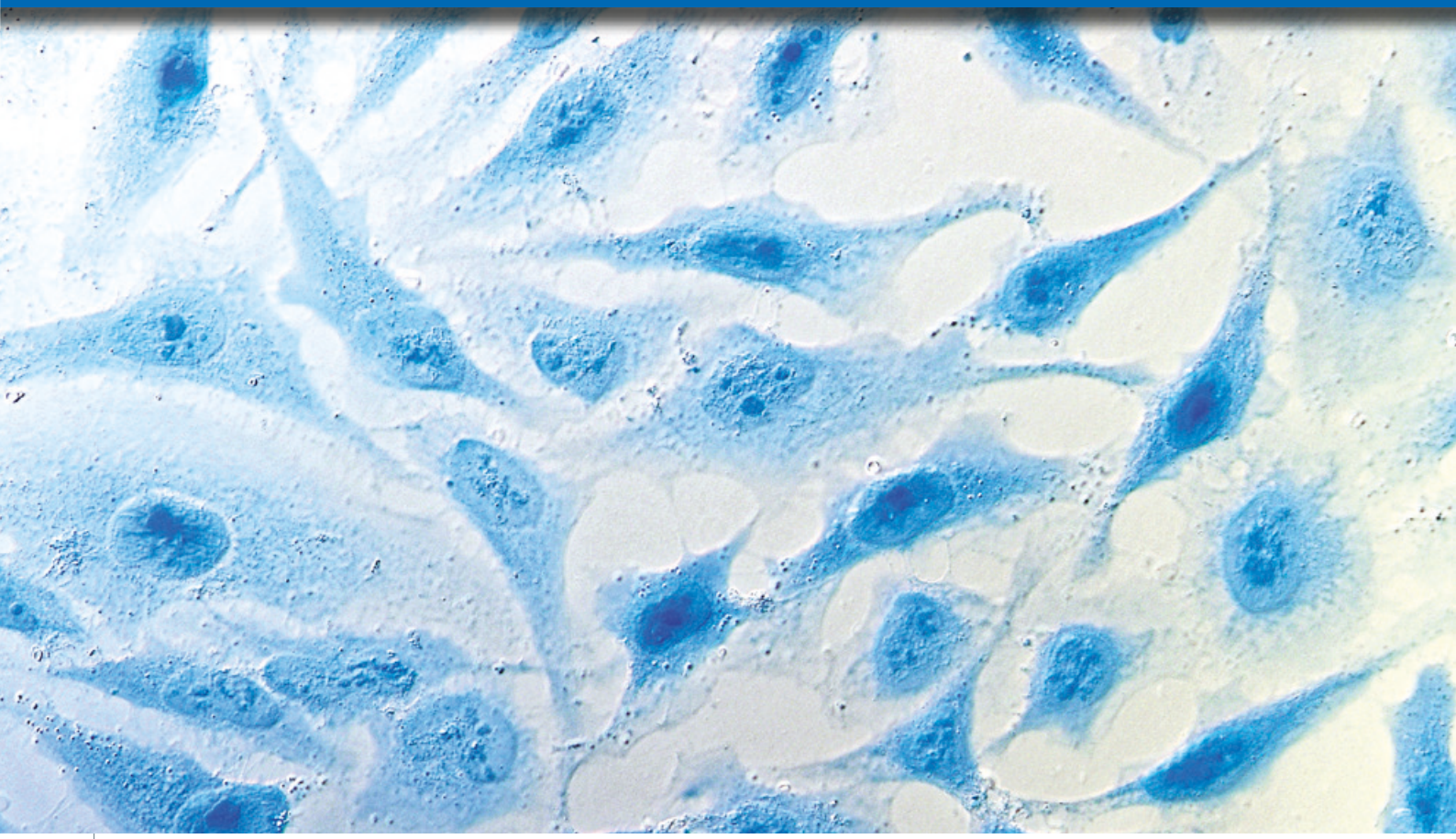

Bruker's nanoElute UHPLC shows excellent performance and ease of use for proteomics

Abstract

Bruker's new nanoElute nanoflow UHPLC offers excellent chromatographic performance in terms of separation and reproducibility with different gradient lengths, and with or without a trap column.

