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Small Scale Peptide and Impurity Isolation Using the ACQUITY UPLC H-Class and Waters Fraction Manager-Analytical Systems

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APPLICATION BENEFITS

- The ACQUITY UPLC® H-Class System configured with the Waters® Fraction Manager-Analytical (WFM-A) reproducibly separates and collects target peptide and its closely-eluting impurities, making isolation at the small scale fast and easy.
- Fast valve switching and movement between vessels, as well as a fraction divert valve with very low dispersion volume enables the collection of narrow peaks, increasing confidence in compound isolation.
- Small scale compound isolation saves time, solvent, and resources promoting faster research and development timelines.

WATERS SOLUTIONS

ACQUITY UPLC H-Class System

ACQUITY UPLC PDA Detector

Waters Fraction Manager-Analytical (WFM-A)

Empower® 3 Software

XBridge® Columns

0.45 µm GHP Acrodisc 13 mm syringe filter

KEY WORDS

Small scale prep chromatography, peptide purification, peptide isolation, ACQUITY UPLC H-Class, Waters Fraction Manager-Analytical (WFM-A), analytical purification

INTRODUCTION

As peptides become more popular in the development of new therapeutics, it is increasingly important to quickly optimize the synthetic and cleavage processes by isolating and identifying both the target peptide and its related impurities. Collecting and analyzing closely-eluting impurities while isolating the target peptide saves time and effort and provides additional information about steps that can be taken to improve the quality and yield of the peptide product. While peptide isolation is routine for groups involved in synthesis and cleavage, peptide isolation is also useful for scientists in research and discovery groups. Whether peptide studies are focused on how these complex molecules affect the body and are metabolized or on how peptides are isolated from naturally-occurring sources,[1] only small amounts are required for initial experiments. In this study, we illustrate the utility of the ACQUITY UPLC H-Class and Waters Fraction Manager-Analytical (WFM-A) Systems for the analysis and isolation of a synthetic peptide and its closely-eluting impurities. This instrument configuration can be adapted for the isolation of constituents from complex synthetic, metabolic, or natural product mixtures at the small scale.

EXPERIMENTAL

Conditions

Analytical column:	XBridge Peptide BEH C_{18}, 4.6 x 50 mm, 5 µm
Flow rate:	1.46 mL/min
Mobile phase A:	0.1% trifluoroacetic acid in water
Mobile phase B:	0.1% trifluoroacetic acid in acetonitrile
Wash solvent:	7:2:1 Acetonitrile/Methanol/Water
Purge solvent	9:1 Water/Methanol
Wavelength:	280 nm
Gradients and injection volumes:	as noted in figures
Column temperature:	30 °C
Sample:	Crude synthetic peptide comprised of the following 16 amino acid residues: 7 polar, 6 nonpolar, 1 acidic, 2 basic; purity 56% by HPLC

Instrumentation

ACQUITY UPLC H-Class System with an
ACQUITY UPLC PDA Detector and Empower 3 Software

Waters Fraction Manager-Analytical

Figure 1. ACQUITY UPLC H-Class System with Waters Fraction Manager-Analytical (WFM-A) System.

RESULTS AND DISCUSSION

The principles of scaling chromatography[2] remain the same whether the objective is to increase the amount of sample isolated at one time on a large column, or to decrease the amount of product based on the immediate need for material to perform experiments which answer pertinent questions quickly. For these studies, another new aliquot of the crude synthetic peptide sample used in previous work[3] was isolated using the same optimized and focused gradient,[4] this time at a much smaller scale – on a 4.6 x 50 mm XBridge Peptide BEH C_{18} Column using the ACQUITY UPLC H-Class System configured with a WFM-A fraction collector. The crude peptide (2.4 mg) was dissolved in dimethylsulfoxide (DMSO) and filtered using a 13 mm Acrodisc GHP syringe filter. Whereas 10 µL was the maximum injection volume that maintained resolution between the peptide and its impurities on the 4.6 x 100 mm XBridge C_{18} Column used in previous work (Figure 2), geometric scaling to the shorter 4.6 x 50 mm column for these experiments reduced the injection volume to 5 µL. Likewise, the reduction in column length automatically reduced the gradient run time from 18 minutes on the 100 mm column to 9 minutes on the 50 mm column. With the target peptide peak and its closely-eluting impurities eluting well before 36% B, the gradient conditions were adjusted to run from 28–32% B in 5 minutes, thus saving time in the method. Because of the complexity of crude synthetic peptide samples, shallow focused gradients with slopes of about 0.2–0.3% change per column volume are useful for resolving more sample constituents.

