Easy-to-Use, Plug-and-Spray Ion Source for Robust and Reproducible Ultra High Pressure Nanoflow LC/MS

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Key Words
EASY-Spray, nanoViper, Peak capacity, Peak resolution, Loading capacity

Introduction

Nanoflow liquid chromatography-mass spectrometry (LC/MS) is widely used for qualitative and quantitative proteomics studies due to the high sensitivity it provides. Nanoflow operation is traditionally unreliable, however. Small imperfections in connections between the tubing, column, high-voltage electrode, and emitter can easily result in poor or irreproducible results. Improper tubing connections cause leaks and large dead volumes that result in substantial peak broadening and thus poor sensitivity. Poor high voltage connections yield poor data due to spray instability.

The Thermo Scientific EASY-Spray nano-electrospray ion source (Figure 1) addresses these issues. It uses Thermo Scientific EASY-Spray columns in which the separation column, column heater, high-voltage electrode, and emitter are combined in a ready-made assembly (Figure 2). EASY-Spray™ columns are easy to install and provide highly reproducible results.

EASY-Spray columns employ Thermo Scientific nanoViper zero-dead-volume (ZDV) fingertight connections that eliminate the assembly of PEEK sleeve connections. nanoViper™ fittings are capable of withstanding pressures up to a 1000 bar and allow tool-free nano-LC connections. Eliminating imperfect connections makes it far easier to achieve state-of-the-art nanospray performance and data.

Chip-based nanoflow systems attempt to solve connections issues through the use of very short microfluidic columns. These columns typically have very limited resolution and loading capacity. By contrast, EASY-Spray columns are available in 15 cm, 25 cm and 50 cm lengths for high-performance separation of complex samples.

Goal

The goal of this note is to demonstrate the ability of the EASY-Spray nanospray source and columns to simplify high-performance nanoflow LC/MS. All important aspects of chromatographic performance are evaluated, including chromatographic resolution, retention time reproducibility, sample loading capacity, and column robustness.

Figure 1. EASY-Spray nano-electrospray ion source with EASY-Spray column.

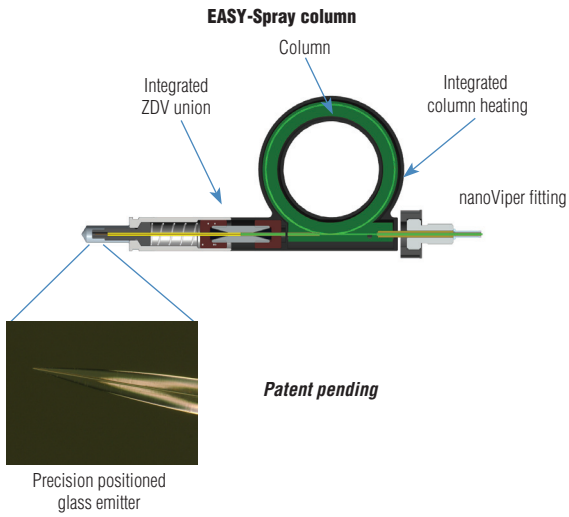

Figure 2. The EASY-Spray column incorporates a column up to 50 cm in length, column heater, high-voltage electrode and integrated emitter, all preassembled with nanoViper fittings to eliminate dead volumes. It provides immediate, stable spray when the column is plugged in.

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Experimental

EASY-Spray Column Concept

Figure 2 shows the design and configuration of the EASY-Spray column assembly. The precisely positioned, diamond-polished, 5 µm i.d. fused-silica emitter allows immediate, stable spray as soon as the column is plugged into the EASY-Spray source[1]. Conversely, when the column is unplugged from the source, the emitter automatically retracts, protecting the tip from damage. Samples can be loaded onto the EASY-Spray column directly or passed through a trap column for desalting before loading onto the EASY-Spray column.

Samples

Simple BSA digests and complex digest mixtures (*E. coli* and human cell) were used for the evaluation.

