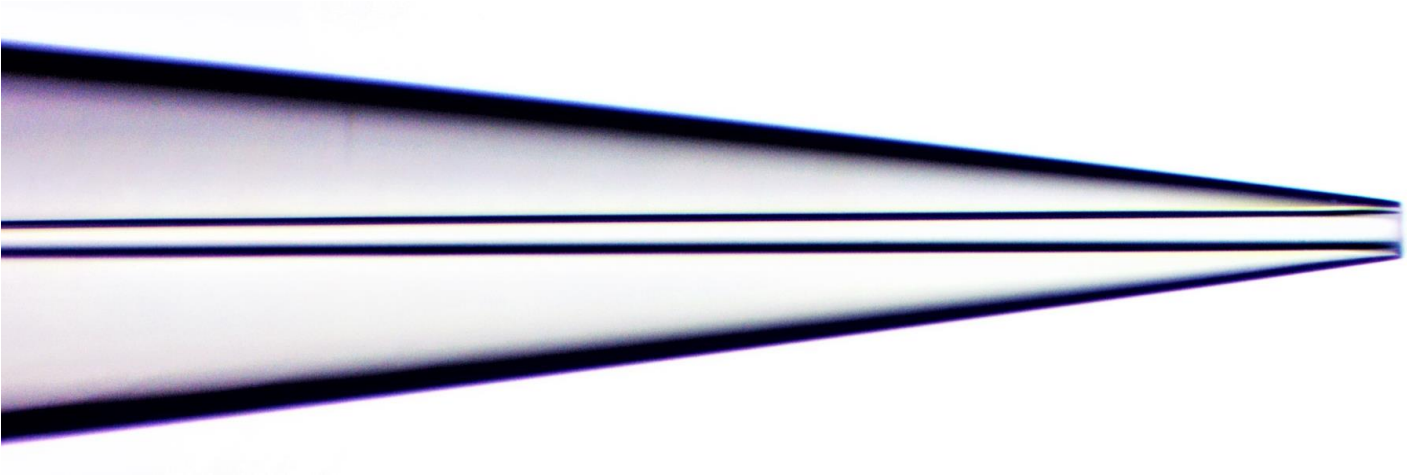
Robust ionization for repeatable proteomics workflows

A run-to-run evaluation of the Sharp Singularity™ nano-ESI emitters in bottom-up proteomics



Nano and micro electrospray provide a very efficient ionization mechanism for the analysis of large biomolecules, but robustness can be an issue. The emitters are so small that micrometric geometric deviations change the meniscus shape, and hence its ionization performance.

The Sharp Singularity™ emitters are mechanically micro-machined and incorporate:

- **Very acute angle.** The meniscus is stable over a wider range of voltages, flow rates, surface tensions, wettability, and conductivities.
- **Well-defined edges** constitute the anchorage line for the meniscus. This stabilizes the meniscus base and improves signal stability and emitter-to-emitter repeatability.
- **Constant ID** reduces the risk of clogging and improves geometric consistence, resulting in better robustness and signal reproducibility.

This technical note studies the run-to-run variability of a standard proteomics workflow and shows the repeatability and the stability of the signals and the protein group identifications.

FIT FOSSILIONTECH

nanoESI **Emitters**

(Technical note)

Methods

The analytical platform consisted of an Ultimate 3000 LC system (Thermo) with a 50 cm µPAC™ column (PharmaFluidics), an Orbitrap Elite™ (Thermo), the nanospray Flex™ Ion Source (Thermo), equipped with the column to emitter interface µPAC™ Flex iON Connect™ (PharmaFluidics). The emitter was 5cm long, 20 µm ID (Fossiliontech Ref.: 20-05)*

The sample was a HeLa cell digest standard spiked with a synthetic peptide retention time standard (Pierce™ retention time calibration, PRTC).