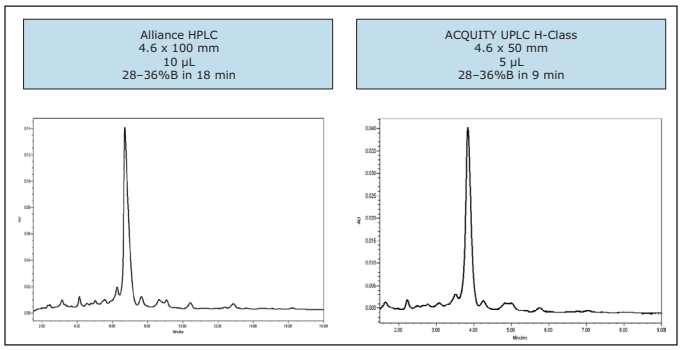
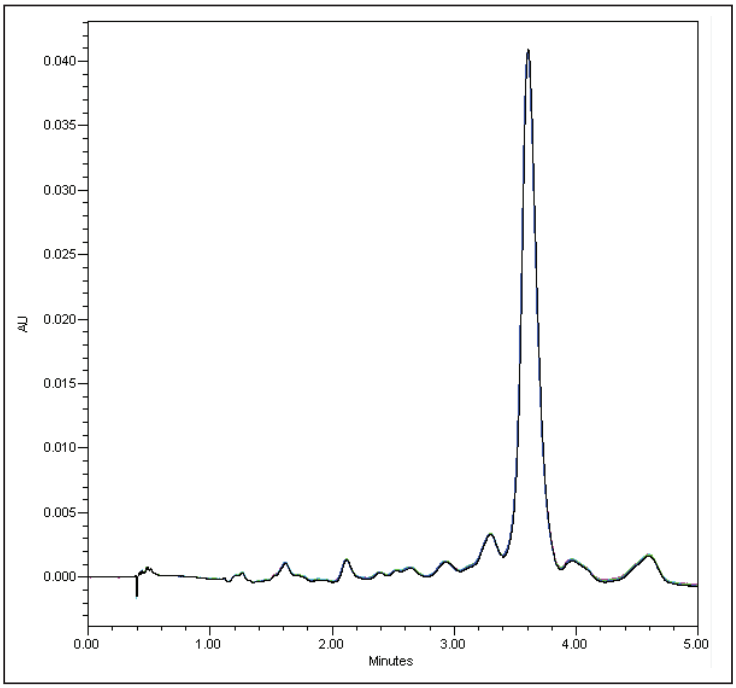


Figure 2. The peptide sample profile was very similar on two different systems. Geometric scaling principles were applied. The sample concentration for work done on the Alliance® HPLC System was 7.2 mg/mL while the sample concentration used on the ACQUITY UPLC H-Class System was 2.4 mg/mL. Column chemistry: XBridge C_{18}, 5 µm.

The ACQUITY UPLC H-Class System, with its low system dispersion,[5] exact control of solvent composition,[6] and accurate sample injection scheme,[7] provided excellent chromatographic reproducibility, as shown in Figure 3, where five peptide injections overlayed exactly. The WFM-A was specifically designed to minimize peak dispersion during collection. Figure 4 emphasizes the benefit of low peak dispersion in the fraction collection valve with the comparison of chromatographic profiles obtained with the WFM-A and with a traditional collector.



ABC peptide sample @ 2.4 mg/mL DMSO
5 µL injection Column temperature: 30 °C
28–32% B in 5 min
A = water with 0.1% TFA
B = acetonitrile with 0.1% TFA
Column: 4.6 x 50 mm XBridge Peptide BEH C_{18}, 5 µm; Flow rate = 1.46 mL/min; 280 nm

Figure 3. Overlay of 5 peptide injections on the 4.6 x 50 mm XBridge Peptide BEH C_{18} Column. Gradient: 28–32% B in 5 minutes, 5 µL injection, 280 nm.