Introduction

Samples in shotgun proteomics are often highly complex and can easily contain thousands of peptides in a narrow mass range which leads to analytical challenges. Therefore peptide separation is commonly performed by nano-flow UHPLC, which brings several advantages, including high peak capacity and ESI compatible solvent composition and flow rate. Despite the increasing speed and sensitivity of mass spectrometers, enhancing their ability to analyze more ions eluting from the column at any given time, an improvement of chromatographic separation still provides a tremendous boost

Keywords: LC-MS, nanoElute, nanoflow, peak area reproducibility, retention time stability, peak width, peak capacity

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in the number of identified proteins. Furthermore, highly reproducible peak areas and retention times are crucial for more sophisticated quantitative analyses. To cover all these demands, a reliable HPLC system is essential.

In this study we evaluated the four essential chromatographic performance indicators of the Bruker nanoElute: retention time variation, peak area variation, peak width and peak capacity, to test the nanoElute for the challenging requirements of sophisticated proteomic analytics.

Experimental

Samples

Aliquots of a commercially available tryptic digest of HeLa cells were diluted with 0.1 % formic acid in water to a concentration of 100 ng/µL.

For chromatographic separation a curved gradient with three different lengths was used, as shown in the table below. The gradient consists of solvent A, 0.1 % formic acid in water and solvent B, 0.1 % formic acid in acetonitrile with the temperature of the separation column maintained at 50 °C and a flow rate of 400 nL/min.

Four technical replicates were made with the 90 min gradient and five technical replicates with the 120 min gradient and the 240 min gradient, each measured using a setup without a trap column, and with a trap column. Switching between setups with and without a trap column is conveniently and easily done in the software.

For MS detection an Impact II QTOF mass spectrometer equipped with a CaptiveSpray nanoBooster Source with pure ACN was used. The instrument was operated in ESI positive mode acquiring full scan MS and MS/MS data using the InstantExpertise™ routine. This is a self-adapting auto MS/MS method which is designed to obtain highest quality results independently of the complexity and concentration of the sample. It uses an advanced parent ion selection procedure combined with a variable MS/MS acquisition rate [1] and is pre-installed in the impact II instrument control software (otofControl).

Data Analysis

For analyzing the chromatographic data Bruker's Compass DataAnalysis was used. Eight peptides, which were evenly distributed in time through the gradient, were selected from the HeLa chromatogram. EICs were made for these peptide masses and the peak

Liquid chromatography

		Gradient Conditions:	90 min Gradient	120 min Gradient	240 min Gradient	Composition B
Instrument:	Bruker nanoElute™					
Column:	Acclaim PepMap™ RSLC; 75 µm x 50 cm		0 min	0 min	0 min	2 % B
Mobile phase A:	Water, 0.1 % formic acid		60 min	90 min	180 min	15 % B
Mobile phase B:	ACN, 0.1 % formic acid		90 min	120 min	240 min	25 % B
Trap column loading:	100 % mobile phase A		100 min	130 min	250 min	35 % B
Flow rate:	400 nL/min		110 min	140 min	260 min	95 % B
Injection volume:	2 µL		120 min	150 min	270 min	95 % B
Column oven:	50 °C					

Mass Spectrometry

Instrument:	Bruker impact II QTOF mass spectrometer	Dry Gas:	3.0 L/min
Ion source:	CaptiveSpray nanoBooster in positive ion mode	Dry Temperature:	150 °C
Capillary:	1600 V	nanoBooster:	0.20 Bar

area, retention time and peak widths were compared between the technical replicates.

Results and Discussion

The gradient length in shotgun proteomics typically varies between 90 min to 240 min. With the pressure limit of the new nanoElute of 1000 bar, combined with a column oven, 50 cm column lengths are possible. The nanoElute Method Editor allows any gradient to be generated with only a few mouse clicks and all typical wash and equilibration steps are added automatically.

Performance

Highly reproducible chromatographic separation is essential in any quantitative analysis. The LC must provide an eluent flow, the gradient mixture and the injection volume precisely to ensure the reproducibility of each chromatographic run. To evaluate the instrument performance, we have compared multiple technical replicates with a 90 min, 120 min and 240 min gradient and determined the most important performance criteria.

As is shown in Figure 2, no matter which setup was used, base peak chromatograms were consistent across all gradient lengths with a time shift only due to the higher dead volume when a trap column was used.

High retention time stability and peak area reproducibility were achieved with both column configurations (Figure 3). Retention time drifts of only a few seconds were obtained even with the long 240 min gradient. With all gradient lengths the retention time variation was less than 0.5 %. Also the peak area reproducibility was extremely good with variations of less than 10 % for most of the comparisons and ≤5 % for all experiments using a trap column (Table 1).

