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Nota de aplicación

Small Scale Purification of Constituents from Complex Natural Product Extracts Using ACQUITY H-Class and Waters Fraction Manager-Analytical Systems

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Abstract

This application note demonstrates several collection features of the Waters Fraction Manager – Analytical (WFM-A), a fraction collector designed to collect very narrow fractions from sub-2-µm colums using three complex natural product extracts without distortion from band broadening making this a useful tool for challenging separations and collections.

Benefits

The Waters Fraction Manager – Analytical (WFM-A) is a fraction collector designed specifically to collect the narrow and closely eluting compounds found with UPLC separations.

Introduction

Extracts from natural product samples can be complex, often containing a large number of diverse compounds. Increased separation performance of the ACQUITY UPLC H-Class System combined with ACQUITY BEH C_{18} sub-2-µm Column technology provides a tool that produces sharp, narrow, and more concentrated peaks. When there is a need to collect narrow peaks from these complex mixtures, traditional preparative HPLC fraction collection instrumentation is not suitable for the collection of these narrow peaks. Collection valves and tubing designed for HPLC fraction collection introduce excessive peak broadening, making target isolation difficult. This application note will demonstrate several collection features of the WFM-A, a fraction collector designed to collect peaks generated from sub-2-µm chromatography using 3 natural product extracts.

Experimental

Extracts from three natural products (rosemary, schisandra berry, and angelica root) were analyzed using an ACQUITY UPLC H-Class System combined with the ACQUITY BEH C_{18} Column. Potential peaks of interest were identified and isolated using the WFM-A. Collected fractions were then analyzed to determine purity.

Extractions

Samples (~25 g) of each of the three plant materials were first extracted using supercritical fluid extraction (SFE) using a Waters SFE 500 System equipped with a 100-mL extraction vessel. The extractions were performed using 99% CO_2 and 1% isopropanol at 50 g/min, 40 °C, and 200 bar (2901 psi) for 60 minutes.

Separations

All separations were done using an ACQUITY UPLC H-Class System equipped with an ACQUITY UPLC PDA Detector along with a Waters Fraction Manager – Analytical controlled by Empower 3 Software. Details of the separation conditions are listed within the figure captions.

Results and Discussion

The Waters Fraction Manager – Analytical is a versatile analytical fraction collector for UPLC systems that minimizes fraction loss and carryover to better manage low-volume peaks and allows for efficient collection of small amounts of material for further assays. The WFM-A under Empower 3 control allows for several modes of collection.

Fraction collection based on time

For the rosemary extract, a simple time-based collection mode was employed (Figure 1). Time based collections are simple and efficient but require precise, reproducible chromatography to be successful. In this example, the 2 largest peaks were collected and pooled over eight injections (Figure 2). Over the eight injections, 558 µL and 479 µL were collected for each of the two peaks, respectively, and analyzed (Figure 3) with peaks showing purities of 94.0% and 97.8%. Cumulative, real-time collection volume and location information can be viewed in the ACQUITY console (Figure 4).

