

NanoSpray[®] III Source and Heated Interface

Easy to Use, Robust, and Flexible Solution for Nanoflow Applications

Xu Guo¹, Andrew James², Matthew M. Champion³, Doug Simmons¹, Christie L. Hunter³
¹AB SCIEX, Canada, ²SLRI, Toronto, Canada, ³AB SCIEX, USA

The combination of mass spectrometry (MS) and nanoflow liquid chromatography (nanoLC) techniques is a key element of proteomics research. The strength of nanoLC/MS is that it enables the analysis of limited amounts of biological sample with high sensitivity. The variety of chromatographic phases and column configurations now available has resulted in widespread use of nanoLC/MS to tackle a broad range of biological problems. However, the low solvent flow rates used in nanoLC make chromatographic optimization more challenging than higher flow-rate applications. It is therefore important that the nanoflow interface to the mass spectrometer be robust, easy to use, and flexible to provide optimum performance on a wide variety of applications.

There are many examples of established and emerging applications that place different requirements on the nanoLC source and interface. The wide distribution of hydrophobic and hydrophilic peptides in protein digests usually requires the use of a broad LC solvent gradient to improve protein sequence coverage. LC peak shape can be improved through the use of nanoLC tapered emitter tips with chromatographic material

packed right in the tip. Additionally, targeted detection of phosphopeptides using precursor ion scanning for the negative ion of phosphate (PTM Discovery experiments) on QTRAP[®] systems require rapid polarity switching of the source. The negative mode precursor ion scan detects just the phosphate fragment from phosphopeptides, and then triggers the acquisition of positive mode MS/MS for phosphopeptide ID. It is therefore essential to have a versatile and stable nanoflow ESI system that is robust to polarity, solvent composition and choice in chromatographic media when analyzing proteomic samples in an automated mode.

Key Features of NanoSpray[®] III Source and Heated Interface

- Simplified emitter tip and column replacement with low dead volume finger tight connections with quick release of spray assembly
- Rail-mounted sliding union allows flexibility to use any emitter tip lengths
- Nebulizer gas and HV connect directly to source. Sprayer assembly removes without the need to disconnect gas or HV
- Improved lighting and cameras for continuous spray visualization (new compact LCD monitors)
- Fixed angle spraying (~25°) for easy spray tuning and limited interface contamination
- Source and interface compatible with all AB SCIEX MS instruments (compatible with current Nanospray II source housing)
- Compatible with all nanoLC systems and column configurations, including sorbent-packed tapered emitter tips for high quality separations

