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EASY-Spray PepMap RSLC C18 Column

# Increased sample throughput with a capillary EASY-Spray column

## Benefits

- Higher sensitivity compared with Analytical scale LC

- Higher throughput than in standard nanoLC experiments

- Robust column design for high throughput proteomics

- Designed for TFA-free LC-MS analysis, minimizing ion suppression effects

- Easy to use format for Capillary LC

## Keywords
Capillary LC, high throughput, proteomics

## Introduction
The Thermo Scientific™ EASY-Spray™ 2 µm 15 cm × 150 µm capillary column raises the bar in proteomics by greatly increasing the number of samples that can be analyzed by proteomics laboratories when compared to classical nanoLC methods. Capillary LC offers advantages in terms of ease of use when compared to nanoLC and also higher sensitivity compared to Analytical LC. The column is fully compatible with Thermo Scientific™ Capillary LC and Thermo Scientific™ Orbitrap™ mass spectrometer systems to offer a seamless integration of speed, sensitivity and robustness required for high throughput proteomics workflows.

The EASY-Spray design is a simple and easy to use "plug and spray" approach designed to improve throughput and is a novel alternative to classic low flow LC columns and sources. Integrating this technology with a 150 µm i.d. column further enhances productivity by the ability to increase throughput capacity for current nanoLC users, or by increasing sensitivity for analytical scale LC users.

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## Column technology

The EASY-Spray 15 cm x 150 µm column is packed with Thermo Scientific™ Acclaim™ PepMap™ C18 2 µm 100Å particles and has a 150 µm inner diameter. It is used for the separation of digested peptides that are used for the identification of proteins in proteomics applications. The Acclaim PepMap column is designed for TFA-free analysis, which minimizes ion suppression effects in mass spectrometry experiments.

## Applications

### Improved sensitivity

There is a clear experimental relationship between flow rate and sensitivity that can be described by a power law function (Figure 1). Thus, major improvements in sensitivity can be achieved at nano and capillary flow rates compared to analytical flow rates.

The EASY-Spray capillary column with 150 µm ID can be operated at a wide flow range from 1 to 3 µL/min, corresponding to linear velocities from 2 to 4 mm/s. Results have shown that capLC-MS can be 40 times more sensitive than analytical flow LC-MS at 450 µL/min and between 2 to 4 times less sensitive than nanoLC-MS at 300 nL/min (Figure 1), when loading the same amount of sample onto the column*.

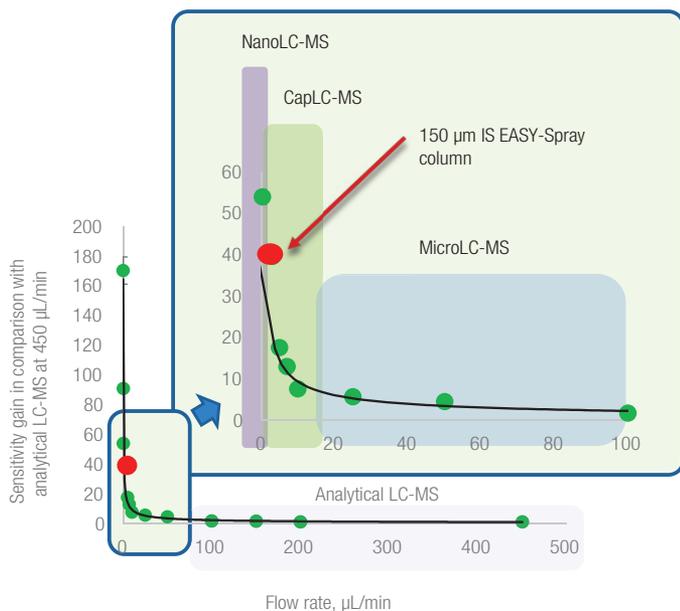* Sensitivity gain or loss are dependent on compound and application



Figure 1. The increase in sensitivity experienced with low-flow LC-MS compared to analytical flow LC-MS

The EASY-Spray 15 cm × 150 µm column has an optimal flow rate of 3 µL/min when used for high-throughput, targeted analysis. When used in profiling of proteomics samples with our capLC-MS platform, an optimal flow rate of 1.2 µL/min was been shown to be ideal for the application used. Typical chromatograms for capLC-MS separations at 3 and 1.2 µL/min (Figure 2 and 3) show that the separation performance is not compromised at low flow rates and is comparable to analytical flow separations. The peak width at half maximum (PWHM) is about 3 s for fast capLC-MS separations at 3 µL/min and less than 15 s for longer gradients at 1.2 µL/min, which results in peak capacities of approx. 300 for both gradients.
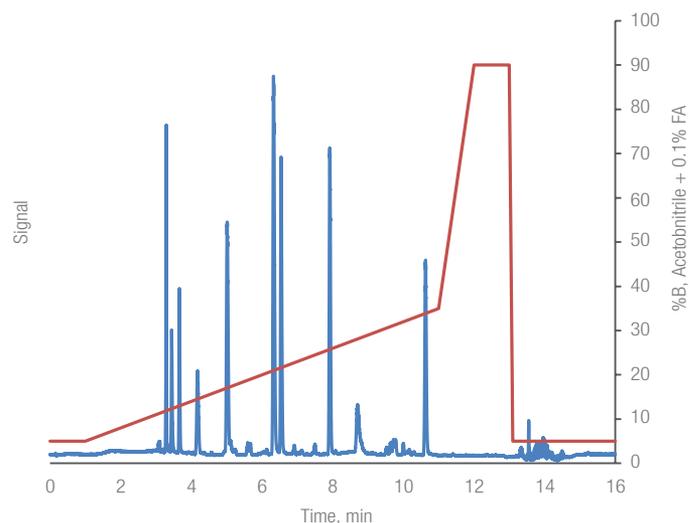


