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Note d'application

Maximizing Chromatographic Resolution of Peptide Maps using UPLC with Tandem Columns

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Abstract

This application note demonstrates that the ACQUITY UPLC System configured with a tandem of 2.1 x 150 mm columns is capable of improving resolution of peptide maps and reducing coelution. This improved resolution is particularly useful for identification and quantification of protein heterogeneities with UPLC-UV.

Introduction

LC-based peptide mapping is extensively employed for protein characterization, from early biotherapeutics development, to quality control of production and monitoring stability and activity after long-term storage. The use of this technology to identify and quantify low levels of impurity proteins, sequence variants, and post-translational modifications is critical for the assessment of protein drugs.

However, due to the complexity of protein enzymatic (often tryptic) digests, peptide coelution and poorly resolved chromatographic peaks are quite common in peptide mapping. In addition to recent advances in MS and particularly MS^E,[1] a good LC assay removes issues of ion suppression and isobaric interferences that may affect sensitive identification and accurate quantification of heterogeneities. Furthermore, a high-resolution LC assay can be more readily transferred for quality control purposes when utilizing UV detectors.

The use of sub-2-µm particles allows UPLC to push the limits of both peak capacity (higher efficiency) and sensitivity (sharper peaks). With UPLC, speed of analysis is also improved due to higher linear velocities.[2] These features make UPLC-based peptide mapping powerful and attractive. Currently, the dimensions of 1.7-µm Peptide Separation Technology BEH C_{18} columns used with an ACQUITY UPLC System include 2.1 x 50 mm, 2.1 x 100 mm, and 2.1 x 150 mm. The pressure generated by these columns is far below the pressure limit (15,000 PSI) of the ACQUITY UPLC System at 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 properly configured.

In this application note, we demonstrate how to maximize ACQUITY UPLC separation power by using a tandem of two 2.1 x 150 mm columns. The resolution of an IgG1 tryptic digest was compared between the tandem column configuration and single columns, with online detection by both a TUV detector and a SYNAPT MS system with MS^E detection mode. The UPLC-UV/MS^E reproducibility of the new configuration was evaluated. BiopharmaLynx 1.2 Software was used for identification of the eluted peptides.

Experimental

LC conditions

LC System:	ACQUITY UPLC with a standard peptide mapping mixer (425 µL)
Column:	Peptide Separation Technology (PST) BEH300 C_{18}, 1.7 µm, 2.1 x 100 mm, 2.1 x 150 mm, or a tandem of two 2.1 x 150 mm columns
Column temp.:	65 °C
Flow rate:	200 µL/min
Sample Injected:	10 µL (100 pmole)
Buffer A:	0.02% TFA in water
Buffer B:	0.018% TFA in ACN
Gradient:	A linear gradient of 1-40% B was scaled with column lengths (100 mm, 150 mm, and 300 mm by coupling two 150 mm columns) for run times of 60, 90 and 180 min, respectively.
Detection:	TUV (214 nm) and MS^E

MS conditions

The SYNAPT MS System and MS^E methods setup and operating conditions were the same as in previous descriptions.[5-6]

Data management

BiopharmaLynx 1.2 MassLynx Application Manager[3,7]

Materials and reagents

Waters MassPREP Enolase tryptic digestion standard was used. The IgG1 Antibody digest was prepared from a commercially available monoclonal antibody (mAb) by a RapiGest-assisted 4-h trypsin digestion protocol.[3-4]

Results and Discussion

A tandem of two 2.1 x 150 mm columns were coupled by capillary metal tubing and installed in the ACQUITY UPLC Column Manager. We first tested the system pressure generated by the configuration and the chromatographic reproducibility. At flow rate 200 µL/min and column temperature 65 °C, the system pressure was ≤7500 psi, far below the pressure limits of both the ACQUITY UPLC System (15,000 psi) and the 425 µL standard peptide mapping mixer (10,000 psi). We also tested the system pressure at different column temperatures, and demonstrated that the configuration with a tandem of two columns was feasible as a working system if the column temperature set above 40 °C.

