Use of New YMC-Triart 1.9µm UHPLC Stationary Phase for Fast Peptide Mapping of Monoclonal Antibodies

Jeffrey Kakaley[1], Takashi Sato[2], Ernest J. Sobkow[1]

1. YMC America, Inc., Allentown, PA USA 2. YMC Co., Ltd., Kyoto, Japan

Introduction

It has become increasingly important for biopharmaceutical companies to improve their characterization methods for mAb products. These needs stem not only from increasing regulatory requirements, but also from intra-organizational initiatives such as reducing runtime, increasing productivity, and reducing laboratory costs. Peptide mapping is an analysis that is typically costly to perform in terms of employee-hours, instrument time, and solvent usage. This poster highlights improvements in speed, resolution, and solvent consumption offered by YMC's Triart 1.9µm stationary phase when used for peptide mapping of monoclonal antibodies.

Experimental

Sample Preparation

Denaturation and Reduction
Monoclonal antibody samples were diluted accordingly with HPLC water to 1mg/mL. 1mL of each was then added to a glass tube with 2.5mL of 8M Guanidine HCl, 200µL 2.5M Tris Base, 400µL 1N HCl, and 12µL β-mercaptoethanol (BME). Samples were mixed well, adjusted to pH=7.5 with 1N HCL or 1N NaOH, and allowed to incubate at 37° C for 1 hour.

Desalting
The equivalent of 300µg (~1.25mL) of each denatured and reduced antibody sample was added to a 10kd cut-off spin filter and desalted with 0.1M Ammonium Bicarbonate. Once desalted, the protein was removed via pipette from the filter and placed in a maximum recovery HPLC vial for trypsin digestion.

Trypsin Digestion
Each Trypsin vial (Promega Corporation) was reconstituted with 20µL of resuspension buffer and 12µL of this solution was added to each sample vial. Each sample was then digested at 37° C for 3 hours. The reaction was stopped by adding 10µL of 1N HCl to each vial.

Method Parameters

Mobile Phases
Mobile Phase A: Water with 0.1% Trifluoroacetic Acid (TFA)
Mobile Phase B: Acetonitrile with 0.1% TFA

All columns were equilibrated with a minimum 10 column volumes of mobile phase prior to 1st injection.

Instrument Parameters

HPLC System:	Waters AcQuity UPLC
Flowrate:	0.2mL/min (5µm column) 0.6mL/min (1.9µm column)
Column Temperature:	40° C
Sample Temperature:	4° C
Detection λ:	215 nm
Injection Volume:	5µL (5µm column) 2µL (1.9µm column)

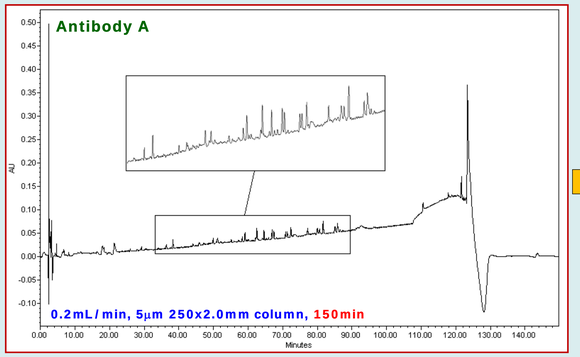
Columns Used
YMC-Triart C18, 250x2.0mm, 5µm, 120Å, P/N: TA12S05-2502WT
YMC-Triart C18, 100x2.0mm, 1.9µm, 120Å, P/N: TA12SP9-1002PT

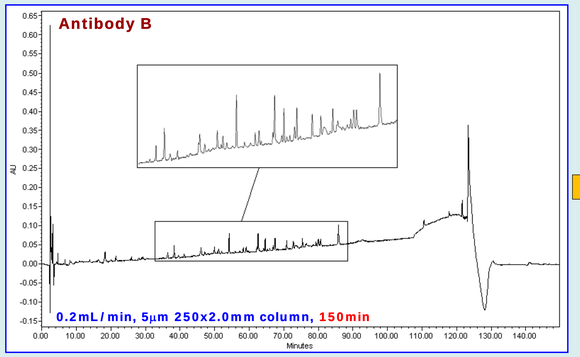
Effects of Particle Size and Column Length on Peptide Mapping

YMC-Triart C18, 5µm, 120Å, 250x2.0mm Flowrate = 0.2mL/min

Gradient:

Step	Time (min)	Flow (mL/min)	%A	%B
1	0	0.2	98	2
2	5	0.2	98	2
3	105	0.2	55	45
4	120	0.2	0	100
5	125	0.2	98	2
6	150	0.2	98	2

