Use of New 1.9μm YMC-Triart C18 and 2.7μm YMC-Meteoric Core C18 BIO Stationary Phases 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

In their quest to increase the speed and efficiency of their analytical methods, today's scientists are increasingly turning to innovative products such as uHPLC totally porous as well as superficially porous core-shell materials. These stationary phases allow for faster analyses and increased throughput while simultaneously providing increased resolution. These advantages are thoroughly evident when applied to peptide mapping runs which are often more than an hour per injection on standard 5μm and 3μm particle size columns. This poster highlights improvements in speed, resolution, and solvent consumption offered by YMC-Triart C18 1.9μm and YMC-Meteoric Core C18 BIO 2.7μm stationary phases 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

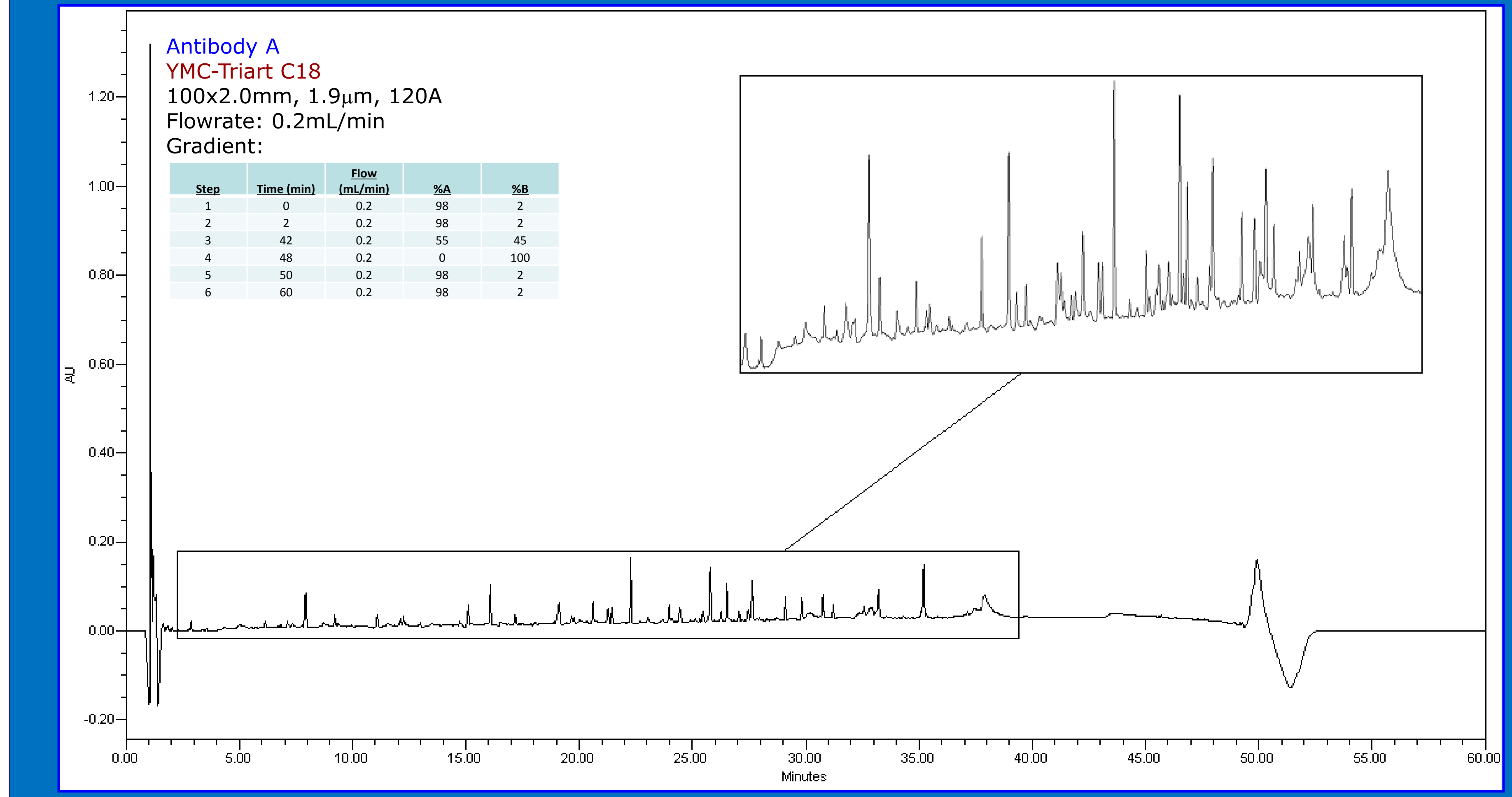
Mobile Phases
Mobile Phase A: Water with 0.1% Trifluoroacetic Acid (TFA)
Mobile Phase B: Acetonitrile with 0.1% TFA