Excellent chromatographic repeatability was observed (Figure 1) in six continuous runs of MassPREP Enolase tryptic digestion standard using a gradient of 1-40% buffer B in 180 minutes. The retention time (RT) fluctuation from injection to injection is within 4 seconds for each eluted peak in the chromatograms. For example, the RT fluctuation observed for the lately eluted enolase tryptic peptide T37 (YPIVSIEDPFAEDDWEAWSHFFK, MW 2827.3 Da, RT around 150.19 min) is 3.3 seconds. The average relative standard deviation (RSD, calculated by standard deviation SD / average RT * 100%) is 0.017%. This proved the configuration did not affect the good reproducibility of the ACCQUITY UPLC System, as has been demonstrated in previous work studying ACCQUITY UPLC system-to-system reproducibility for peptide mapping.[8]

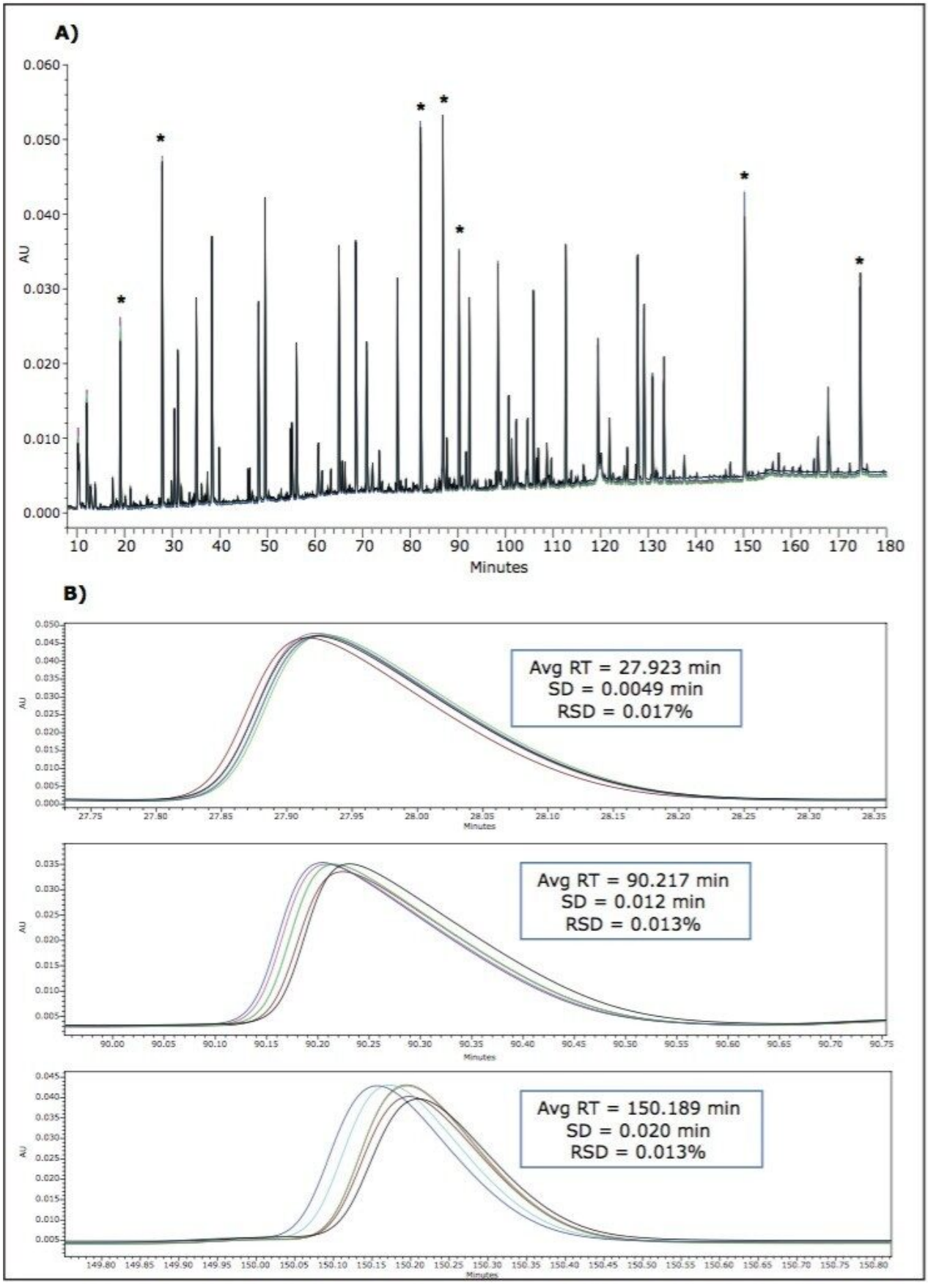

Figure 1. Reproducibility of six continuous injections of Waters MassPREP Enolase tryptic digest. A) UPLC-UV

In order to evaluate the performance of the new configuration, we next compared the peptide maps of the I

Figures 3 and 4 further demonstrate the improved resolution of the tandem column configuration compared

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