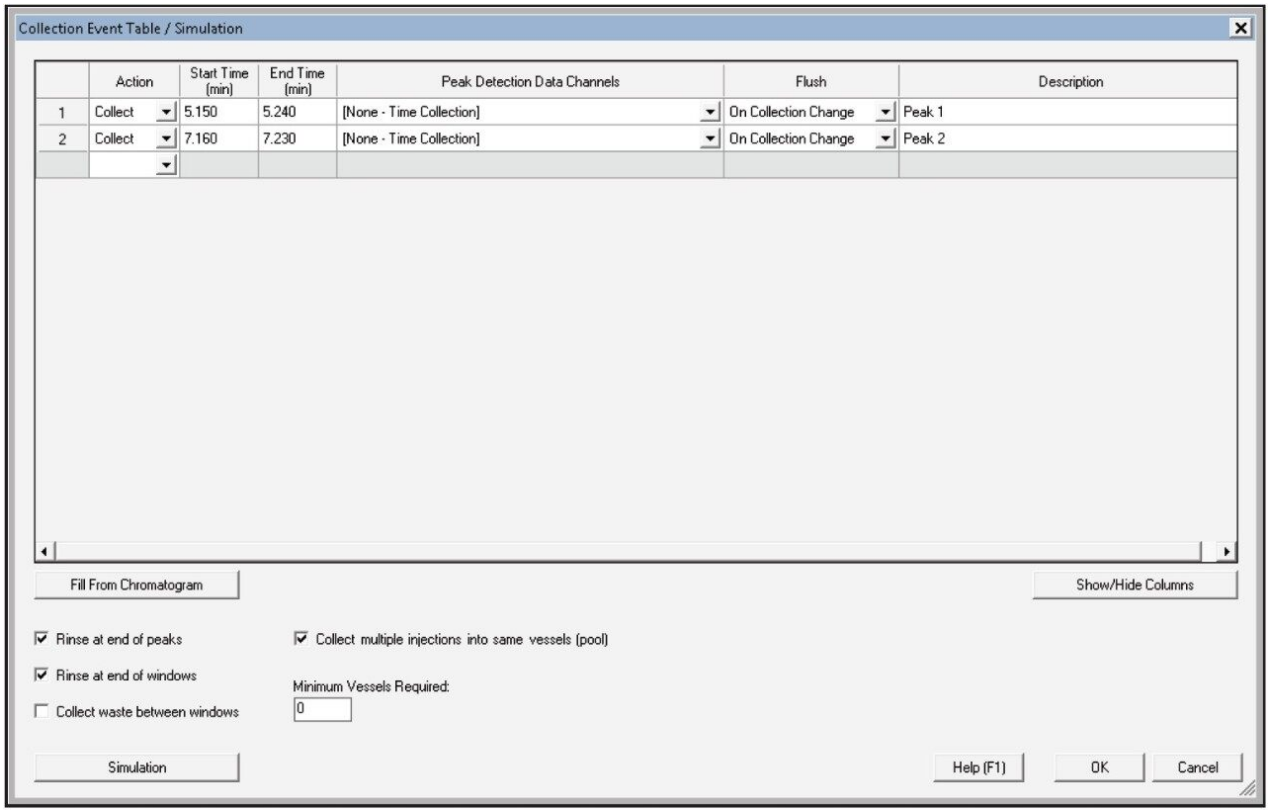

Figure 1. Empower 3–WFM-A method editor showing a simple time-based fraction collection.

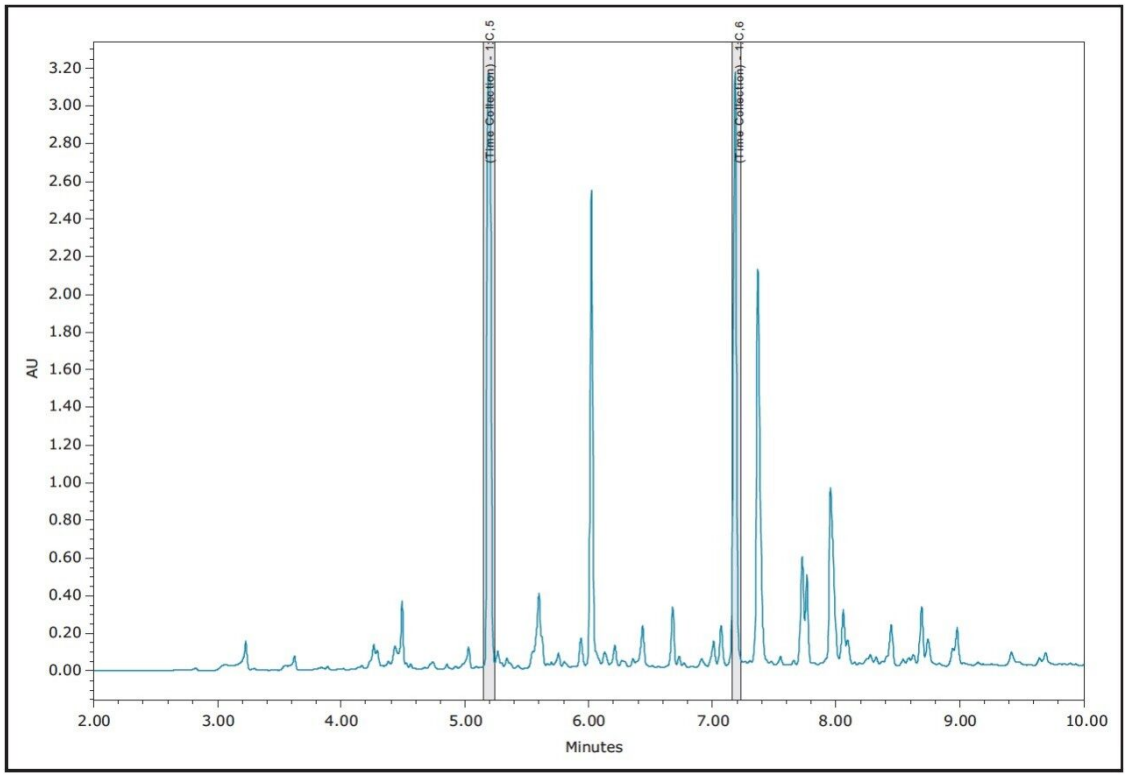

Figure 2. Separation of a rosemary extract with time-based fraction collection. Mobile phase – 0.1% formic acid in water, 0.1% formic acid in ACN gradient, 99:1 to 1:99 over 10 min, temperature – 50 °C, flow – 0.50 mL/min, UV at 254 nm, Column – ACQUITY BEH C_{18} , 1.7 µm, 2.1 x 100 mm.