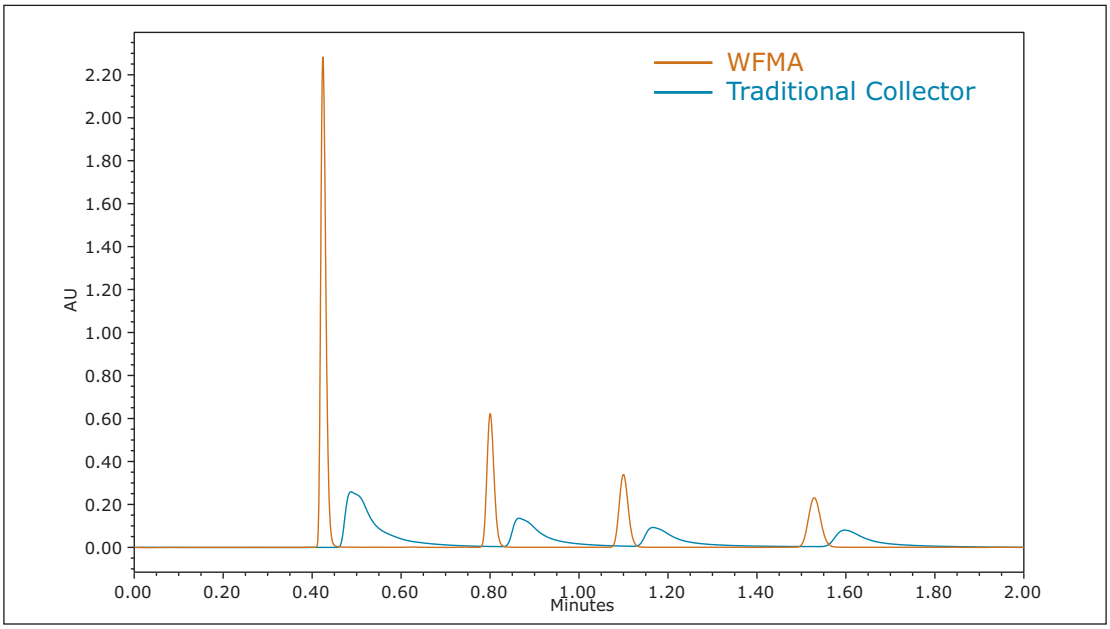


Figure 4. The WFM-A is specifically designed to address the challenge of peak dispersion. As shown, the WFM-A allows for the collection of concentrated narrow peaks of interest with the highest recovery possible.

Narrow, concentrated peaks are easily identified and collected with higher recovery when peaks are clearly defined. Fractions can be collected by time, slope, threshold, or any combination of the three. While the collection starting and ending times may be manually entered in the WFM-A method editor if desired, it is also possible to populate the WFM-A method automatically using the processed results from an analytical injection (Figure 5). Selecting the result displays the integrated peaks with their retention times, start times, and end times, which are then automatically filled into the collection event table (Figure 6).

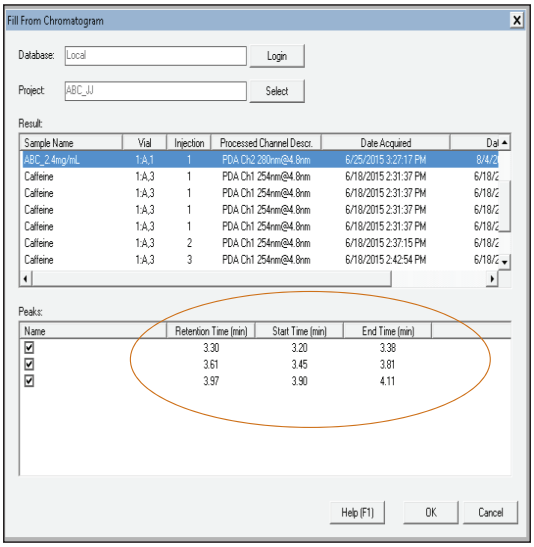


Figure 5. The sample result selected populates the peak table with the retention time as well as the start time and end time for each peak. Selecting OK fills in the Collection Event Table.