As expected the 90 minute gradient produced the narrowest peaks for both column setups, 11 s full width half maximum (FWHM) (Figure 4). The FWHM values increased proportionally with the gradients length and resulted in a peak capacity between 500 and 700 (Figure 5). This ensures the best separation to enable the mass spectrometer to analyze as many precursors as possible.

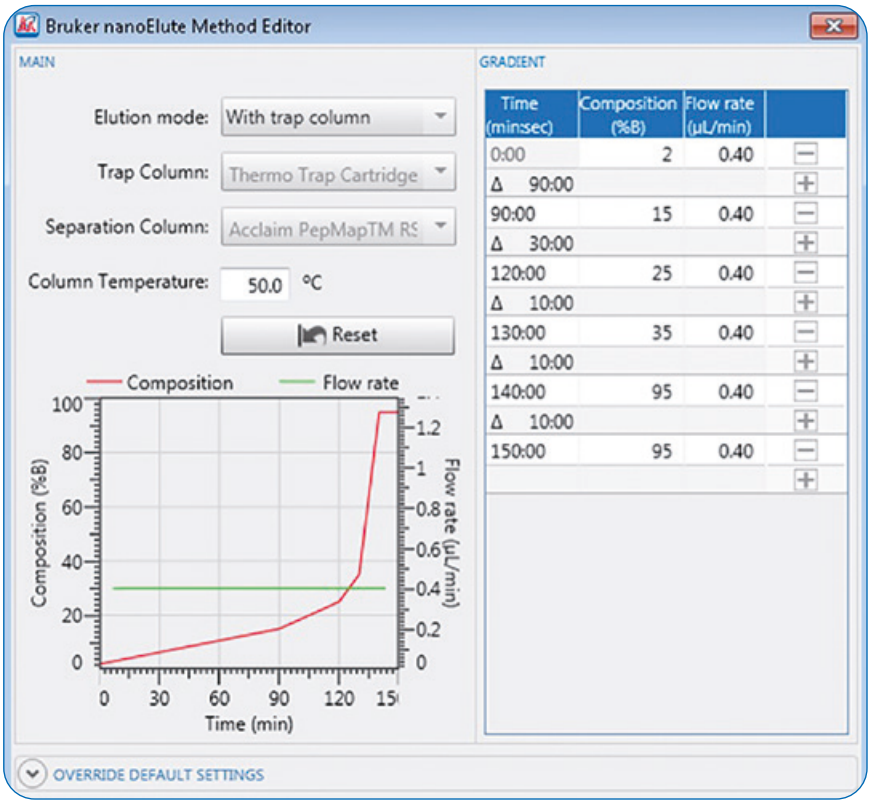

Fig. 1: Bruker nanoElute Method Editor

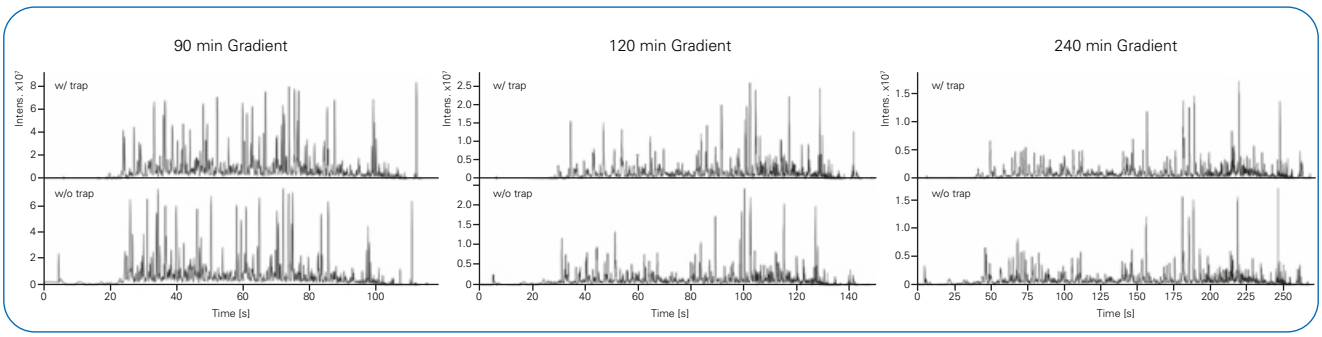

Fig. 2: Representative Base Peak Chromatograms for all gradient lengths and column setups. Chromatograms in the upper row are obtained by using a trap column and in the lower row without a trap column.