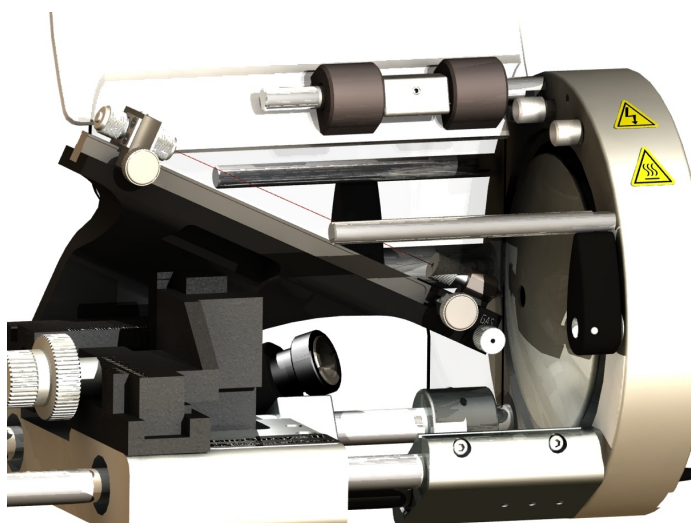
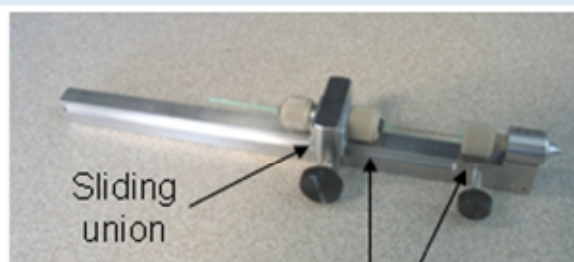
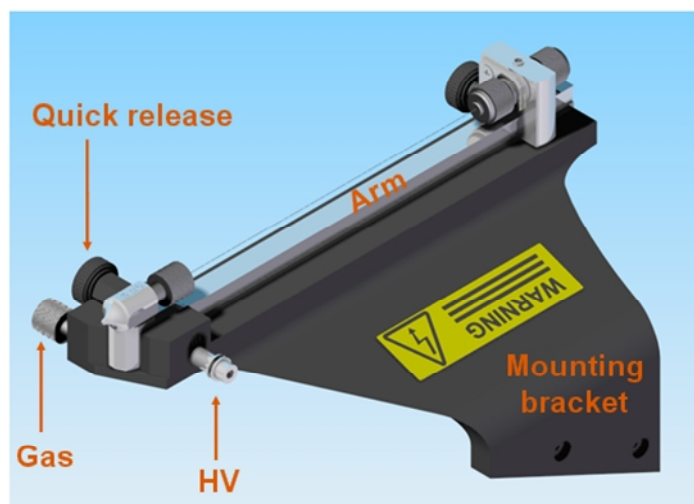


Figure 1. The NanoSpray[®] III Source and Heated Interface. The design provides an easy to use, robust and flexible solution for all nanoflow applications. Cameras and illumination apparatus are not shown for clarity.

New Mounting Bracket and Arm

The NanoSpray[®] II Source can be upgraded to the NanoSpray III Source by replacing the mounting bracket and arm (Figure 2). Simply pull back the X-Y-Z positioning unit and remove the old mounting bracket by removing two screws, then mount the new bracket on the ion source. Both the ionization voltage line and the nebulizing gas line are connected directly to the new bracket and will not have to be disconnected during tip replacement. Finally, the arm containing both the sprayer head and separate liquid union is slid into place. The bracket holds the sprayer assembly at a 25° fixed angle for maximum sensitivity and minimum interface contamination.



Finger tight connections

Figure 2. Mounting Bracket and Arm. Fixed angle bracket ensures robust spray angle and reduces contamination. Removable arm provides flexible configurations and easy tool-free access to change tips and columns.

New Camera and Light source for Spray Visualization

The spray from the emitter tip is now directly visualized on the monitors with the addition of new high-gain cameras and laser light mounted on the NanoSpray III Source. The color camera employs digital signal processing for image control, and results in a clear, high contrast-ratio picture. The laser beam is positioned directly on the volume of the spray plume at the optimal spray tip position for easy visualization. This also provides a simple way of ensuring the the correct spray tip position is used for every acquisition. This light diode has a built-in collimator lens in order to generate a beam that is more focused, in order to maximize the power density of the beam at the location of the spray tip. Fine adjustment of both camera and laser position is easily achieved through the addition of tool-free compression fittings that persist in place.