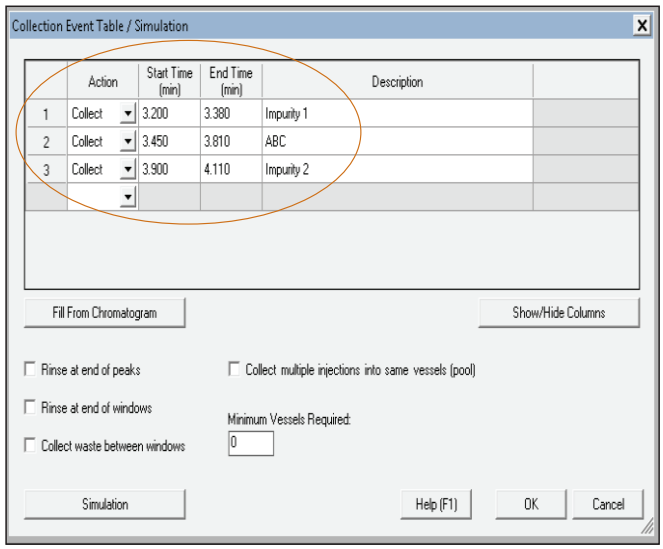


Figure 6. Collection Event Table in the fraction method.

With the collection method developed, the peptide and two closely-eluting impurities were isolated from the crude sample in a total of ten injections. Because the ACQUITY UPLC H-Class System is so reproducible, all of the sample purification chromatograms were identical. Figure 7 shows a representative chromatogram with the shaded areas indicating where fraction collection occurred. The fraction volumes were essentially identical for each of the compounds in each of the isolations (impurity 1, 0.29 mL; peptide, 0.55 mL; impurity 2, 0.33 mL). All of the fractions of each type were pooled. An aliquot of each pool was immediately analyzed using two different gradients.

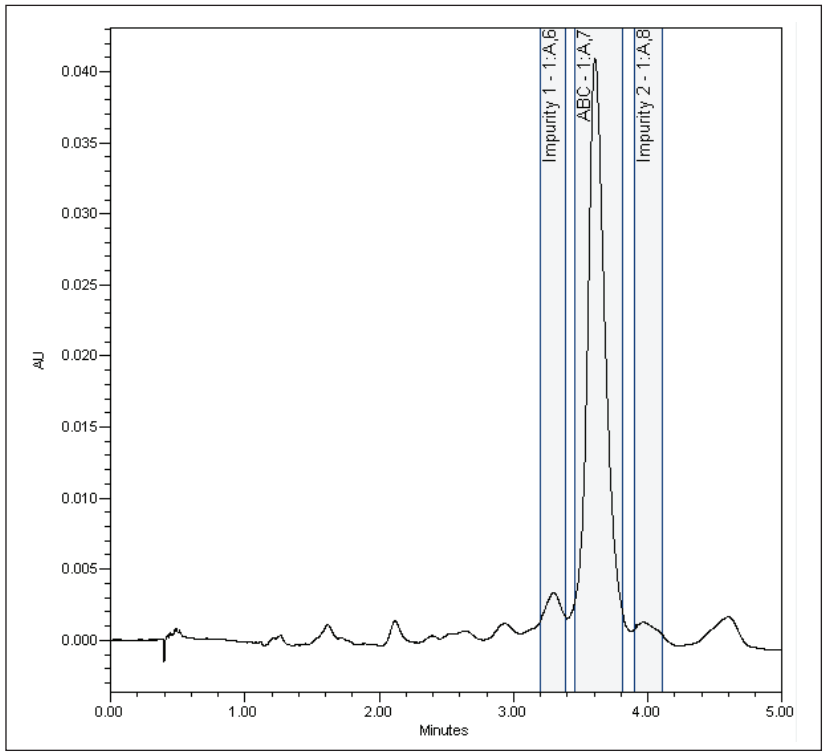


Figure 7. The peptide product and two closely-eluting impurities were collected into a 48-well plate containing 2 mL vials. Gradient: 28–32% B in 5 minutes on a 4.6 x 50 mm XBridge Peptide BEH C_{18} Column, 5 µm; 5 µL injection; 280 nm.

The peptide product purity was 100% as determined by both the fast gradient (Figure 8) and the shallower focused gradients (Figure 9) used for fraction analysis. Slight differences in the purities of both of the contaminant peaks were evident as shown by the two gradients. While the fast gradient (3.38% change per column volume) showed impurity 1 to be about 83% pure, the shallow focused gradient (0.30% change per column volume) resolved yet another coeluting peak and reduced the estimated purity to about 77%. Impurity 2 had a purity of 98% using the fast gradient and 80% using the focused gradient, again due to better resolution of compound constituents. If higher purity contaminant fractions were required for subsequent studies, further method development would likely be needed.

