New NanoESI Source for Increased NanoLC-MS Performance in a Plug-and-Spray Configuration

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Overview

Purpose: Evaluate the performance of a new nanospray ion source and nanoflow column assemblies that incorporate a nanoflow column, column heater, high-voltage electrode, and emitter into an integrated unit. Key aspects of chromatographic performance are evaluated: chromatographic resolution, retention-time reproducibility, sample loading capacity, and column robustness.

Methods: A prototype nanospray source was installed on the mass spectrometer and connected to a nanoLC pump. Column assemblies were tested to evaluate column-to-column and run-to-run reproducibility. Complex peptide mixtures and a simple digest mixture were used for the evaluation. The retention-time reproducibility, peak shape, resolution and peak capacity, in different temperature ranges, were evaluated. In order to test the device flexibility, several flow rates from 150 nL/min to 500 nL/min were used.

Results: A high spray stability was achieved over all LC runs. The chromatographic resolution, sensitivity, and reproducibility obtained matched other state-of-the-art data without need for adjustments, intervention or other actions beyond simply inserting the column/sprayer assembly into the nanospray source. More than 1000 proteins were identified with increased component detection in the complex protein digest mixtures. The column-to-column retention-time reproducibility for the targeted peptides in the simple digest mixture was excellent.

Introduction

Nanoflow LC-MS is widely used for qualitative and quantitative proteomics studies due to the high sensitivity it provides. However, inconsistency and irreproducibility have historically been common due to imperfect connections between components and difficulty in achieving optimum alignment in the ion source. Improper connections can result in leaks and large dead volumes that cause substantial peak broadening and poor sensitivity. Poor high-voltage connections cause spray instability resulting in poor data. Incorrect alignment reduces sensitivity.

Chip-based nanoflow systems have attempt to solve connection issues through the use of very short microfluidic columns. These systems solve some of the connection and alignment issues, but the columns typically have very limited resolution and loading capacity.

In order to address the connection and alignment issues without sacrificing chromatographic resolution and capacity, a new nanoelectrospray ion source and integrated column assemblies were developed. Each column assembly incorporates a separation column, column heater, high-voltage electrode, and emitter into a unit that is easy to install and operate. Column lengths up to 50 cm are available. Zero-dead-volume fingertight connectors that eliminate the need for PEEK sleeved connections and withstand pressure up to 1000 bar are used. The column assembly installs in the ion source with no tools and requires no alignment.

Methods

Sample Preparation

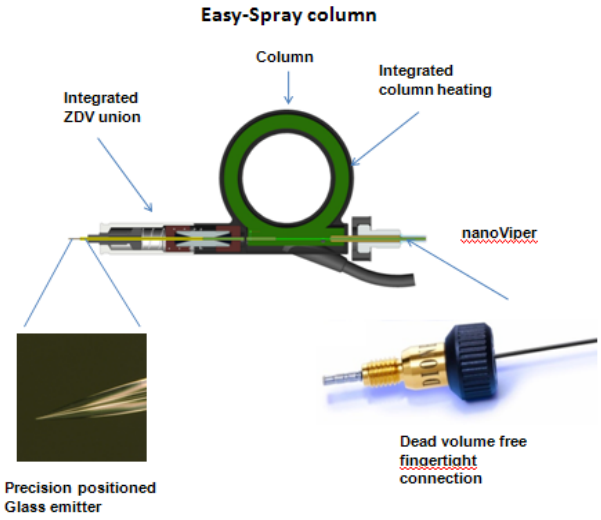
Simple BSA digest and complex digest mixtures (*E.coli* and human cell) were used for the evaluation.