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 (60min runtime)
0.6mL/min (20min runtime)
Column Temperature: 40° C
Sample Temperature: 4° C
Detection λ: 215 nm
Injection Volume: 10μL

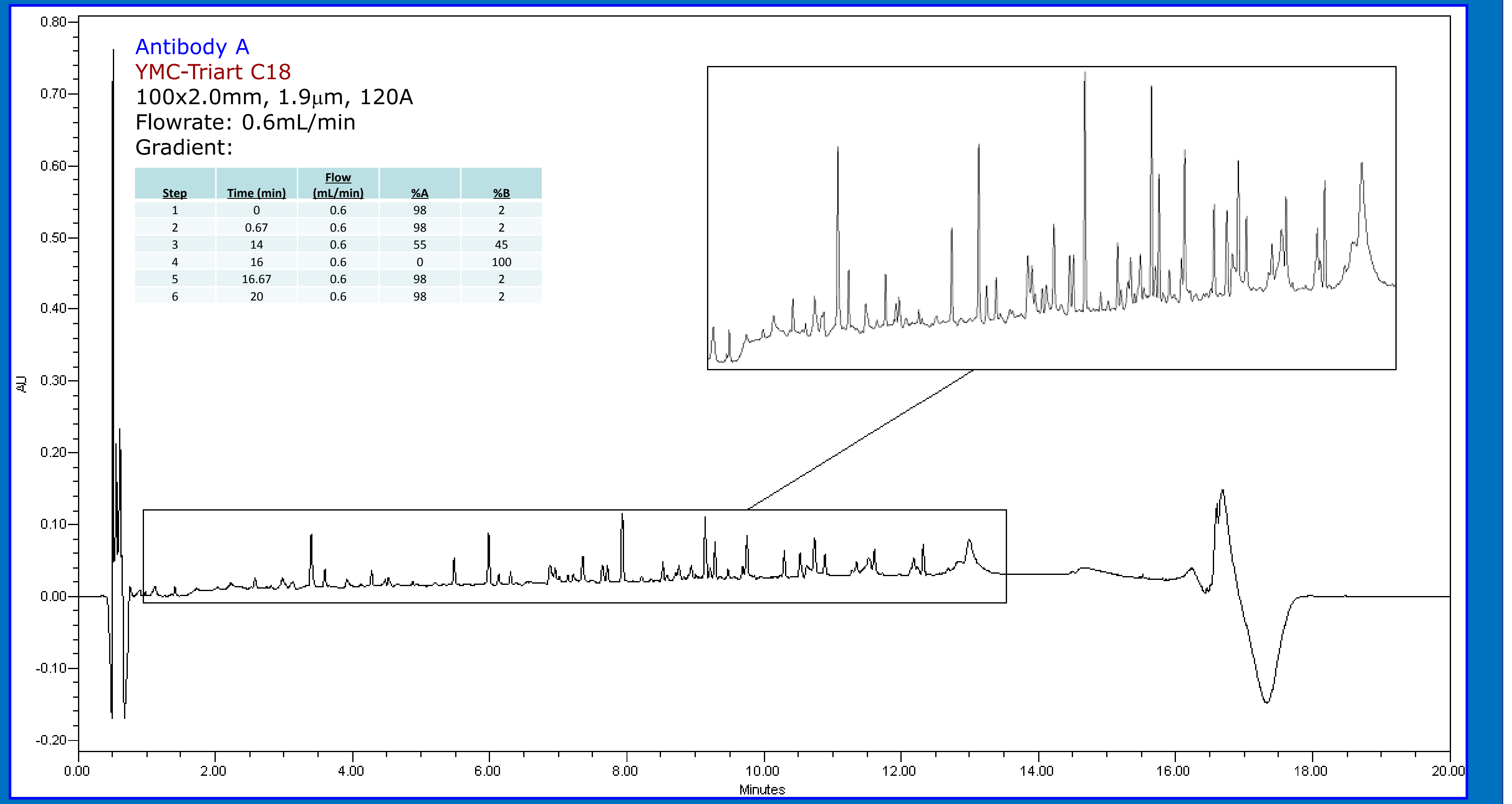
Columns Used
YMC-Triart C18, 100x2.0mm, 1.9μm, 120Å, P/N: TA12SP9-1002PT
YMC-Meteoric Core C18 BIO, 100x2.0mm, 2.7μm, 160Å,
P/N: CAW16S07-10Q1PT

Increased throughput on sub-2μm and core-shell materials


Antibody A
YMC-Triart C18
100x2.0mm, 1.9μm, 120A
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