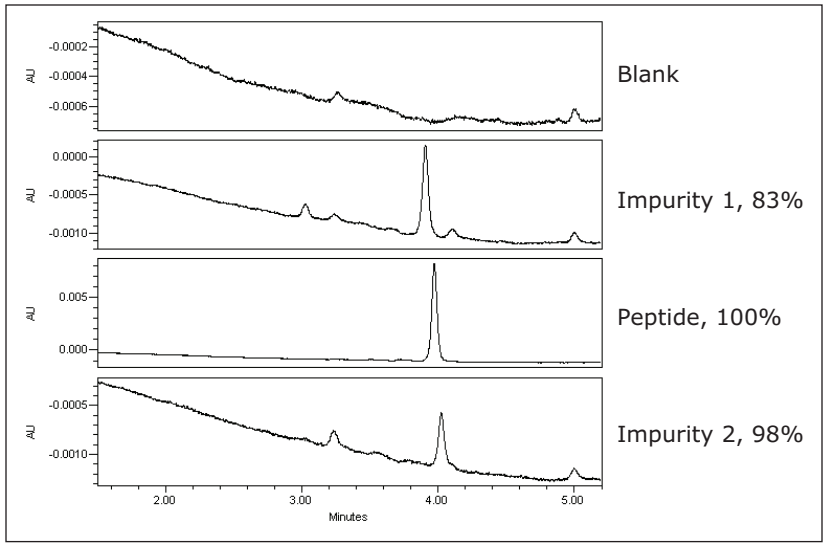


Figure 8. Approximate compound purities after subtracting the peaks present in the blank. Fraction analysis gradient: 5–50% B in 5 minutes, rate of gradient change 3.38%/column volume, injection volume 40 µL, 280 nm.

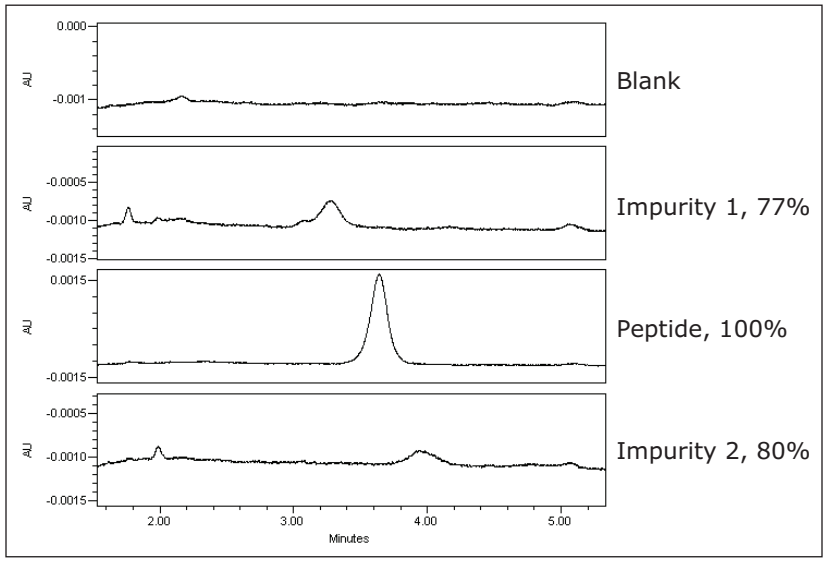


Figure 9. Approximate compound purities after subtracting the peaks present in the blank. Fraction analysis gradient: 28–32%B in 5 minutes, rate of gradient change 0.30%/column volume, injection volume 40 µL, 280 nm.

CONCLUSIONS

- The ACQUITY UPLC H-Class System configured with the Waters Fraction Manager-Analytical (WFM-A), with its very low system dispersion, exact control of solvent composition, accurate sample injection, and precise fraction collection, allows scientists to perform small scale peptide and impurity isolation with assurance.
- Fast valve switching and movement between vessels, as well as a fraction divert valve with very low dispersion volume, facilitates narrow target peak collection and increases confidence in compound isolation.
- Small scale peptide isolation saves sample, time, and resources, promoting efficiency in the purification process.
- The ACQUITY UPLC H-Class System configured with the WFM-A can be adopted for the isolation of compounds from complex synthetic, metabolic, or natural product mixtures at the small scale.

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