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Applikationsbericht

Maximizing Resolution in Peptide Mapping Analysis Using UPLC with a 300-mm Column

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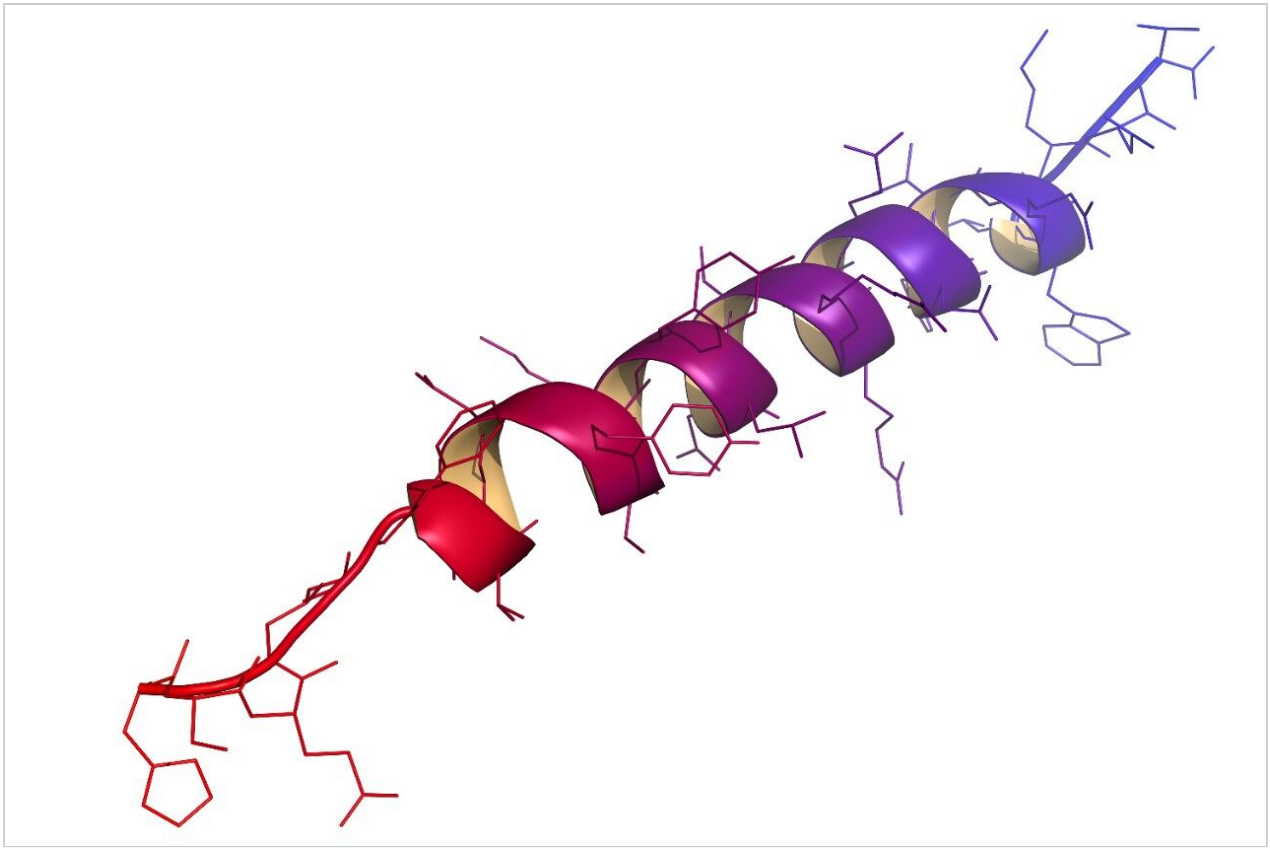

Abstract

This work demonstrates that ACQUITY UPLC separation power can be greatly enhanced by using columns with an increased column length of 2.1 x 300 mm.

Benefits

Use of the 300-mm column can reduce method development time for some challenging separation problems and increase the productivity and efficiency of the workflow.