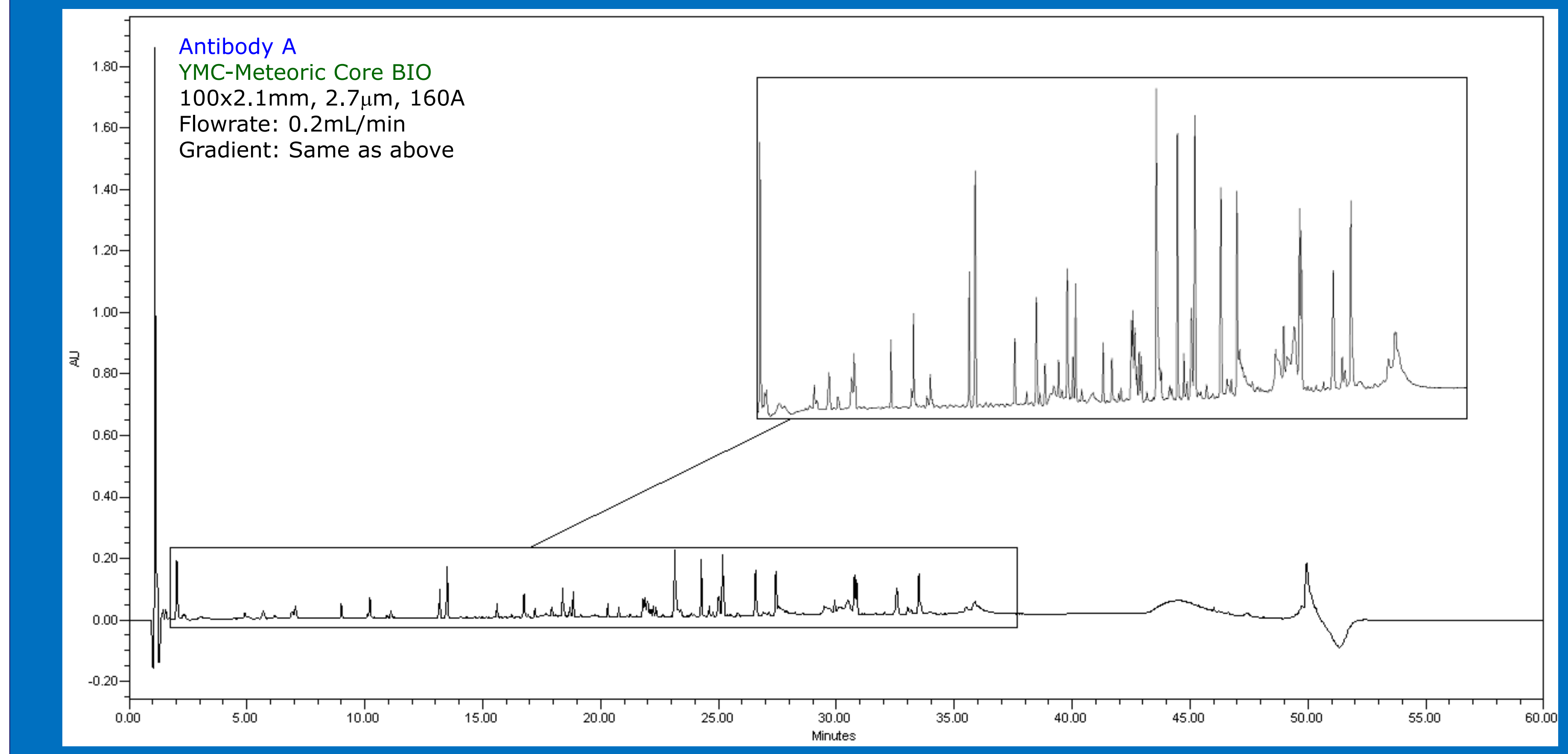
Same Column, 3X Flow


Antibody A
YMC-Triart C18
100x2.0mm, 1.9μm, 120A
Flowrate: 0.6mL/min
Gradient:

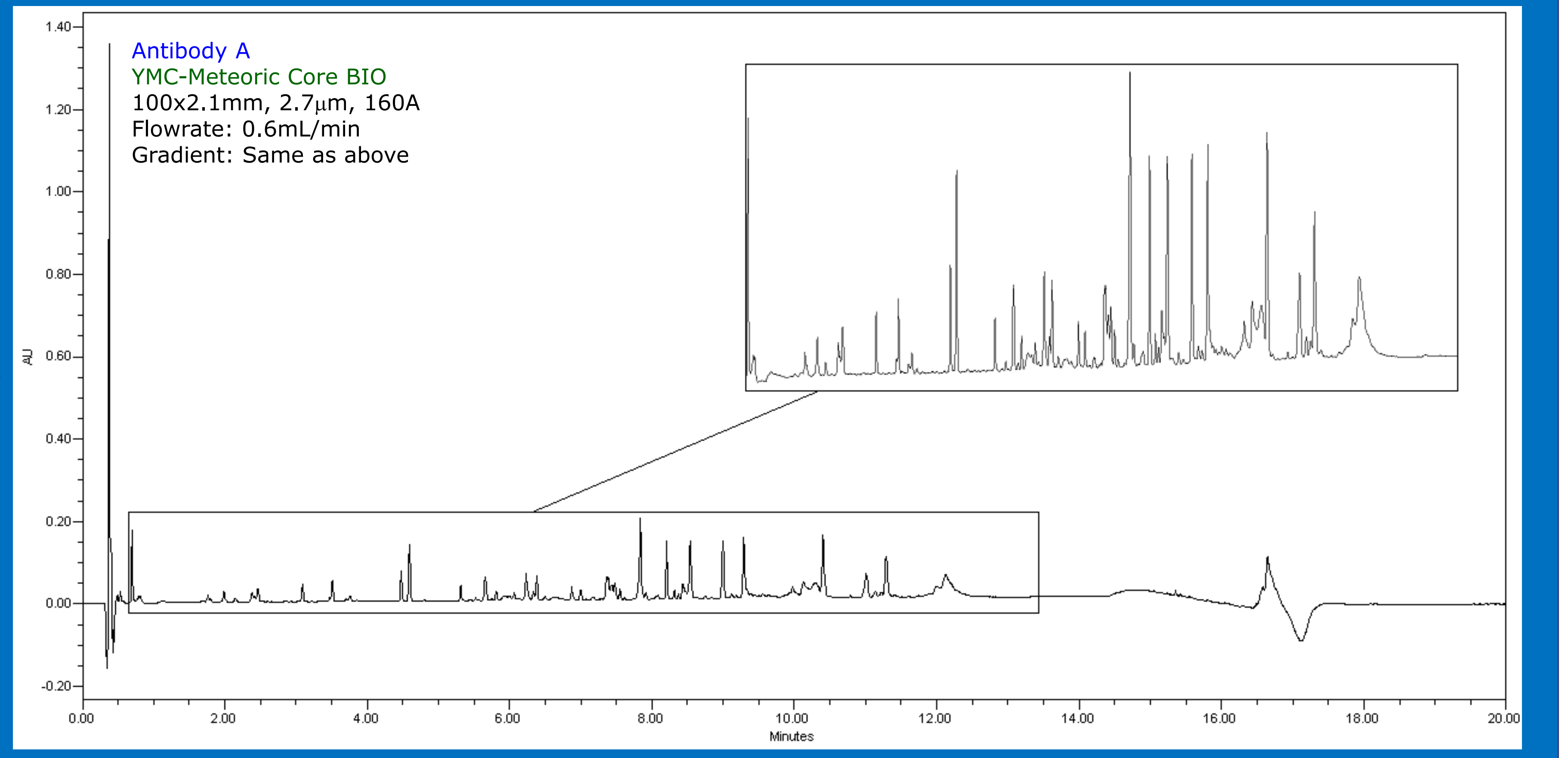
Step	Time (min)	Flow (mL/min)	%A	%B
1	0	0.6	98	2
2	0.67	0.6	98	2
3	14	0.6	55	45
4	16	0.6	0	100
5	16.67	0.6	98	2
6	20	0.6	98	2

Same Conditions

Same Conditions


Antibody A
YMC-Meteoric Core BIO
100x2.1mm, 2.7μm, 160A
Flowrate: 0.2mL/min
Gradient: Same as above

Same Column, 3X Flow


Antibody A
YMC-Meteoric Core BIO
100x2.1mm, 2.7μm, 160A
Flowrate: 0.6mL/min
Gradient: Same as above

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 original application began as a typical peptide mapping analysis run on a 5μm 250x2.0mm C18 column, using a linear gradient spanning 150 minutes (data not shown). The method was then transferred to a 1.9μm 100x2.0mm YMC-Triart C18 column as well as a 2.7μm 100x2.0mm YMC-Meteoric Core C18 BIO column. The injection volume and gradient were scaled down to account for the change in column length. This shortened run time 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 and the 2.7μm core shell 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 shortening of column length allows for decreased run times at the same linear velocity.
- Flow rate is then increased, thereby increasing linear velocity by 3X and shortening run times further, increasing throughput and decreasing solvent usage by more than half.
- YMC-Triart C18 1.9μm and YMC-Meteoric Core BIO are good choices for scaling down lengthy peptide mapping runs.

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