- BSA: 100 fmol/µL
- *E. coli* digests: 500 ng/µL
- Human cell (k569) digests: 1 µg/µL

Nano-LC

A Thermo Scientific EASY-nLC 1000 nano-LC system was used. Two different types of EASY-Spray columns (50 µm i.d. × 15 cm and 75 µm i.d. × 50 cm) packed with 2 µm Thermo Scientific Acclaim PepMap RSLC C18 beads were used. Multiple flow rates (150 nL/min, 300 nL/min, 400 nL/min and 500 nL/min) and column temperatures (ambient, 35 °C, 45 °C and 55 °C) were used to test peak capacity, resolution, retention time reproducibility, sample loading capability and column robustness. The samples were loaded directly onto the column for all experiments. One microliter of each sample was injected per run.

Mobile phase:

A: Water containing 0.1% formic acid (0.1% FA water)

B: Acetonitrile containing 0.1% formic acid (0.1% FA ACN)

Mass Spectrometry

The EASY-Spray source was tested on Thermo Scientific Orbitrap Velos Pro and Orbitrap Elite hybrid ion trap-Orbitrap mass spectrometers. For ionization, a spray voltage of 1800 V and capillary temperature of 250 °C were used.

Orbitrap Elite™ MS: One full MS scan (60,000 resolution) followed by 20 rapid CID MS/MS scans

Orbitrap Velos Pro™ MS: One full MS scan (30,000 resolution) followed by 15 CID MS/MS scans

Data Processing

Thermo Scientific Proteome Discoverer software version 1.3 was used for database search with the Mascot search engine with a 1% FDR.

Results

High Column Efficiency and Better Chromatographic Resolution

The combined column/sprayer fitted with nanoViper fittings eliminated dead volumes and offered high column efficiency and resolution. This resulted in ultra-sharp 3.5 s peaks (full width half maximum – FWHM) for 100 fmol of BSA (Figure 3).

Figure 4 shows a base peak chromatogram of a 500 ng *E. coli* digest. The data were collected on an Orbitrap Elite instrument at 300 nL/min. Over 1000 unique proteins were identified from this single 60 minute gradient run. The high column resolution resulting in maximized peptide coverage was sustained over lower and higher flow rates (Figure 5).

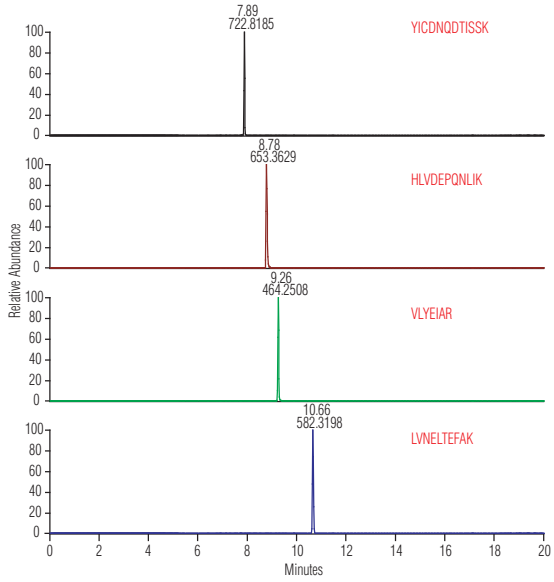

Figure 3. Extracted peptide peaks from a 100 fmol BSA sample analyzed on an Orbitrap Elite instrument. Ultra-sharp peaks (3.5 s FWHM) were observed. A 50 µm i.d. × 15 cm column was used for the separation. The gradient was 5% B to 30% B in 15 minutes at 300 nL/min. The column heater was set at 35 °C.