To evaluate the spray quality and stability within a typical bottom-up proteomics workflow, a series of 24 consecutive reversed phase LC-MS runs was performed over a period of 24h. Each run time was 60 minutes, where the gradient lasted 30, and the remaining time was used to flush and prepare the column. Table 1 shows more details on the experimental conditions.

We calculated the main properties of the nano-electrospray by inputting the physical properties of the solvent, the emitter and the spray settings in the 'nano-Electrospray calculator' tool available at www.fossiliontech.com/nano-esi.
This spreadsheet agglutinates analytical and empirical estimations based on available electrospray literature to provide a rapid estimation of the type of spray one can expect to have.

Experimental	
LC	
LC system	Thermo Scientific™ Ultimate 3000 LC system
Analytical column	PharmaFluidics µPAC™ 50cm nanoLC C18
Mobile phase	A: Water (100%) with 0,1% (v/v) FA
	B: Water/acetonitrile (20/80) with 0,1% (v/v) FA
Loading buffer	Water/acetonitrile (99/1) with 0,1% (v/v) TFA
Flow rate	500 nL/min
Gradient profiles	non-linear 1-45% B in 30 min gradient
Temperature	50 °C
Sample	100 ng/µL HeLa cell digest - 100 fmol/µL PRTC
Injection	1 µL user defined prog injection (direct injection)
Ion source	
ESI source	Thermo Scientific™ nanospray Flex™ ion source
LC - ESI Interface	µPAC™ Flex iON Connect
Polarity	Positive
Capillary temperature	275°C
nano-electrospray	
ESI emitter	Fossiliontech, The Sharp Singularity™, nano-ESI emitter Ref, 20-5
ID	20 µm
Semiangle	7.5°
OD	365 µm
Length	5 cm
Spray voltage	2100 V
MS settings	
MS instrument	Thermo Scientific™ Orbitrap Elite™
Acquisition mode	DDA
MS1	
Resolution	240,000
Maximum IT	30 ms
Scan range	300 - 1600 m/z
MS2	
AGC target	1.00E+05
Maximum IT	250 ms
TopN	20
Isolation window	2.0 m/z
NCE	35
Charge states rejected	unasigned and 1
Charge not rejected	2+,3+,4+
Dynamic exclusion	20 sec
Lock mass	445.120020 Da (polysiloxane peak) (internal calibration)

Table 1, LC-nESI MS Experimental conditions

*µPAC™ & iON Connect™ are trademarks of PharmaFluidics; Orbitrap Elite™, Pierce™ & nanospray Flex™ , are trademarks of Thermo Fisher Scientific; Sharp Singularity ™ is a trademark of Fossil Ion technology.