1) BSA digest: 100 fmol/µL
2) *E.coli* digest mixture: 500 ng/µL
3) Human cell (k569) digest: 1 µg/µL

Nano-LC

A Thermo Scientific EASY-nLC 1000 was used. Figure 1 shows a schematic of the new Thermo Scientific EASY-Spray column. Two different EASY-Spray™ columns (50 µm i.d. x 15 cm and 75 µm i.d. x 50 cm) packed with 2 µm Thermo Scientific Acclaim PepMap C18 beads were used in these tests. Flow rates of 150 nL/min, 300 nL/min, 400 nL/min and 500 nL/min, and column temperatures of ambient, 35 °C, 45 °C and 55 °C, were used to test peak capacity, resolution, retention-time reproducibility, sample loading capacity and column robustness. Samples were loaded directly onto the column in all experiments. One microliter of each sample was injected per run. The mobile phases were 0.1% formic acid/water and 0.1% formic acid/acetonitrile. A 15 min linear gradient was used for the BSA digest separation. 60 min and 240 min linear gradients were used for the *E. coli* and human cell separations, respectively.

FIGURE 1. The new EASY-Spray column incorporates up to a 50 cm column, column heater, high-voltage electrode, and integrated emitter. The high-pressure end of the assembly is fitted with Thermo Scientific nanoViper fittings to eliminate leaks and dead volumes in the connection to the nanoLC. A trap column can be used for desalting if needed.



Patents pending

MS

The new Thermo Scientific EASY-Spray source was installed on a Thermo Scientific Orbitrap Elite hybrid ion trap-Orbitrap mass spectrometer. Source settings: spray voltage of 1800 V and capillary temperature of 250 °C.

Mass spectrometer parameters: One full MS scan (60,000 R) followed by 20 rapid collision-induced dissociation (CID) MS/MS scans.

Data Processing

Thermo Scientific Proteome Discoverer software verion1.3 was used for database search with the Mascot™ search engine and a 1% FDR.

Results

High Column Efficiency and Better Resolution

The integrated column/sprayer eliminated dead volumes and offered very high column efficiency and resolution. This resulted in ultra-sharp peaks (3.5 s full-width half-maximum for 100 fmol BSA, Figure 2). Figure 3 shows a base-peak chromatogram of the 500 ng *E.coli* digests collected on the Orbitrap™ Elite system at a 300 nL/min flow rate. Over 1000 unique proteins were identified over a single 60 min gradient run (Figure 3, insert). The high column resolution maximized peptide coverage sustained over the lower and higher flow rates (Figure 4).

Column efficiency and sample loading capacity were increased using the longer 50 cm column. Figure 5 shows a representative base-peak chromatogram of 1000 ng human cell (k569) digests. More than 4000 unique proteins were identified in a single 240 min run.

Outstanding Retention Time Reproducibility

Five hundred nanogram injections of an *E.coli* digest were used to test retention time precision. To evaluate the retention-time reproducibility of the EASY-Spray column at various conditions, different flow rates (per “Methods”) with 35 °C column temperature, and different column temperatures (per “Method”) with 300 nL/min flow rate, were used. Each condition was run 20 or more times. Figure 6 shows the base peak chromatograms of the *E.coli* digests at the beginning, middle and the end of the 45 runs. Excellent retention time reproducibility was observed across all 45 runs. For the detected *E.coli* peptide EAVNQVIALLDSGALR, Figure 7 shows that the coefficient of variation (CV) of retention time over 20 runs was only 0.4% at room temperature, and 0.3% at the heated-column temperatures. Similar retention time precision was observed for the runs at different flow rates. For the detected *E.coli* peptide EAVNQVIALLDSGALR, the retention time CVs were less than 0.4% for each 20-repeat run set at the various flow rates (Figure 8).

Longevity

Figure 9 shows the outstanding longevity of the EASY-Spray column (Acclaim® PepMap™ C18, 50 µm i.d. x15 cm, 2 µm) over 200 injections.

Column-to-Column Reproducibility

Three EASY-Spray columns (Acclaim PepMap C18, 50 µm id x15 cm, 2 µm) were used for testing the column-to-column reproducibility. Figure 10 shows the base peak chromatograms of a 500 ng E.coli digest sample collected from the three columns. Similar column resolution and retention times were observed for all three columns.