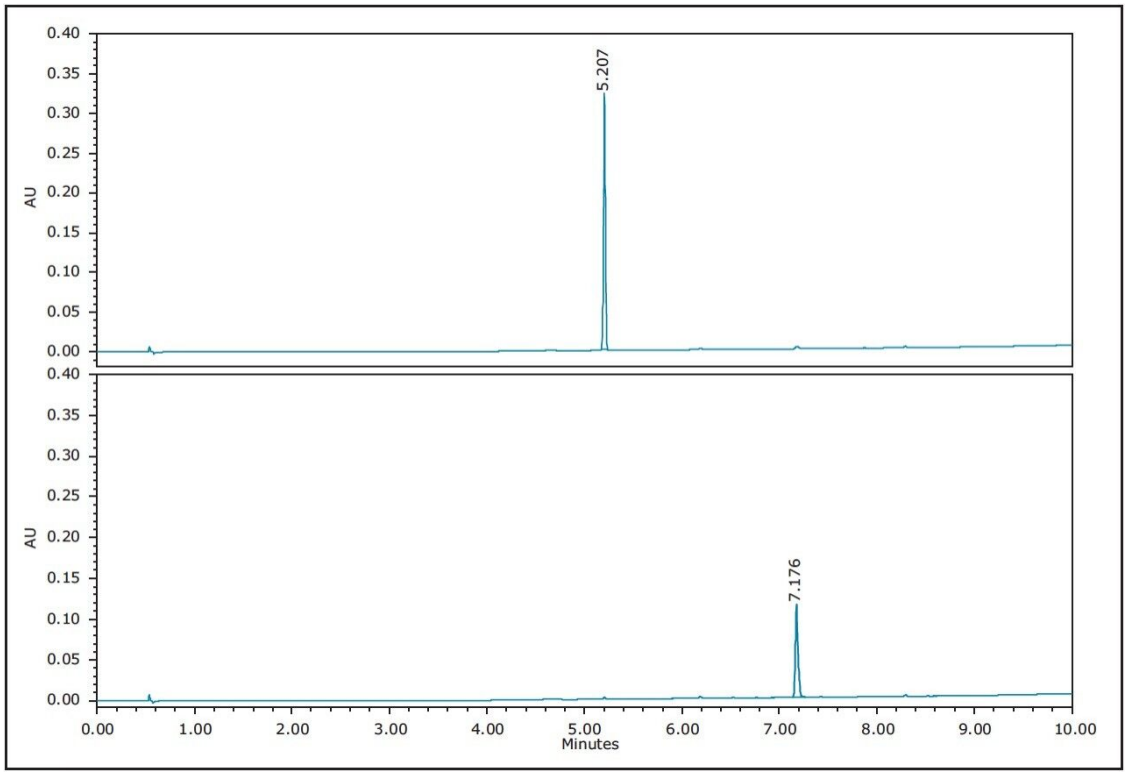

Figure 3. Analysis of pooled, collected fractions from a rosemary SFE extract. Mobile phase – 0.1% formic acid in water, 0.1% formic acid in ACN gradient, 99:1 to 1:99 over 10 min, Temperature – 50 °C, Flow – 0.50 mL/min, UV at 254 nm, Column – ACQUITY UPLC BEH C_{18} , 1.7 µm, 2.1 x 100 mm.