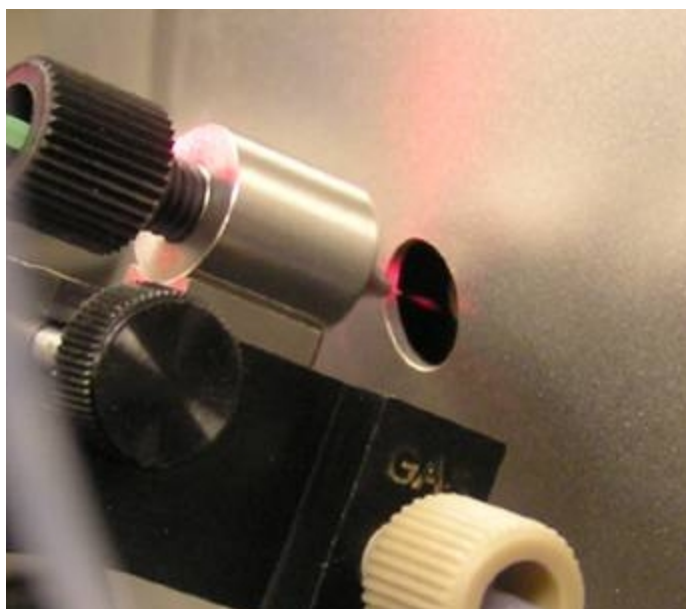


Figure 3. Continuous Visualization of nanoLC Spray. The CCD camera and laser light source enable the direct visualization of spray, to facilitate spray tuning and troubleshooting.

Importance of Using the Heated Interface

The Heated Interface of the NanoSpray Source is a particle discriminator interface (PDI), which provides maximum sensitivity for nanoLC applications². As shown in Figure 3, the heated interface includes a heated laminar flow chamber located between the curtain plate and gas conductance-limiting orifice¹. In addition to the drying effects of the Curtain Gas™ Interface, the laminar flow chamber may be heated from 80 to ~250 °C to ensure sufficient desolvation across a wide liquid flow range. The flow of gas minimizes solvent introduction into the vacuum system. Since the laminar flow chamber does not restrict the gas flow into the first vacuum stage of the mass spectrometer, its internal diameter is selected to consume a large portion of the ion plume. A Teflon spacer ensures a gas-tight seal between the laminar flow chamber and the orifice, establishing laminar flow streamlines that converge upon the orifice to optimize ion transport into the vacuum system.

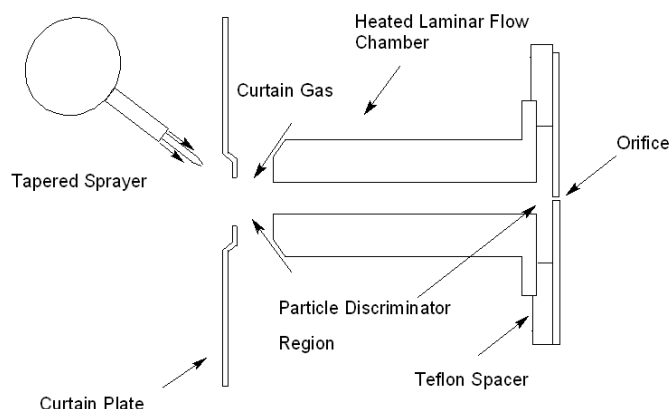


Figure 4. Schematic of Heated Interface. Ions are generated within a few millimeters of the entrance to the heated laminar flow chamber. Two stages of desolvation are provided with the Curtain Gas™ interface and heated chamber. More favorable gas dynamics are provided by reduction of the curtain gas flow speed and establishment of laminar flow prior to the gas conductance limiting orifice.