FIGURE 2. Extracted peptide peaks from 100 fmol BSA. Sharp, symmetrical peaks of 3.5s (FWHM) were observed. A 50 µm i.d. x15 cm column, packed with 2 µm Acclaim PepMap C18 beads, was used for the separation. The gradient was 5% B to 30% B in 15 min at 300 nL/min flow rate. The column heater was set at 35 °C.

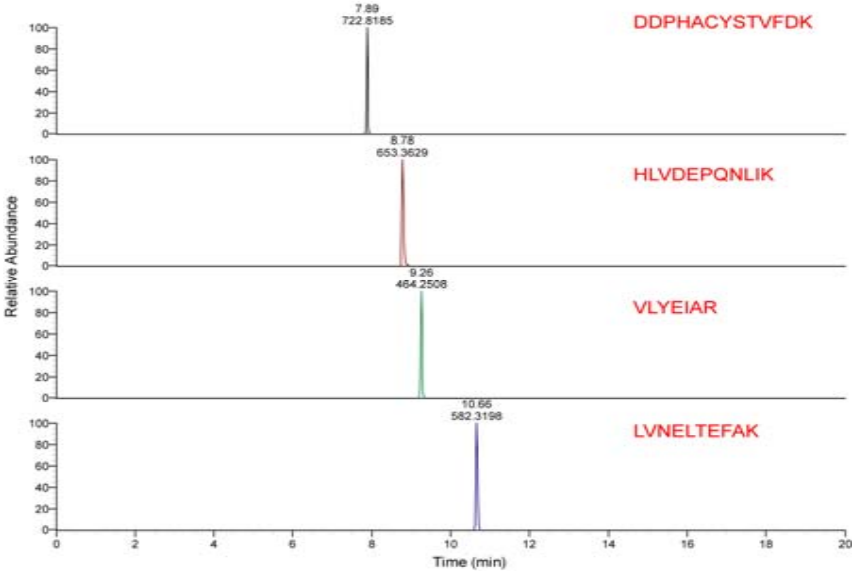


FIGURE 3. Representative base-peak chromatogram of 500 ng *E. coli* digests.

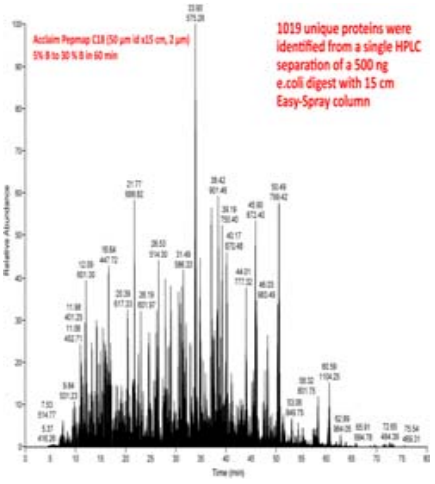


FIGURE 4. Representative number of unique proteins identified in a 500 ng *E. coli* digest collected at different flow rates

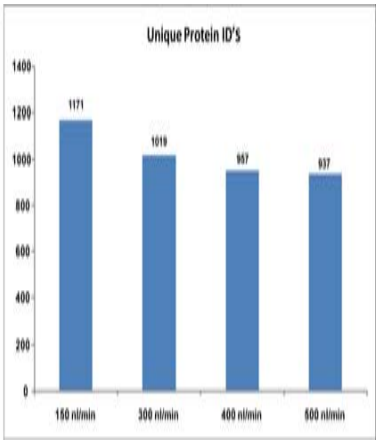


FIGURE 5. Representative base peak chromatogram of one 1000 ng human cell (k569) digest. The insert shows the database search results from this single LC-MS run.