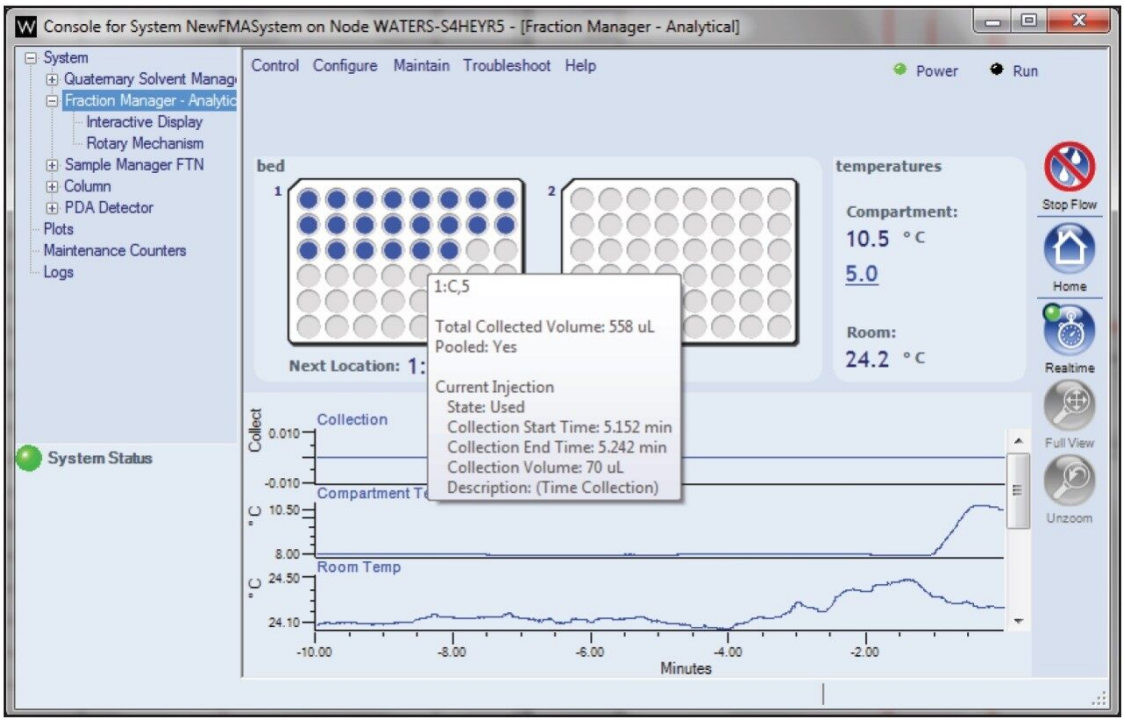

Figure 4. ACQUITY Console showing real-time fraction collector bed status.

Fraction collection based on threshold

Fraction collection for the angelic root (Figure 5) was achieved through peak detection using threshold. In this mode, fraction collection is triggered based on peak intensity. When you specify a start threshold value, peak collection starts when the UV detector data channel level rises above the preset value. When you specify an end threshold, collection continues until the data channel level falls below the end threshold value. This mode is useful when chromatography is unstable or there is a desire to collect multiple peaks within a single time window. Using this collection mode requires the use of an optimized, low dispersion delay coil which allows the software time to make the correct collection decision. Despite this delay coil, it is still possible to accurately collect closely eluting peaks (inset, Figure 5). Analysis of the collected fraction indicated purity (based on UV) of 89.4% and 94.1% for peaks 1 and 2, respectively (Figure 6).