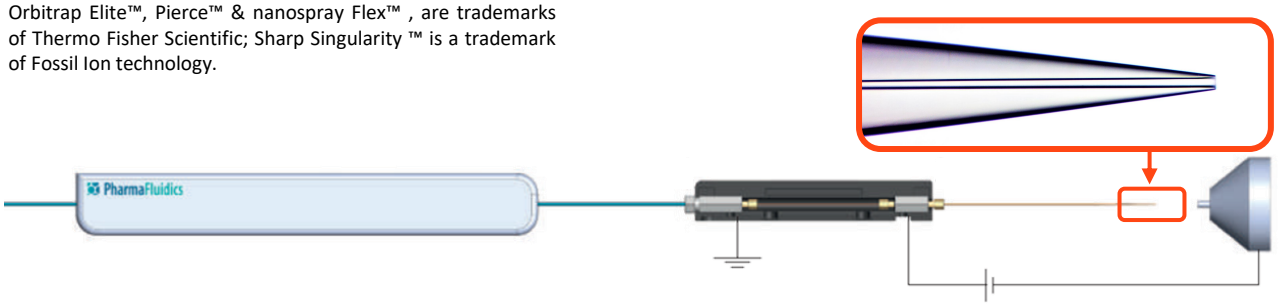


Figure1, LC-nESI MS Experimental assembly

Electrospray characteristics

Table 2 is a direct capture of the input & output data of the 'nano-Electrospray calculator' tool. For simplicity, we introduced only the data corresponding to the end of the LC gradient. The anchorage diameter refers to the diameter of the base of the electrospray meniscus, which is larger than the ID of the emitter and depends on the shape of the tip. In the case of the Sharp Singularity™ emitters, the anchorage diameter is the edge defined at the intersection between the sharp cone and the front surface of the emitter (see Figure 2). The emitter to surrounding distance is the average distance between the center of the emitter (not its tip) and the base of the ion source.

The meniscus evaporation rate (18.6 nl/min) indicates the minimum flow rate this spray can handle before liquid concentration is substantially distorted. The LC flow rate (500 nl/min) is well above this limit, which means evaporation concentration at the tip can be neglected.

The minimum voltage required to form the spray is the spray onset voltage (1.29 kV) plus the emitter voltage drop (0.23kV). Applying 2.1 kV provides a comfortable margin to ensure the spray is stable and ions are pushed forward. The current detected by the MS was (~1µA). The discrepancy between this and the calculated spray current (~300nA) is mostly due to the current evacuated through the grounding fitting.

The first droplet diameter is 385nm. These droplets require 1-2 coulombic fission steps to reach the sub-10nm level and produce gas-phase ions. This indicates that the ionization efficiency could be improved by reducing the flow rate.

The electric field strength at the jet provides an indication of discharge formation. When the field strength at the jet surface exceeds 1V/nm, ions evaporate directly from the jet surface and are rapidly energized, causing a pseudo-discharge that distorts the spray and complicates the spectra. In this spray, with 0.1 V/nm, this undesired effect is negligible.

Input arguments		
Parameter	Units	Value
Liquid		
Conductivity	[S/cm]	5.00
Surface tension	[mN/m]	30.00
Density	[g/cm^3]	1.00
Relative electric permitivity	[-]	40.00
Viscosity	[mPa·s]	0.60
Vapor pressure @ spray temperature	[mBar]	100.00
Molar mass	[g/mol]	41.05
Vapor diffusivity	[cm^2/s]	0.10
Emitter		
inner diameter (ID)	[µm]	20.00
Length (L)	[cm]	5.00
anchorage diameter	[µm]	30.00
Emitter-surrounding ground distance	[cm]	4.00
Spray Settings		
Flow rate	[nL/min]	500.00
Voltage	[KV]	2.10
Tip to counterelectrode distance	[mm]	4.00
Other parameters		
Vacuum permitivity	[F/m]	8.85E-12
R (gas constant)	[J/(K·mol)]	8.31
Spray temperature	[K]	300.00
Results		
Spray characteristics		
Emitter pressure drop	[Bar]	0.64
Meniscus evaporation rate	[nL/min]	18.61
Flow available for the jet	[nL/min]	481.39
Spray onset voltage	[KV]	1.29
Spray current	[nA]	294.87
Emitter voltage drop	[kV]	0.23
Jet / first droplet diameter	[nm]	384.47
No. of Coulomb fissions to reach 10nm	[-]	1.58
Electric field strength at jet	[V/nm]	0.09
Characteristic times		
Charge relaxation time	[ns]	7.08
Meniscus mechanical oscillation time	[µs]	23.56
Emitter resistive-capacitive time	[ms]	0.56