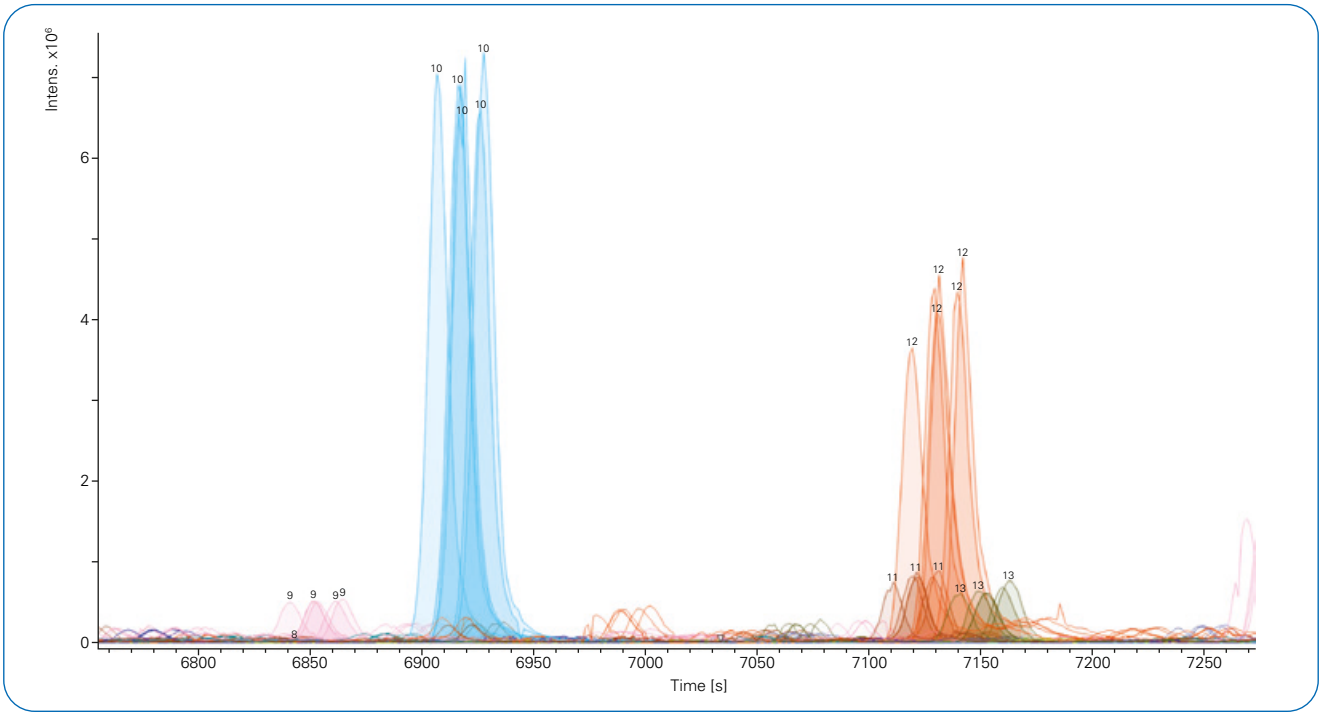

Fig. 3: Extracted Ion Chromatograms showing retention time reproducibility of selected peptides across 5 technical replicates using a 120 min gradient.