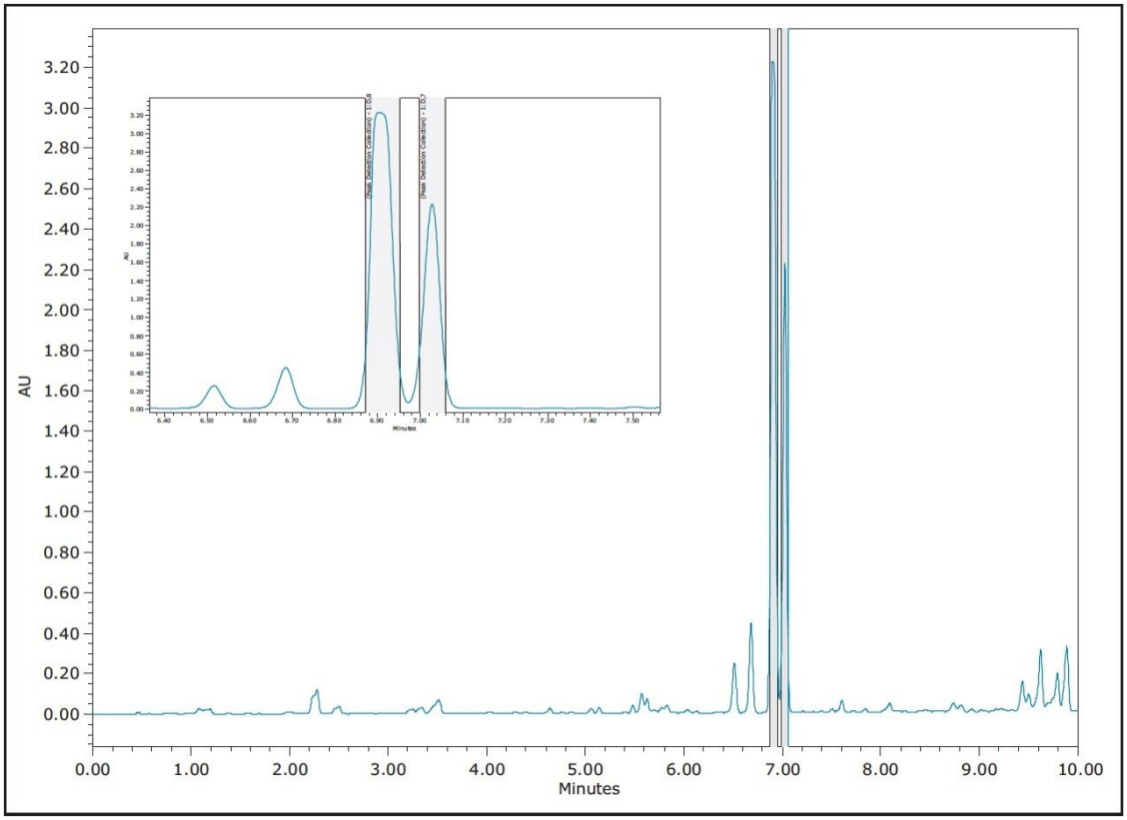

Figure 5. Separation of an angelica root extract with peak detection (threshold) based fraction collection. Mobile Phase – 0.1% formic acid in water, 0.1% formic acid in ACN, gradient – 80:20 to 30:70 over 10 min, temperature – 50 °C, flow – 0.50 mL/min, UV @ 254 nm, column – ACQUITY UPLC BEH C_{18} , 1.7 µm, 2.1 x 100 mm.

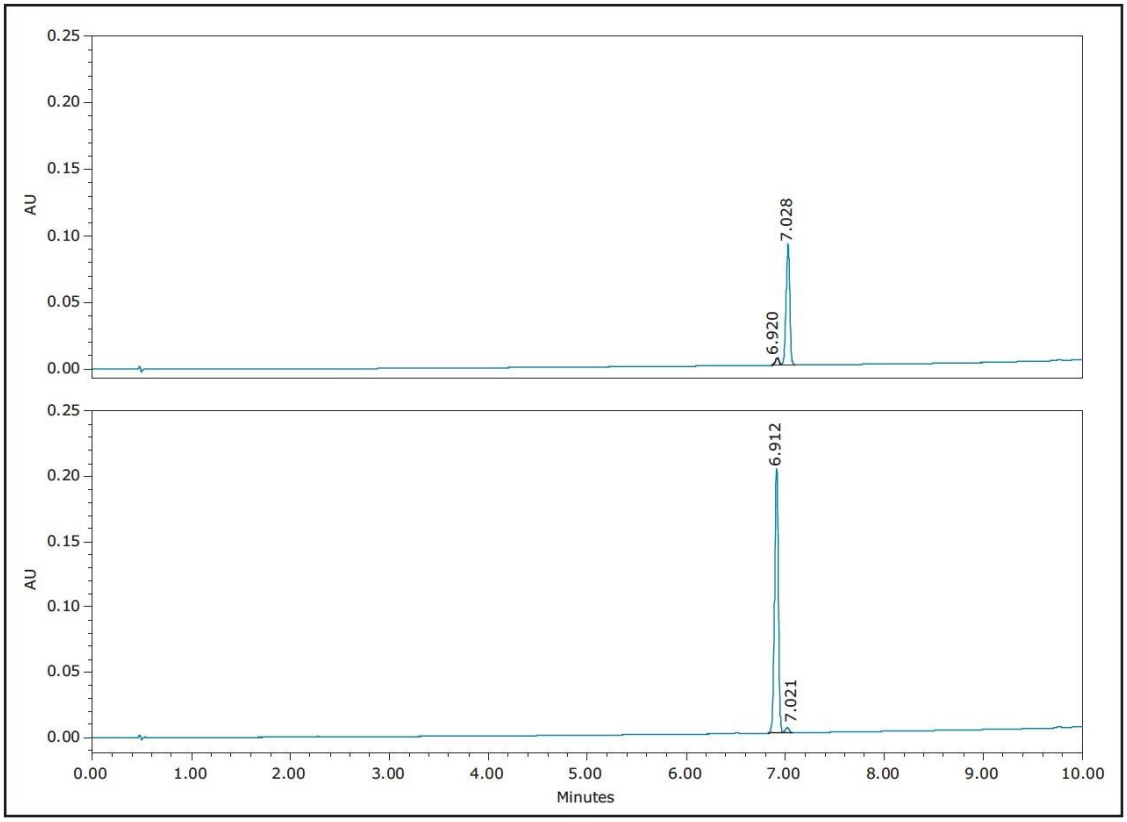

Figure 6. Analysis of collected fractions from angelica root SFE extract. Mobile phase – 0.1% formic acid in water, 0.1% formic acid in ACN, gradient – 80:20 to 30:70 over 10 min, temperature – 50 °C, flow – 0.50 mL/min, UV at 254 nm, column – ACQUITY UPLC BEH C_{18} , 1.7 µm, 2.1 x 100 mm.