Table 2, nano-electrospray calculator data

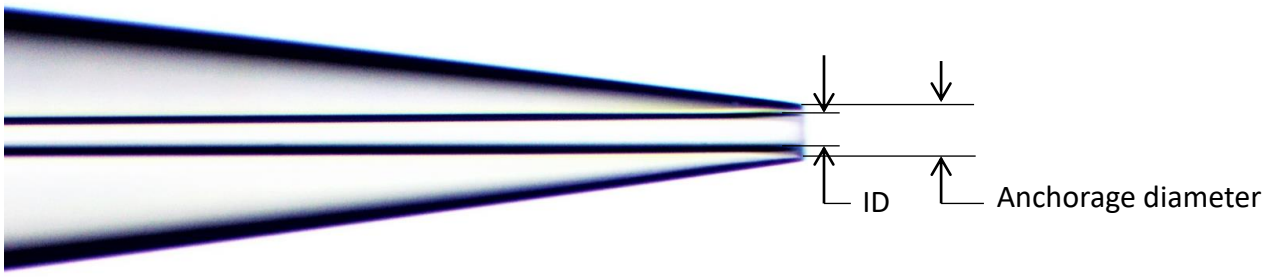


Figure2, ID and anchorage diameter for a 20µm ID Sharp Singularity™ emitter

The result: consistent signal intensity and peak quality

Figure 3A illustrates a chromatogram for the HeLa digest and the peptide calibration mixture. Similar data was acquired in 24 consecutive runs. The conditions for this experiment were selected to emulate a typical experiment where sensitivity throughput and robustness are balanced. Hence, the 500nl/min is in the upper range of the nLC flow range to enabling fast gradients while still maintaining a good sensitivity. For each run, the number of protein group identifications (916 protein IDs were found in average; note that the Orbitrap Elite used was released in 2011, this number would be higher with a more modern spectrometer) and the averaged chromatographic peak width (5.5s in average) was calculated with Proteome Discoverer 2.4 apQuant node (Thermo Fisher). Figure 3B shows the No. of protein IDs and the peak area of the calibration peptides for the consecutive runs. No degradation trend can be observed, showing that the ionization efficiency was consistent. Similarly, figure 3C shows the evolution of the average peak width (FWHM) and the peptide elution time. These flat curves show that the quality of the peaks stays constant for the consecutive runs.