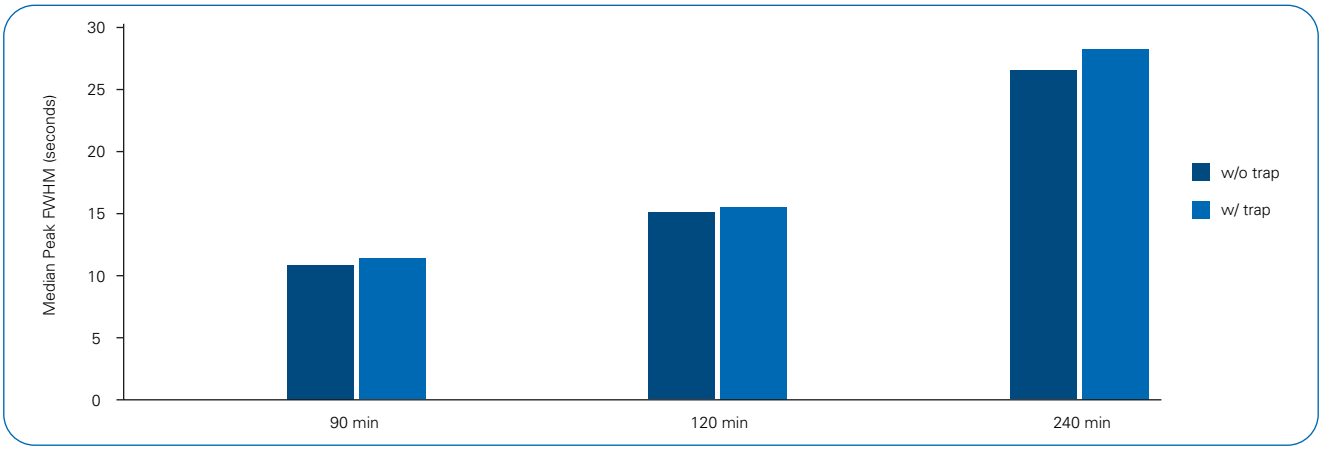

Fig. 4: Median Peak FWHM of 8 peptides across each gradient

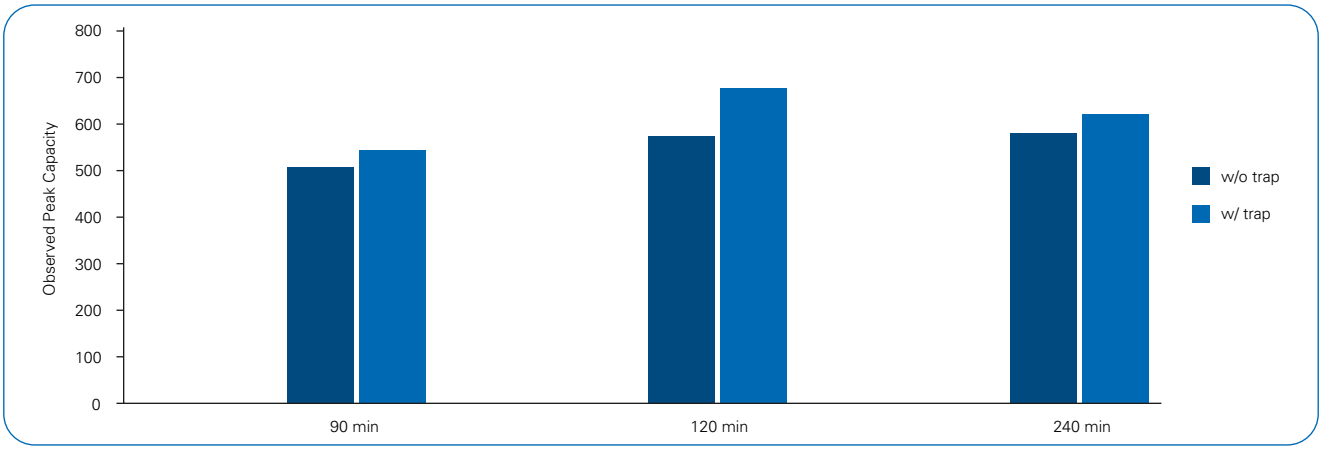

Fig. 5: Peak Capacity of 12 peptides across each gradient

Table 1: Summery of chromatographic performance values

Median Retention Time Variation

90 min		120 min		240 min	
w/o trap	w/ trap	w/o trap	w/ trap	w/o trap	w/ trap
0.16%	0.05%	0.18%	0.35%	0.11%	0.26%

Median Area Variation

90 min		120 min		240 min	
w/o trap	w/ trap	w/o trap	w/ trap	w/o trap	w/ trap
13%	4%	8%	5%	3%	4%

Median Peak FWHM (seconds)

90 min		120 min		240 min	
w/o trap	w/ trap	w/o trap	w/ trap	w/o trap	w/ trap
11	11	15	16	26	28

Observed Peak Capacity

90 min		120 min		240 min	
w/o trap	w/ trap	w/o trap	w/ trap	w/o trap	w/ trap
514	546	577	676	581	620

Conclusion

The nanoElute is the perfect companion to the impact II to perform high performance proteomics experiments. It outperforms state of the art nano-UHPLC systems in terms of performance and reliability combination. At the same time the nanoElute offers easy to use method configuration and sophisticated diagnostic procedures with a push of a button.

It allows for:

- High retention time stability
- High Peak Area reproducibility
- Narrow peaks
- High peak capacity
- Simple gradient configuration
- Automatic diagnostic tool
- Trap column switchable with a push of a button

Learn More

You are looking for further Information? Check out the Link or scan the QR Code.

www.bruker.com/nanoelute-video

References
[1] Bruker App Note LC-MS 81
Introducing New Proteomics Acquisition Strategies with the compact™ – Towards the Universal Proteomics Acquisition Method

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