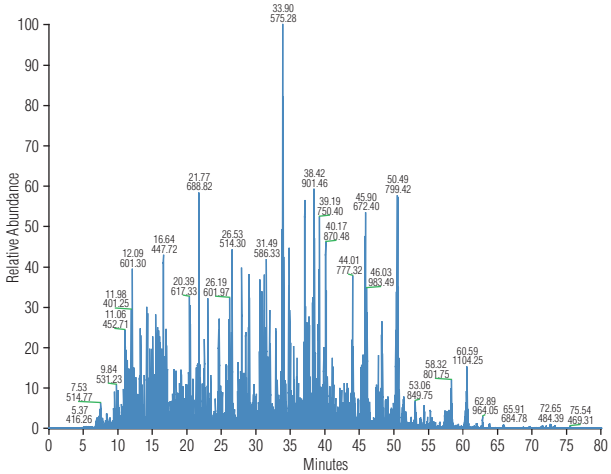

Figure 4. Base peak chromatogram of a 500 ng *E. coli* digest collected on an Orbitrap Elite instrument. This particular run identified 1019 unique proteins. A 50 µm i.d. × 15 cm column was used for the separation. The gradient was 5% B to 30% B in 60 minutes at 300 nL/min. The column heater was set at 35 °C.

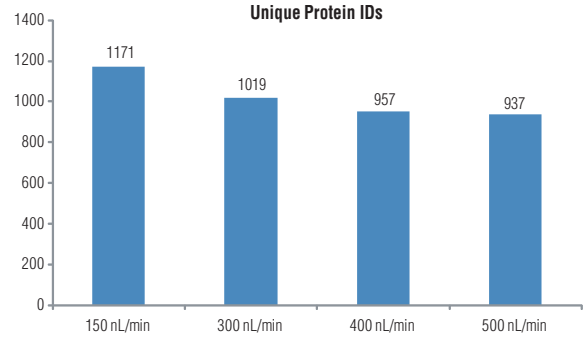

Figure 5. The number of unique proteins identified in a 500 ng *E. coli* digest at different flow rates. A 50 µm i.d. × 15 cm column was used for the separations. The gradient was 5% B to 30% B in 60 minutes. The column heater was set at 35 °C.

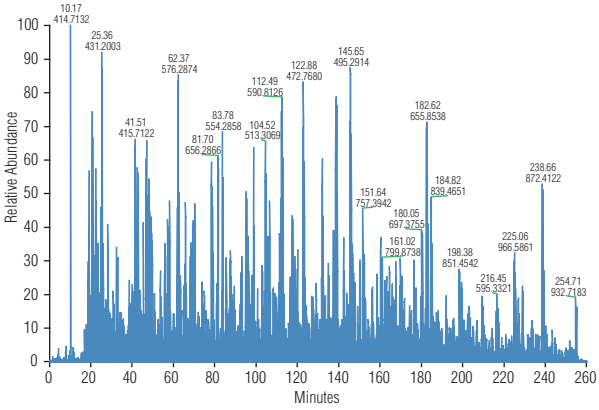

Figure 6. Base peak chromatogram from a 1000 ng human cell (k569) digest acquired on a Orbitrap Elite instrument. A 75 µm i.d. × 50 cm column was used for the separation. The gradient was 5% B to 30% B in 240 minutes at 300 nL/min. The column heater was set at 35 °C.

Even higher column efficiency and larger sample-loading capacity were achieved by using the longer 50 cm column. Figure 6 shows a representative base peak chromatogram of a 1000 ng human cell (k569) digest collected on an Orbitrap Elite instrument. More than 4000 unique proteins were identified from a single 240 minute run.

Outstanding Retention Time Reproducibility

A high degree of retention time reproducibility is essential for label free quantitation workflows. To evaluate retention time reproducibility of the EASY-Spray columns, analyses of 500 ng samples of an *E. coli* digest were performed at multiple flow rates (150 nL/min, 300 nL/min, 400 nL/min, 500 nL/min) with a fixed column temperature (35 °C) and multiple column temperatures (ambient, 35 °C, 45 °C, 55 °C) with a fixed flow rate (300 nL/min). Each condition was run 20 or more times.