Introduction

Reverse-phase liquid chromatography is extensively used for the characterization and quality control of protein products. However, peptide coelution and incompletely resolved chromatographic peaks are frequently observed in peptide mapping analysis due to the complex nature of protein digests. Poor resolution can hinder the detection and accurate quantification of critical components that are important to the quality attributes of protein therapeutics, especially when only ultra violet (UV) detection is employed.

Although improved separation resolution can be achieved by optimizing LC operating parameters, this approach is time consuming, and targeted resolution is not necessarily guaranteed for complex peptide digests such as monoclonal antibody (mAb) tryptic digests. Thus, a column with higher peak capacity can be a more efficient alternative under those circumstances.

The use of sub-2-µm particles allows an ACQUITY UPLC System to produce higher peak capacity (higher efficiency) and better sensitivity (sharper peaks). With UPLC, the speed of analysis can also be improved due to the higher linear velocity. These features make a UPLC-based peptide mapping approach powerful and attractive.

Currently, the dimensions of Waters Peptide Separation Technology BEH-based 1.7-µm column offerings include 2.1 x 50 mm, 2.1 x 100 mm, and 2.1 x 150 mm. The pressure generated by these column is far below the pressure limit (15,000 PSI) of the ACQUITY UPLC System at a typical operating conditions (flow rate 0.2 mL/min, column temperature 20 to 65 °C), which leaves room for achieving further separation if longer columns are employed.

Experimental

Here we demonstrate the ability to enhance UPLC separation power with an ACQUITY UPLC BEH 130 C_{18} 1.7-µm, 2.1 x 300 mm column (P/N 186005792). The resolution of a protein digest was compared based on the separation achieved using columns with the same column packing materials but different column lengths, 2.1 x 150 mm vs 2.1 x 300 mm. All chromatography was performed with the same sample, eluents, and method on an ACQUITY UPLC System with online detection by both a TUV detector and a SYNAPT MS System with MS^E detection mode.

The performance of the newly developed 300-mm column was evaluated using Waters MassPREP enolase tryptic digest (P/N 186002325) based on the peak capacity achieved in a typical condition for peptide mapping analysis.

The ACQUITY UPLC System pressure generated by the 300-mm column was first investigated. At a flow rate of 200 µL/min and column temperature 40 °C, the maximum system back pressure generated by the tandem columns was ~10,000 PSI, well below the system's pressure limit (15,000 PSI). We also tested the system pressure at different column temperatures, and demonstrated that the configuration with a 300-mm column was feasible as a working system if the column temperature set ≥ 40 °C.

Results and Discussion

Examples of selected peptide maps were examined to illustrate the resolution obtained with the two columns at the same gradient slope. Improved peptide component resolution with a reduction in coelution was observed with use of the 300-mm column. This improved resolution is particularly useful for identification and quantification of protein heterogeneities with UPLC-UV.

The data were acquired at the same gradient slope using a linear gradient of 5 to 45% B in run times of 90 and 180 min respectively to match with column volumes. A good scalability and an apparently better separation were observed for the 300-mm column (Figure 1). The calculated peak capacities at 4σ for the 300-mm and the 150-mm column in this peptide mapping experiment are about 700 and 500, respectively. Peak capacity (P) was calculated and averaged from six repeated analyses.