Mixed mode fraction collection

As a demonstration, fraction collection for the schisandra berry was achieved through a mix oftimed and threshold collection (Figure 7). The WFM-A method editor software allows for the use of different collection modes within the same chromatographic run. Washing functions are also available to reduce sample to sample collection carryover.

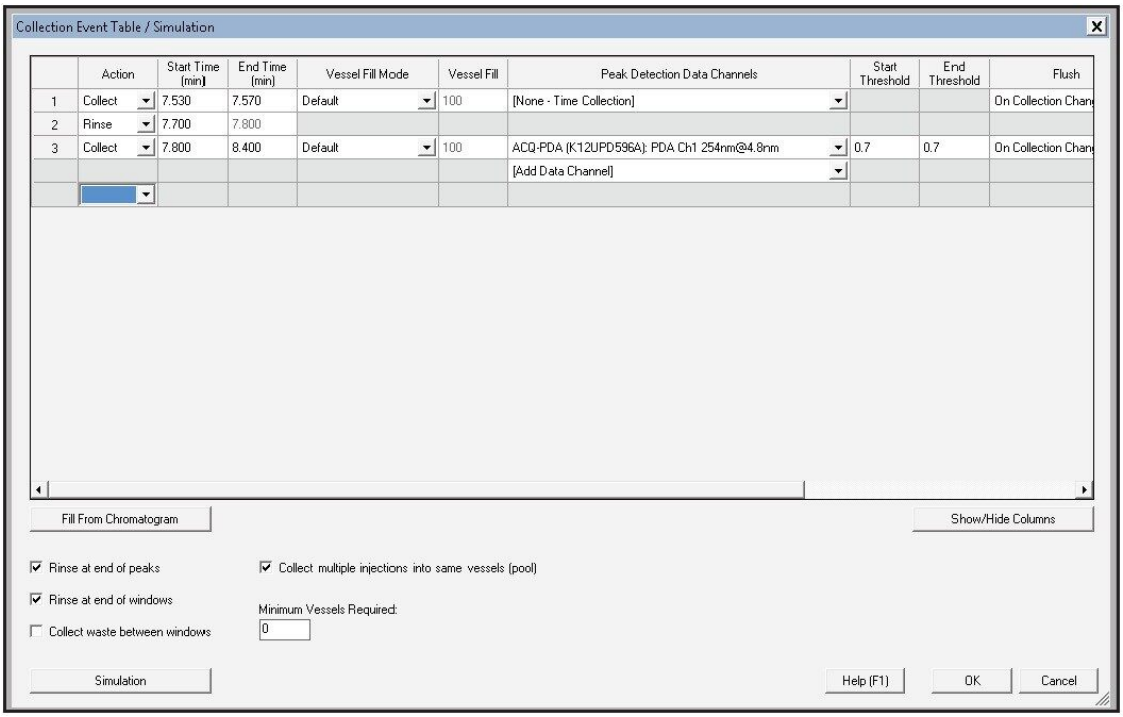

Figure 7. Empower 3 WFM – A method editor showing a time based fraction collection for peak 1 along with a threshold collection within a time window.

For the schisandra berry extract, a peak was cut out of a co-eluting group four peaks using a time collection (Figure 8), two subsequent peaks were collected using threshold collection in a time window. Analysis of the three fractions (Figure 9) showed purity (by UV) for the three peaks of 70.6%, 85.9%, and 98.9% respectively.