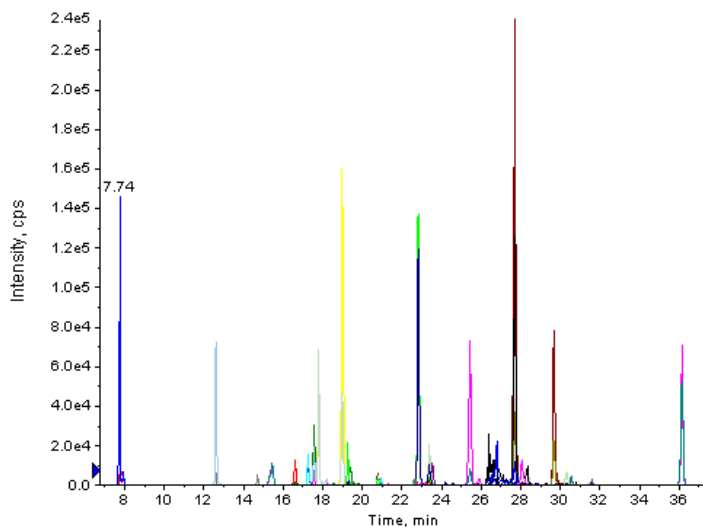


Figure 5. Flexible Configuration of NanoSpray III Source. The NanoSpray II source enables use of sorbent packed tapered emitter tips. Emitter tips packed with Zorbax C18 chromatography phase (75 μm ID x 15 cm) were used by passing the column through the nebulizer head and applying the voltage pre-column at the liquid junction. Very good MRM peak widths (10 sec wide at half height) were obtained with this configuration for MRM transitions to BSA peptides (30 fmol on column).

Flexible Configuration for Use with Any Column Type

The flexible configuration of the NanoSpray III source simplifies emitter tip and column replacement with advanced finger-tight fittings and conductive union connections. With the unique design of union mounting rail, the union can be fixed at any position along the rail to accommodate all types of emitter tips, including different lengths of sorbent packed tapered emitters. In the last couple years, the combination of tapered, fritted fused-silica tips packed with reverse-phase media has been used in an effort to improve the LC peak shape by minimizing post-column junctions and dead volumes. The example shown in Figure 5 is an LC Multiple Reaction Monitoring (MRM) experiment using a tapered emitter tip packed with Zorbax C18 reverse-phase media. The observed peak widths are very good, with widths of ~10s at half height. The conductive assembly is also compatible with the precolumn pressures generated by UHPLC configurations.

Using Normal Columns

For applications where convenience, flexibility, and ease-of-use are top priorities, commercially packed columns are an excellent solution. These provide robust, reliable separation and are available with a selection of packing media for various sample types. The NanoSpray® III source allows users to extract the maximum chromatographic performance from commercial columns while offering outstanding usability. The movable finger-tight union provides a consistent, low-dead-volume connection between the column and emitter tip to give optimum chromatographic performance and run-to-run reproducibility (Figure 6). Columns can be installed and disconnected easily without removing the spray assembly or the emitter tip. Similarly, the finger-tight union enables tool-free replacement of emitter tips without disturbing the column connection.

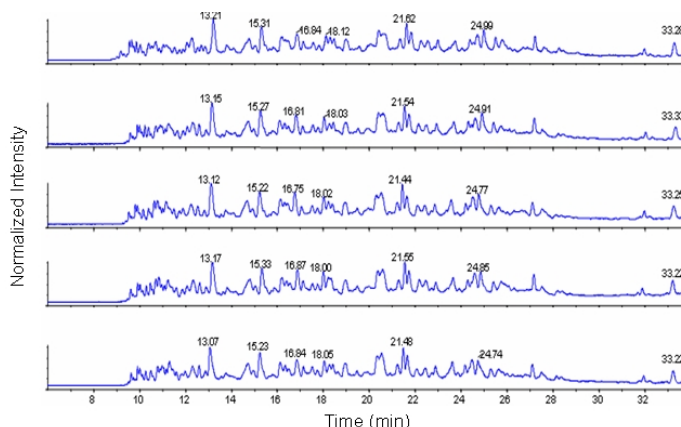


Figure 6. Reproducibility of Ionization. Replicate injections of a 20-protein mixture was performed and the MS TIC was compared across the samples. A commercial C18 nanoLC column (150 × 0.075 mm, 350 nL/min) was used with a 10 µm ID emitter tip (length: 7cm). Good TIC reproducibility was observed.