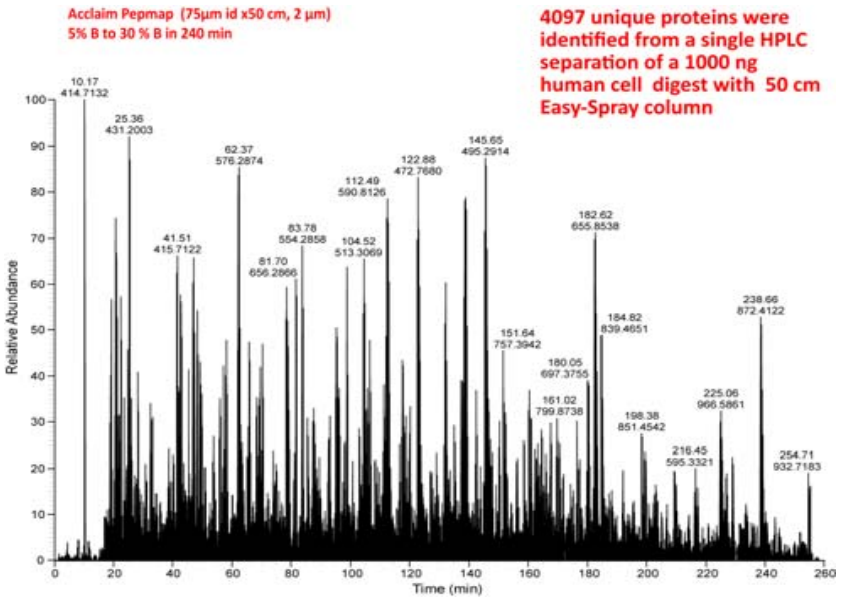


FIGURE 6. Overlays of selected base peak chromatograms of 500 ng *E. coli* digests collected on the Orbitrap system over 45 repeat runs

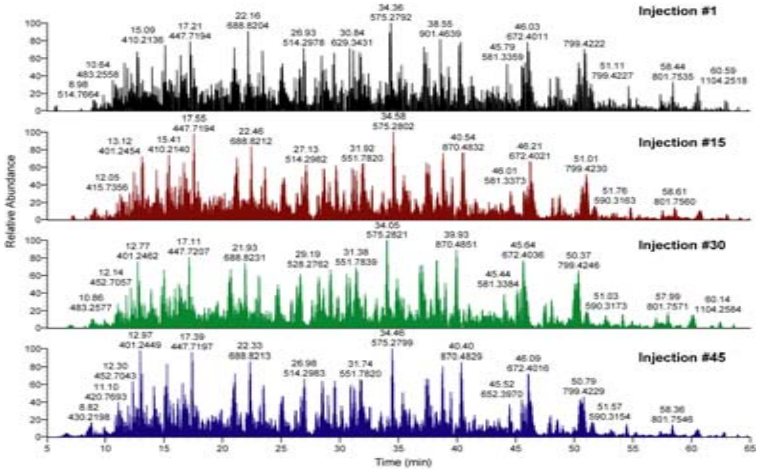


FIGURE 7. The calculated % CV of retention time for the *E. coli* peptide EAVNQVIALLDSGALR over 20 injections at different column temperatures.

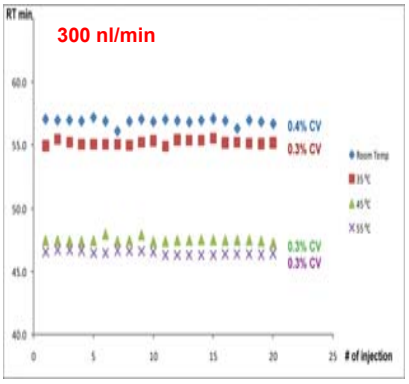


FIGURE 8. The calculated % CV of retention time for a *E. coli* peptide GNFDLEGLER over 20 injections at different flow rates.

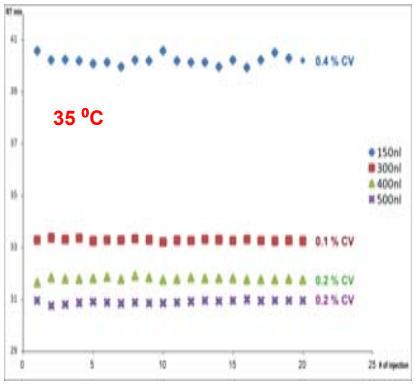


FIGURE 9. Longevity test on the EASY-Spray column (Acclaim PepMap C18, 50 µm i.d. x 15 cm, 2 µm).