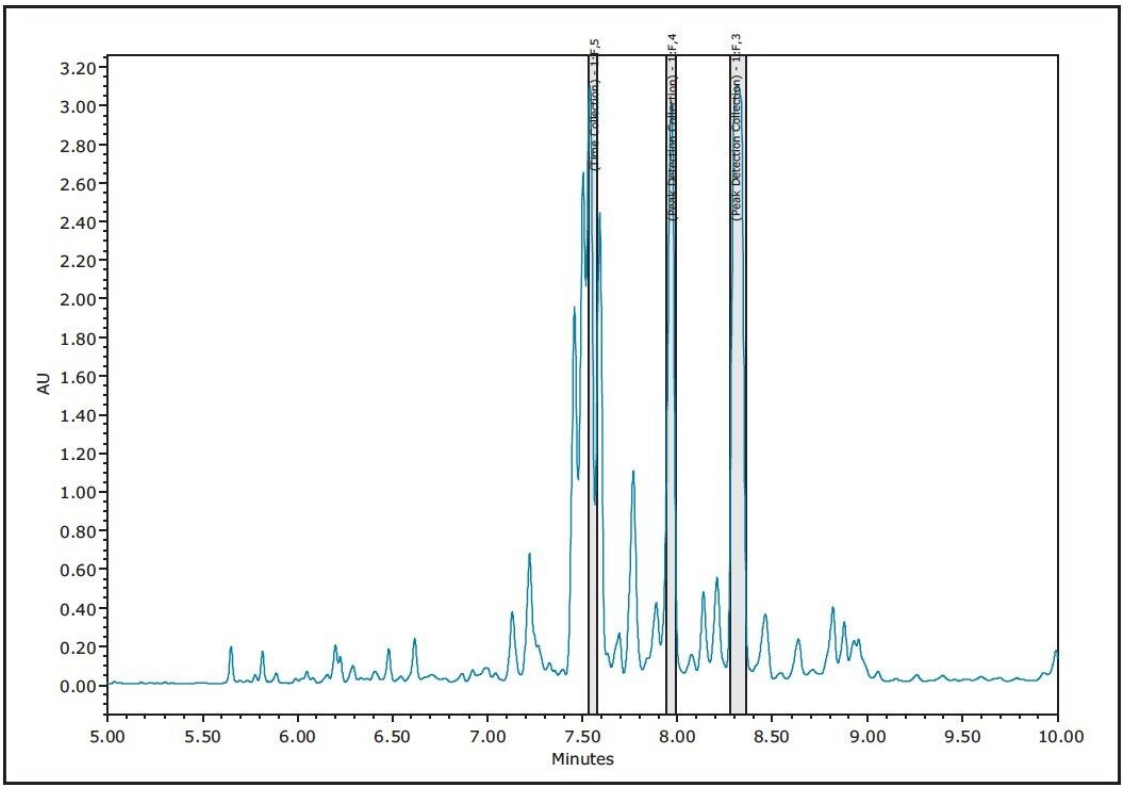

Figure 8. Separation of a schisandra berry SFE extract with time based fraction collection for peak 1 and threshold triggering for peaks 2 and 3. Mobile phase – 0.1% formic acid in water 0.1% formic acid in ACN gradient – 99:1 to 1:99 over 10 min, temperature – 50 °C, Flow – 0.50 mL/min, UV @ 254 nm, column–ACQUITY UPLC BEH C_{18} , 1.7 µm, 2.1 x 100 mm.