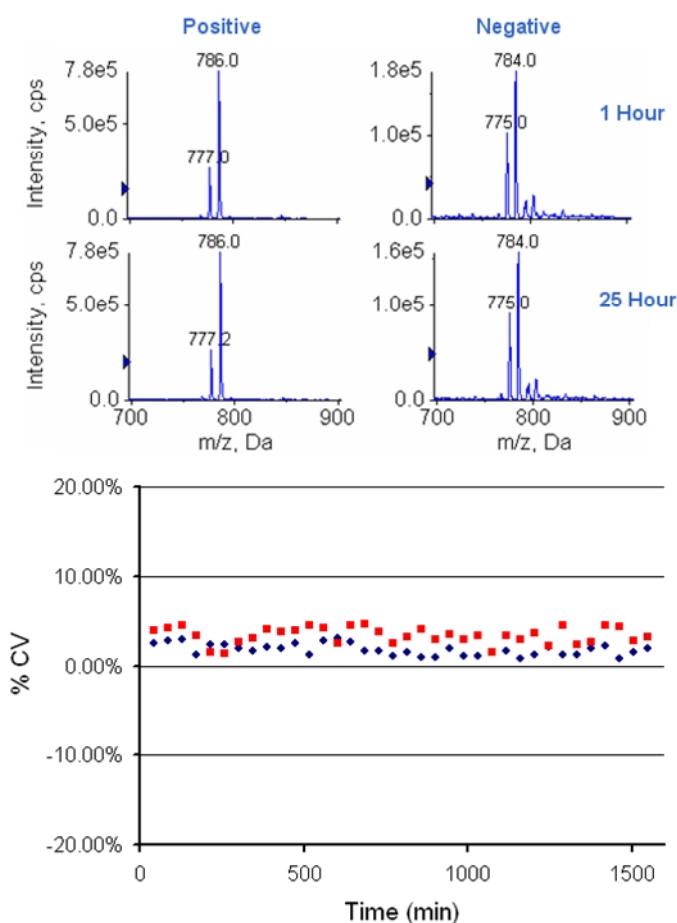


Figure 7. Stability of Polarity Switching. Shown in top panel are the Q1 spectra for Glu-Fibrinopeptide B in + and – mode during 3 sec polarity switching cycles. Below are the identical spectra after 25 hours of continuous polarity switching experiments. The %CV from the positive (blue circles) and negative (red squares) signals from 0-25h throughout the experiment are plotted in the bottom panel.

Used in conjunction with the heated interface, the extremely consistent ‘sweet-spot’ of the interface allows for effortless and reproducible positioning of the sprayer for optimum sensitivity. With the quick, tool-free column attachment and emitter tip replacement, the NanoSpray III source offers unmatched ease-of-use and performance with no compromise.

Polarity Switching and Spray Stability

One common workflow in nanoLC applications involves rapid polarity switching during PTM Discovery experiments, where precursor ion scans are used to determine sites of phosphorylation. In order to assess the stability of the NanoSpray III Source during polarity switching, a 25 hour nano-flow injection acquisition (FIA) experiment was performed using Glu-Fibrinopeptide (GFP), where Q1 was continuously toggled between + and – polarity every four seconds. As shown in Figure 7, the reproducibility of the signal across the 25 hour injection series was extremely high. The top panel shows the mass spectra of Q1 (+/-) for GFP during the 1st injection (hour 1) and the 18th injection (hour 25). More than 21,000 independent polarity switches occurred during this experiment. Throughout, the quality and intensity of the peak shape are identical, and the variance within the experiment was excellent (bottom panel).

Conclusions

The NanoSpray III Source significantly improves the ease of nanoflow applications on all AB SCIEX MS instruments, while still maintaining all the flexibility to address the diverse applications in proteomics. The easily adjustable arm enables a wide use of tip and column configurations. The improved spray visualization provides constant monitoring of spray for easy source optimization. Finally, the preset angle reduces risk of instrument contamination for long term stability.

References

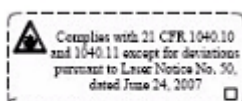
1. Schneider, B.B., et al. J. Am. Soc. Mass Spectrom., 14, 2003, 1236-1246.
2. Schneider, B.B., et al. J. Am. Soc. Mass Spectrom., 16, 2005, 1545-1551.



Label identifying laser classification and specifications.



Laser radiation warning label.



Compliance label



Label identifying location of aperture through which laser beam emerges.

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