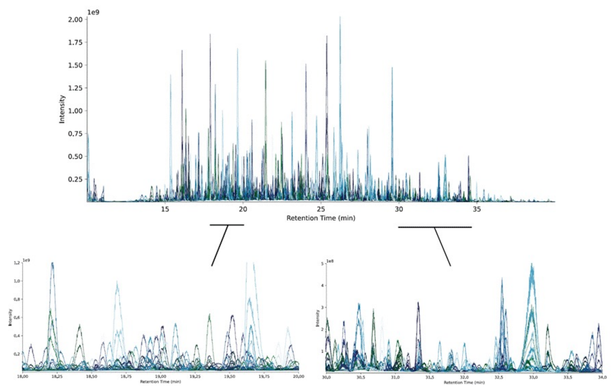


Flow Rate = 500 nL/min; Emitter Diameter = 30 µm
Inlet Distance 4.5 mm; All 0.1% Formic Acid

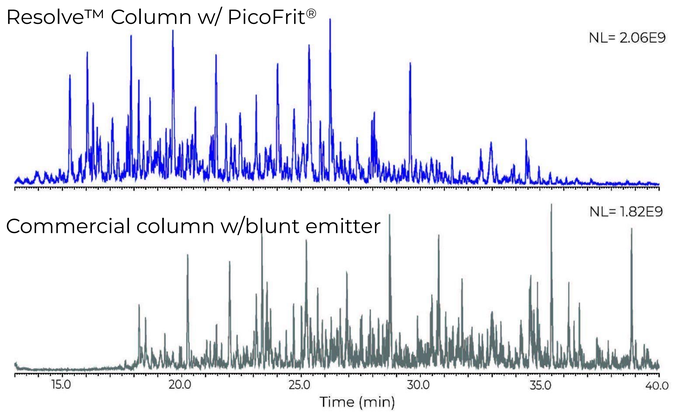
Visualization of spray morphology across a gradient and different percent organic mobile phase composition.



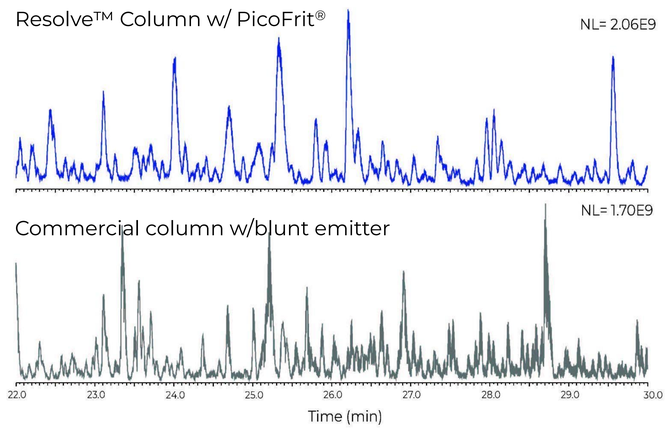
Packed emitter column geometry: New Objective PicoFrit® (10 µm OD) versus commercial column having a blunt ended emitter column (25 µm OD) with long taper.



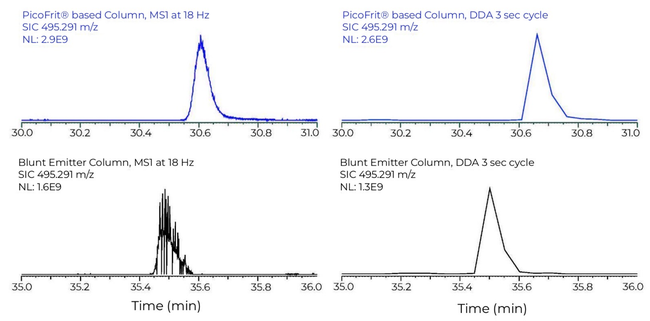
PicoFrit® demonstrating stability through a fast gradient. Rolling SIC (top 20 ions) reconstructed chromatogram from MS1-only data.



The top chromatogram was collected with the sub 2 µm C18 packed PicoFrit® column material and the bottom chromatogram was collected with the sub 2 µm packed column with blunt end emitter. Identical LC and ESI conditions. 18Hz acquisition speed.



Expanding the 22-minute to 30-minute range, with sub 2 µm particle columns with different emitter styles. 18Hz acquisition speed. Note the transition to spray instability as the gradient passes through the emitter on the bottom trace.



Detail of spray instability late in the elution profile: Selected ion chromatograms (SIC) of a tryptic peptide (495.291 m/z) acquired at different acquisition rates. The left-hand data was extracted from fast MS1 acquistion (18 Hz) and the right-hand data was collected in data-dependent (DDA) mode (3 Hz). Slow acqusition masks spray instability.

Conclusion

- Electrospray spray stability is critical for performant LC-MS experiments, as clinical research depends on robust quantification to produce statistically interpretable results.
- Smaller particles, compressed gradients, and increased MS acquisition speeds place ever increasing demands on spray stability.
- Older methods such as data-dependent acquisition, mask spray stability deficiencies.
- Emitter geometry plays a critical role in establishment and delivery of a stable ESI signal.
- A sharp, fritted emitter maintains stability with changes in mobile phase composition.
- PicoFrit® based columns provide the stability and robustness needed to maintain consistent data quality.

Reference

Valaskovic, G. A.; Murphy, J. P.; Lee, M. S. “Automated Orthogonal Control System for Electrospray Ionization.” J. Am. Soc. Mass Spectrom. *2004*, **15**, 1201–1215.

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