Figure 7 shows the base peak chromatograms of the *E. coli* digests from the beginning, middle and end of 45 runs. Excellent retention time reproducibility was observed across all 45 runs. The coefficient of variation (CV) of retention time over 20 runs was only 0.4% at room temperature and 0.3% at heated column temperature of 35 °C, 45 °C and 55 °C for the detected *E. coli* peptide EAVNQVIALLDSGALR (Figure 8).

Similarly good retention time precision was observed for the repeated runs at different flow rates. The retention time CVs were less than 0.4% for each 20 repeat run set at various flow rates for the detected *E. coli* peptide EAVNQVIALLDSGALR (Figure 9).

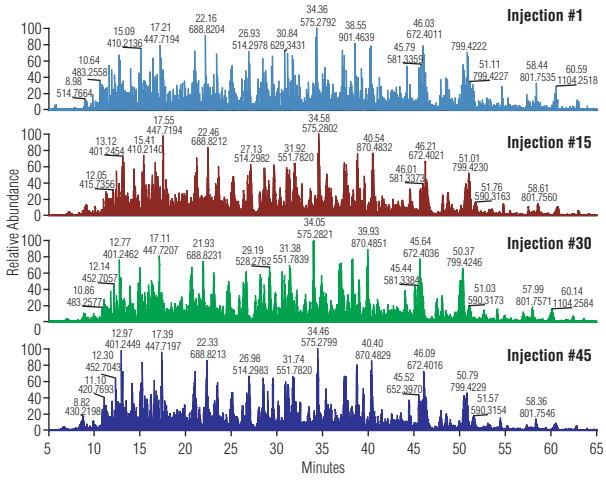

Figure 7. Selected base peak chromatograms from 500 ng samples of *E. coli* digest analyzed on an Orbitrap Velos Pro. A 50 µm i.d. × 15 cm column was used for the separation. The gradient was 5% B to 30% B in 60 minutes at 300 nL/min. The column heater was set at 35 °C.

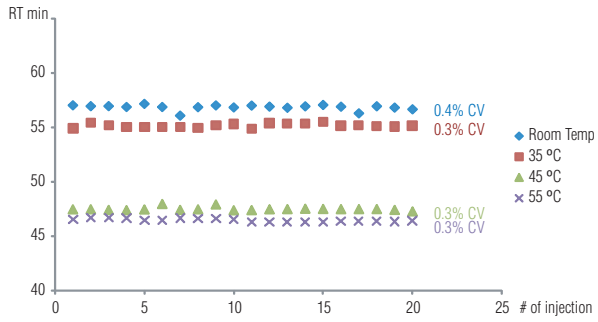

Figure 8. The calculated %CV of retention time for over 20 injections at different column temperatures a detected *E. coli* peptide EAVNQVIALLDSGALR. A 50 µm i.d. × 15 cm column was used for the separation. The gradient was 5% B to 30% B in 60 minutes at 300 nL/min.

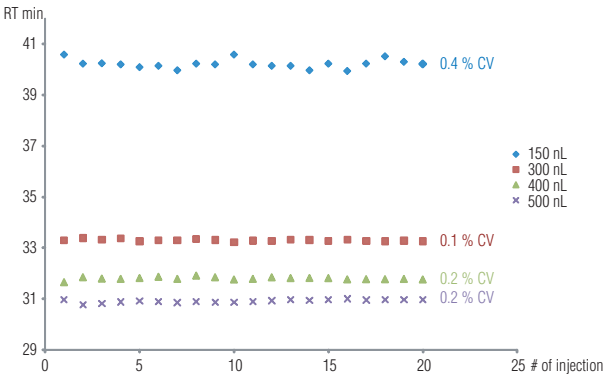

Figure 9. Calculated %CV of retention time over 20 injections at different flow rates for a detected *E. coli* peptide GNFDLEGLER. A 50 µm i.d. × 15 cm column was used for the separation. The gradient was 5% B to 30 % B in 60 minutes. The column temperature was 35 °C.