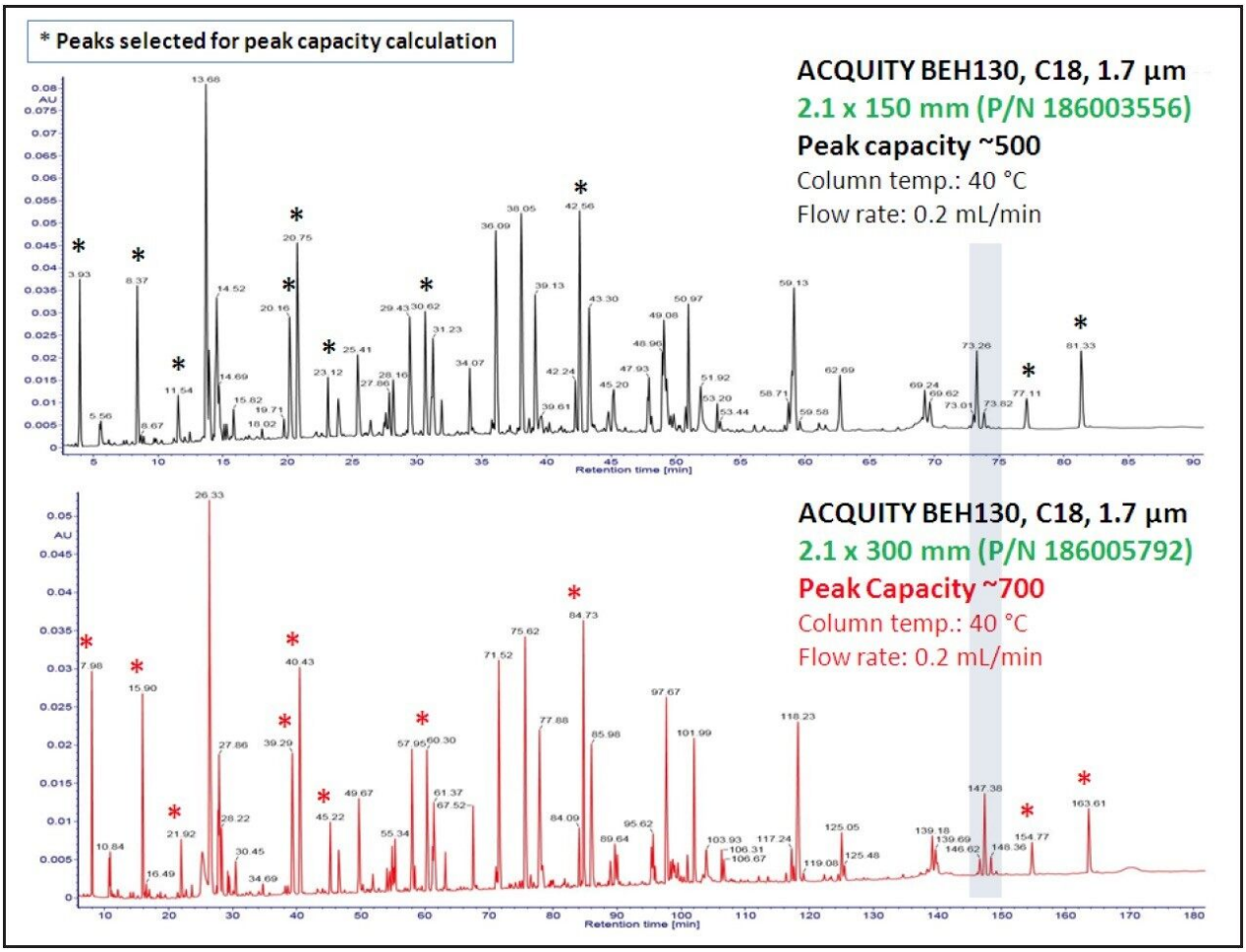

Figure 1. Comparing peptide maps of tryptic digest using 2.1 x 150 mm (top) and 2.1 x 300 mm (bottom) columns. Good scalability and an apparently better separation were observed with the 300-mm column. Ten peaks, marked with an asterisk, were chosen for the peak capacity calculation.

Ten peaks (marked with asterisk in Figure 1) were chosen for the peak capacity calculation. To make a clear and direct comparison for the peak shape and peak width for the two columns, peaks in the region highlighted in shadow is shown in Figure 2 from the two columns. It clearly shows that non-chromatographically resolved peaks by the 150-mm column are now completely separated by the 300-mm column, due to significantly improved peak capacity.