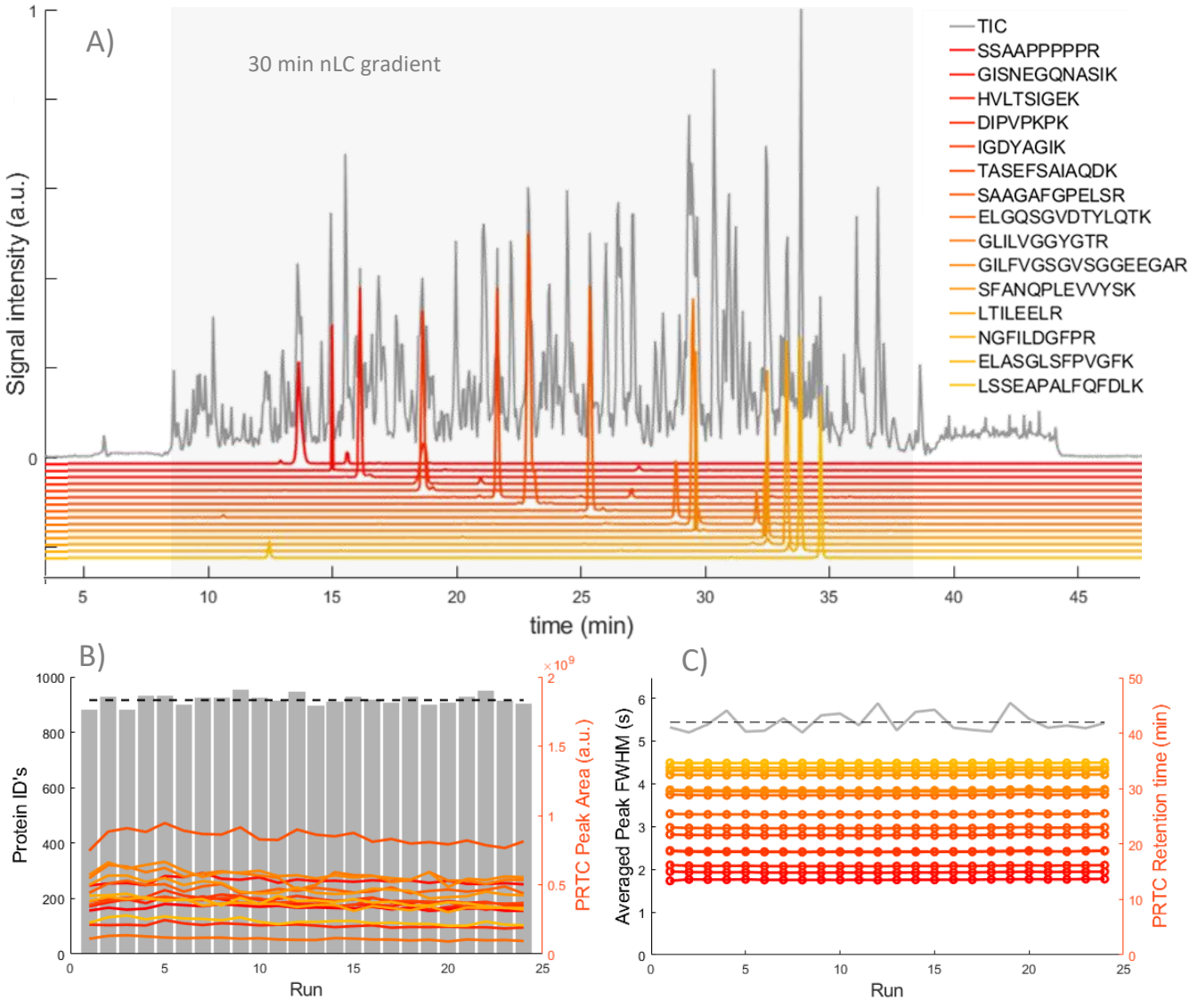


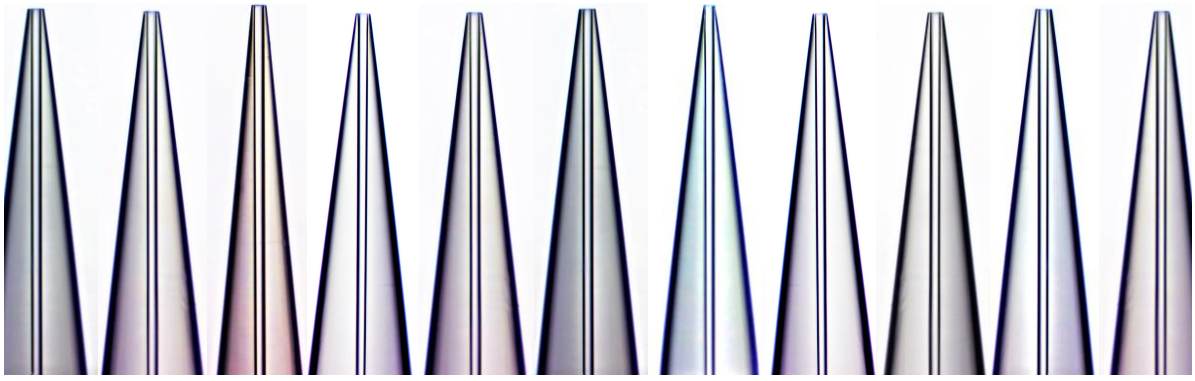
Figure 3, LC-MS data for HeLa and harp Singularity emitter peptide standard.

nanoESI **Emitters,** the Sharp Singularity

(Technical note)

Robust and repeatable nano-electrospray ionization for High quality data:

- very acute angle,
- well-defined edges,
- constant ID,
- geometric reproducibility
- full traceability and quality control



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Find more information on available dimensions and pricing in www.fossiliontech.com/nanoesi-emitters or write to info@fossiliontech.com to order your emitters