Column Robustness

Two hundred injections of the 500 ng *E. coli* digest sample were carried out to test the life time of the EASY-Spray column (Acclaim™ PepMap™ RSLC C18, 50 µm i.d. × 15 cm, 2 µm). Figure 10 shows stacked base peak chromatograms from the first run and the two hundredth run. The column resolution and retention time remained constant over 200 injections.

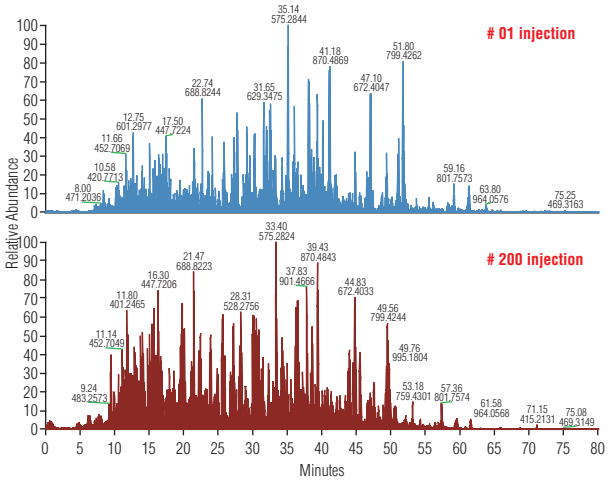

Figure 10. Longevity test on EASY-Spray column (Acclaim PepMap C18 RSLC, 50 µm i.d. × 15 cm, 2 µm)

Column-to-Column Reproducibility

Three EASY-Spray columns (Acclaim PepMap RSLC C18, 50 µm i.d. × 15 cm, 2 µm) were used for testing the column-to-column reproducibility. Figure 11 shows stacked base peak chromatograms of *E. coli* digest samples collected from the three columns. Very similar column resolution and retention time were observed for all three columns.

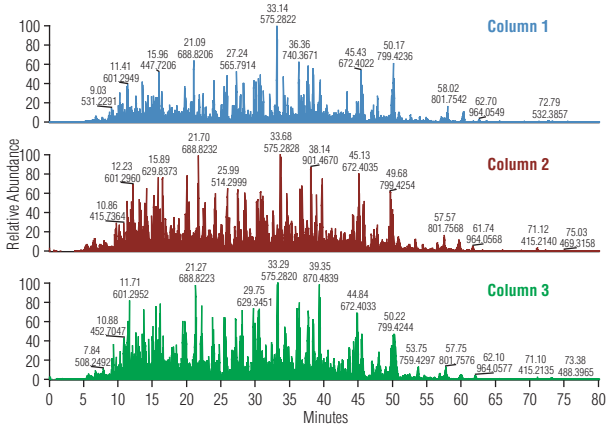

Figure 11. Base peak chromatograms of 500 ng *E. coli* digest samples, showing column-to-column reproducibility on EASY-Spray column (Acclaim PepMap C18 RSLC, 50 µm i.d. × 15 cm, 2 µm)

Conclusion

The EASY-Spray concept provides an integrated, temperature-controlled column-emitter solution that requires a user to make a single nanoViper connection between the LC and the MS source to achieve exceptional nanoflow LC/MS performance. EASY-Spray columns eliminate dead volumes, assuring narrow peaks and excellent results. EASY-Spray columns are available in 15 cm, 25 cm and 50 cm lengths for high separation efficiency and resolution to maximize peptide coverage. In contrast, chip-based microfluidic systems use short columns with limited resolution and loading capacity and may lack temperature control.

The new EASY-Spray column design provides excellent retention time reproducibility over wide range of flow rates and column temperatures as tested on a single column and in column-to-column tests. The EASY-Spray emitter also demonstrated high robustness. With its ease-of-use, robustness and reliability, the new EASY-Spray source provides state-of-the-art nanoflow HPLC performance for both MS experts and non-experts.

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