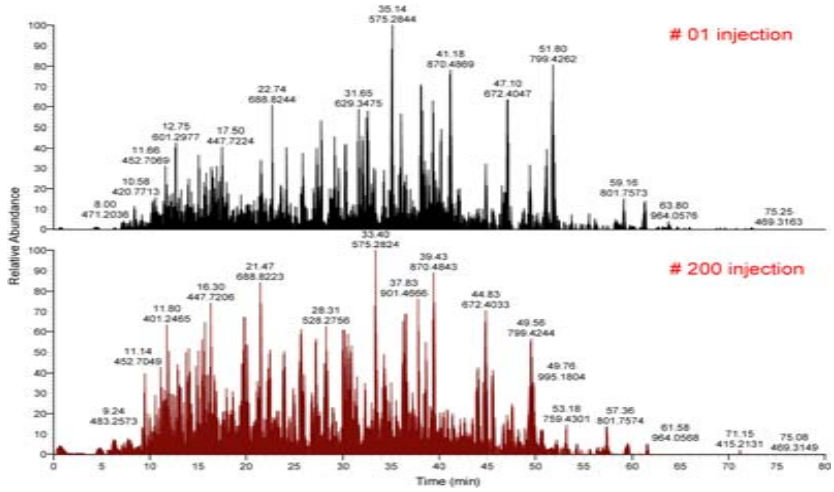
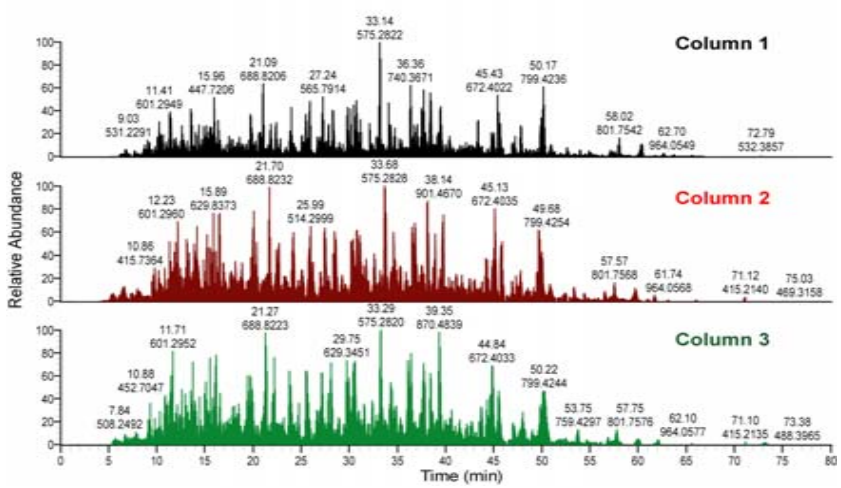


FIGURE 10. Column-to-column reproducibility on the EASY-Spray column (Acclaim PepMap C18, 50 µm i.d. x 15 cm, 2 µm).



Conclusion

- Integrated EASY-Spray columns install in the EASY-Spray source without tools and require no adjustments to alignment.
- The EASY-Spray columns require only one nanoViper™ fingertight zero-dead-volume connection between the LC and the MS source.
- The EASY-Spray columns provided excellent chromatographic separation with ultra-sharp peaks (3.5 s full-width half-maximum for 100 fmol BSA in a 15 min run). Thousands of proteins were identified from complex samples in single 60–240 min runs.
- Excellent retention-time reproducibility was observed over a wide range of flow rates (150–500 nL/min) and column temperatures (ambient to 55 °C) as tested in replicate single-column and column-to-column tests.
- The system exhibited a high level of robustness.

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