Figure 2. Typical chromatogram for 1 pmol of CytC protein digest on column. Flow rate: 3 µL/min backpressure: 450 bar



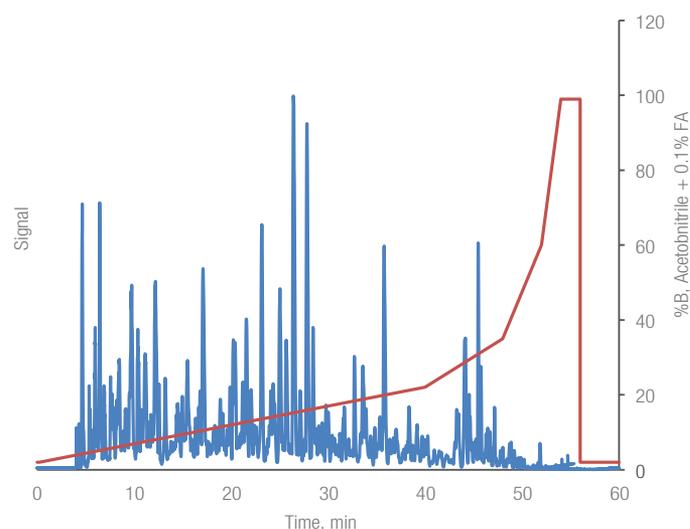Figure 3. Typical chromatogram for 1 µg of HeLa cell lysate digest on column. Flow rate: 1.2 µL/min backpressure: 200 bar

*Robustness*

The EASY-Spray 15 cm × 150 µm column was monitored over an extended injection sequence. A Thermo Scientific™ capillary-flow UltiMate™ 3000 RSLCnano system capLC system was configured for direct injections and evaluated over 8 days of operation (Figure 4a). Over this time period, an excellent retention time stability (RSD < 1%) was observed for 350 injections.

The peak area stability was evaluated on MS1 level for 150 consecutive injections conducted between day 5 and 8 (Figure 4b). The peak areas achieved RSD values of less than 10% and were obtained even without internal standard correction during long term testing.
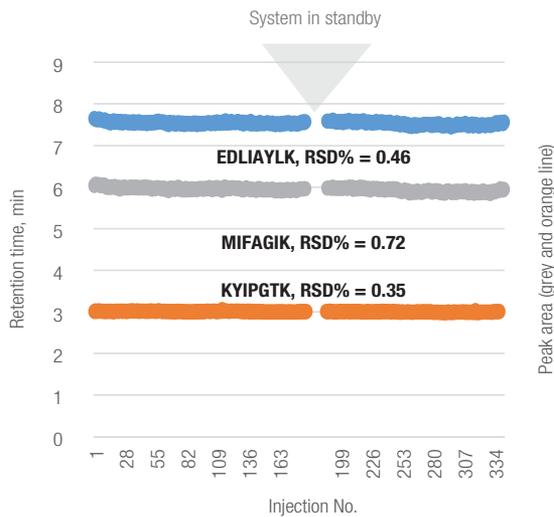


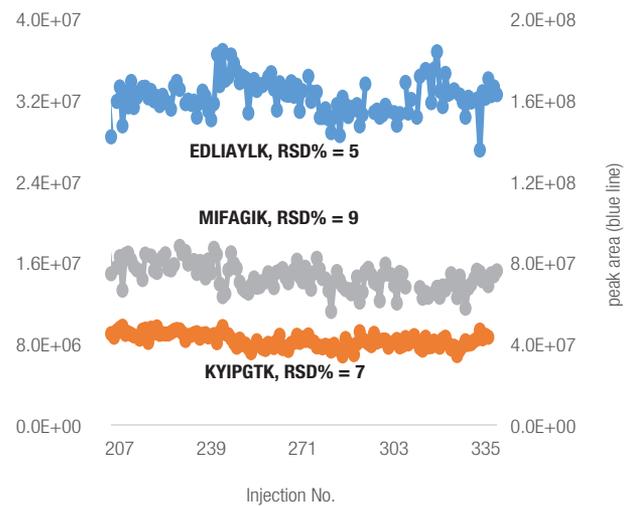Figure 4a. Retention time stability for cap LC-MS analysis of CytC protein digest.



Figure 4b. Peak area stability for cap LC-MS analysis of CytC protein digest.

## Operational specifications

| Parameter | Recommendation |
|---|---|
| Typical applications | Peptide analyses |
| Base material | Spherical silica, 100 Å pores |
| Particle size | 2 µm |
| Chemistry | C18, endcapped |
| Column dimensions | 150 µm i.d. × 15 cm |
| Recommended flow | 1–3 µL/min |
| Recommended temperature | 25–60 °C |
| Maximum pressure | 1000 bar |
| Recommended sample quantity[1] | 5 µg |
| pH stability | 2–8 |
| Solvent compatibility | All common RP solvents |

[1] The recommended sample quantity of the Acclaim PepMap100 150 µm i.d. columns is given in weight amount of a protein digest of bovine serum albumin.

## Ordering Information

| Description | Particle size (µm) | Part number |
|---|---|---|
| EASY-Spray PepMap RSLC C18 15 cm × 150 µm | 2 | ES906 |

## Find out more at **thermofisher.com/EASYspray**

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