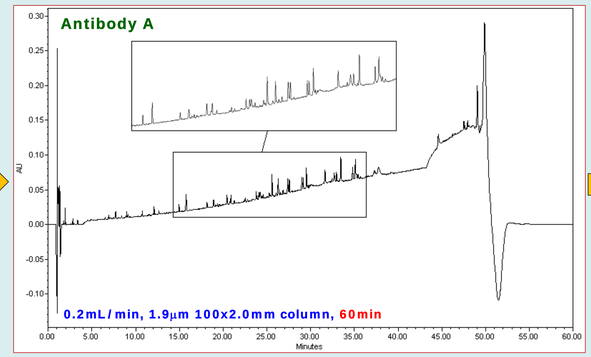


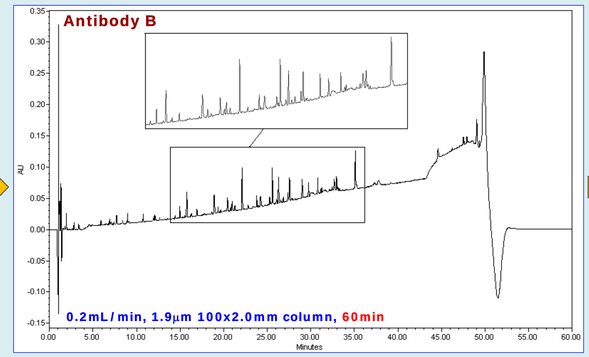


YMC-Triart C18, 1.9µm, 120Å, 100x2.0mm Flowrate = 0.2mL/min

Gradient:

Step	Time (min)	Flow (mL/min)	%A	%B
1	0	0.2	98	2
2	2	0.2	98	2
3	42	0.2	55	45
4	48	0.2	0	100
5	50	0.2	98	2
6	60	0.2	98	2

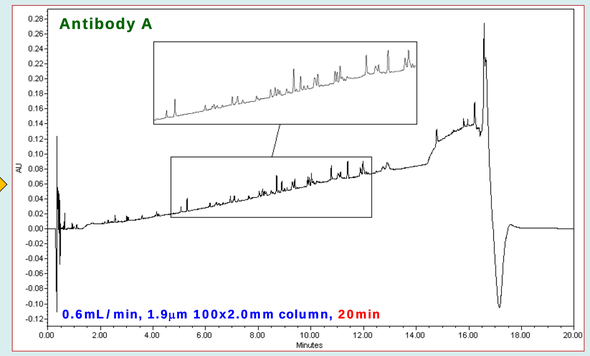


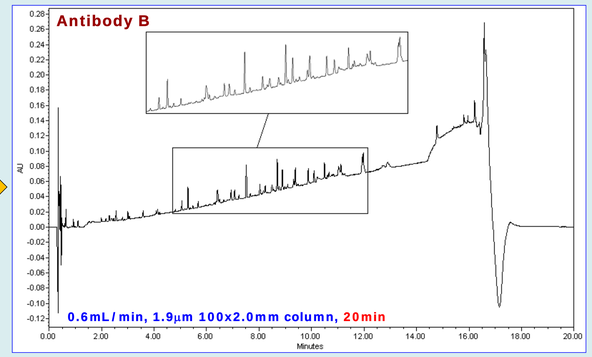


YMC-Triart C18, 1.9µm, 120Å, 100x2.0mm Flowrate = 0.6mL/min

Gradient:

Step	Time (min)	Flow (mL/min)	%A	%B
1	0	0.6	98	2
2	0.67	0.6	98	2
3	14	0.6	55	45
4	16	0.6	0	100
5	16.67	0.6	98	2
6	20	0.6	98	2





Results and Discussion

YMC's Triart 1.9µm particle was evaluated for use in scaling down a monoclonal antibody RP-peptide mapping application to uHPLC. The application began as a typical peptide map run on a 5µm 250x2.0mm Triart C18 column, using a linear gradient spanning 150 minutes. The method was then transferred to a 1.9µm 100x2.0mm Triart C18 column, scaling down the injection volume and gradient to account for the change in column length. This shortened runtime and decreased solvent usage by more than half. Linear velocity was then increased 3-fold in order to take advantage of the resolving power of the 1.9µm particle. This resulted in a runtime of 20 minutes, saving 130 minutes per injection and decreasing solvent usage from 30mL down to 12mL per injection.

Conclusions

- The use of the YMC Triart C18 1.9µm particle provides increased resolution, allowing column length to be shortened.
- The shortening of column length allows for decreased runtimes at the same linear velocity.
- The resolution gains allow for flowrate to be increased, increasing linear velocity by 3X and shortening runtimes further thereby increasing throughput and decreasing solvent usage by more than half.
- YMC Triart C18 1.9µm is a good choice for scaling down lengthy peptide mapping runs.