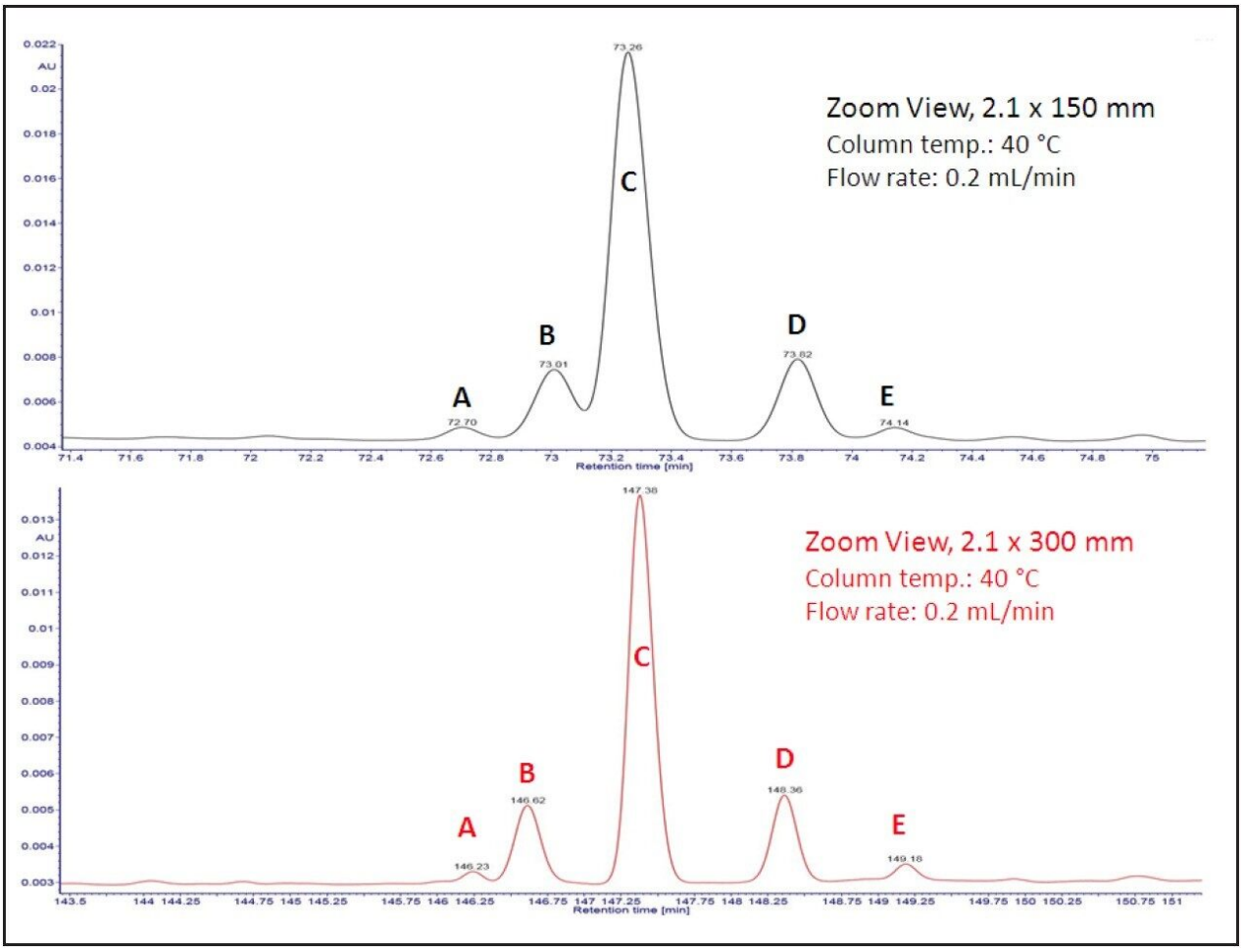

Figure 2. Zooming in on the highlighted area of Figure 1 shows a clear and direct comparison of the peak shape and peak width for the two columns. Peaks that were not chromatographically resolved by the 150-mm column are now completely separated by the 300-mm column due to significantly improved peak capacity.

Conclusion

In a comparison of the peptide mapping performance of an ACQUITY UPLC BEH 130 C_{18} 1.7-µm column in lengths of 2.1 x 300 mm and 2.1 x 150 mm, we found that the use of a 300-mm column results in improved chromatographic resolution and greater peak capacity. The enhanced performance is particularly useful for the identification and quantification of protein heterogeneities when a peptide mapping experiment is based on an LC-UV method.

The longer column configuration maintains the excellent gradient separation reproducibility that an ACQUITY UPLC System can achieve and operates well below the system's maximum 15,000 pressure limit under the detailed conditions. Use of the 300-mm column can reduce method development time for some challenging separation problems and increase the productivity and efficiency of the workflow.

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