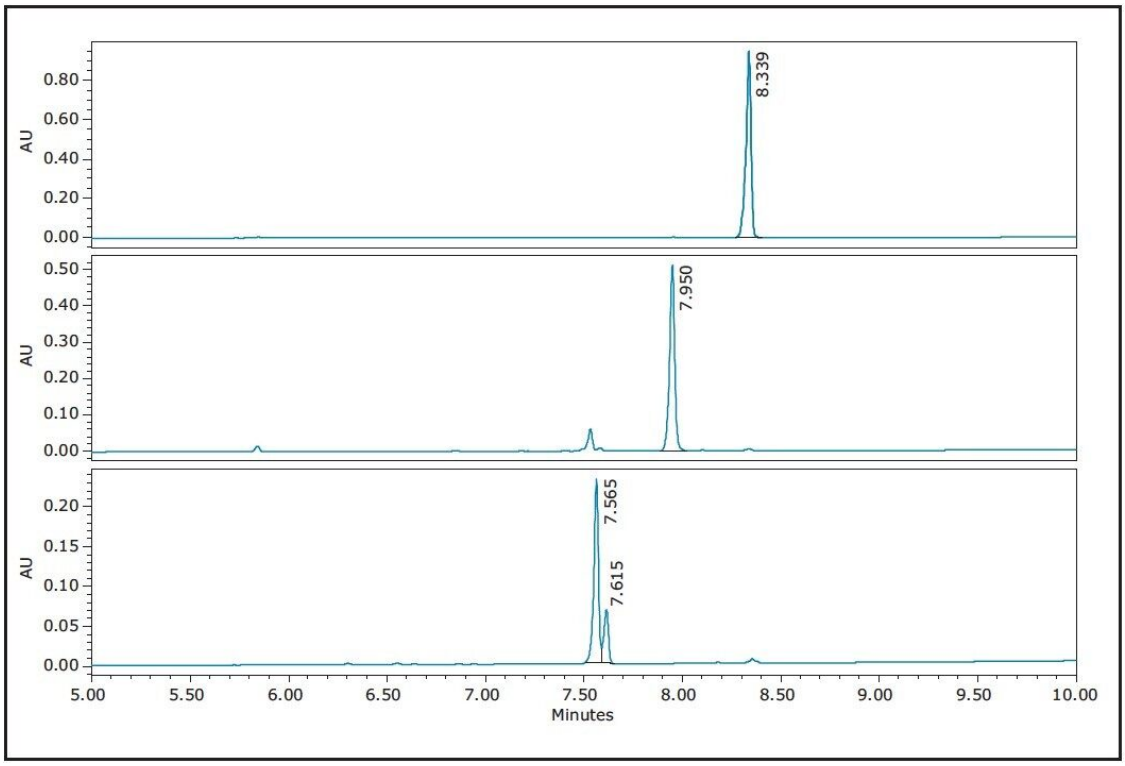

Figure 9. Analysis of collected fractions from schisandra berry SFE extract. Mobile phase – 0.1% formic acid in Water 0.1% formic acid in ACN gradient – 99:1 to 1:99 over 10 minutes, temperature – 50 °C, flow - 0.50 mL/min, UV at 254 nm, column - ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 100 mm.

Along with collection mode flexibility, the software also provides a method simulator (Figure 10). The simulator can take any previously run chromatogram and apply the collection method parameters to it. This allows users to make edits to the collection method and instantly see how the modification will affect subsequent collections.

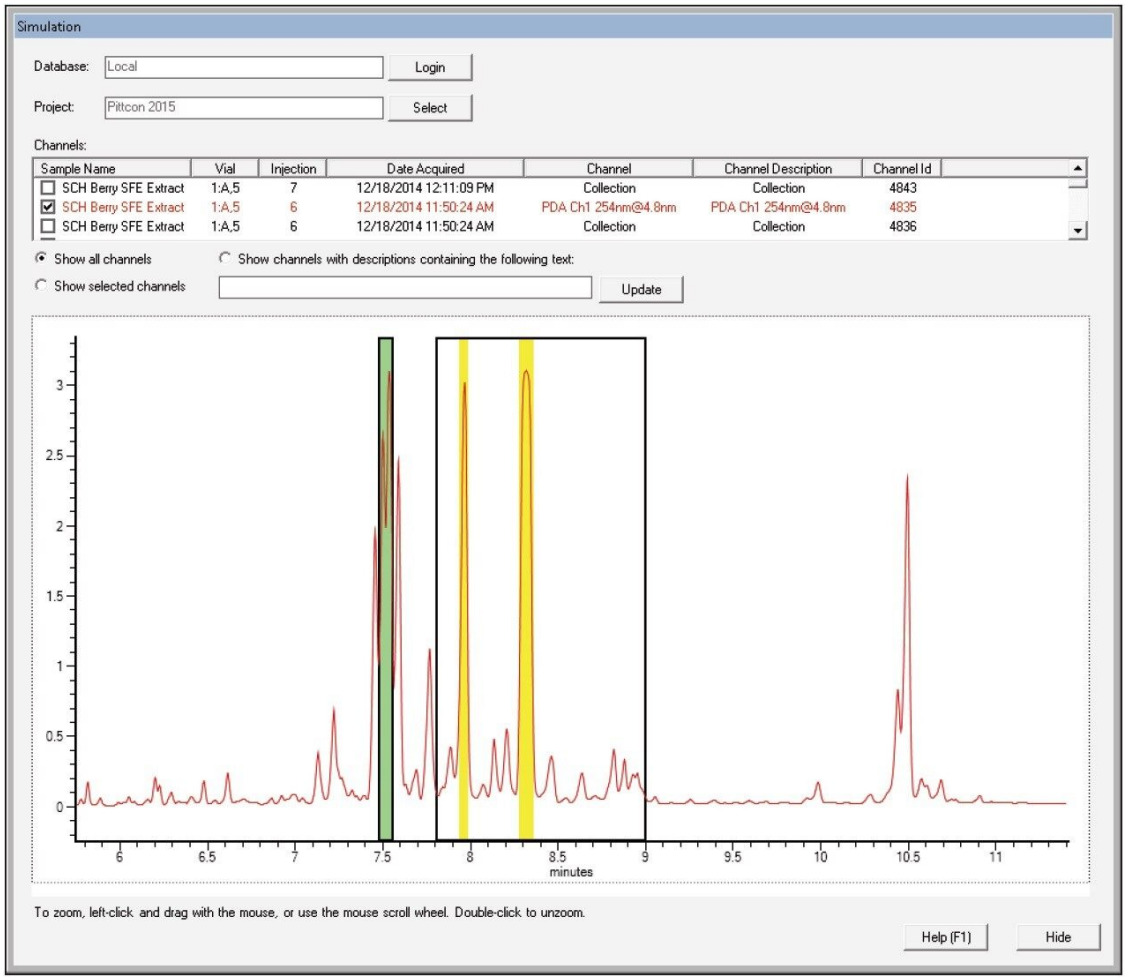

Figure 10. WFM-A method editor collection simulation.

Conclusion

- Using the Waters Fraction Manager – Analytical (WFM-A) peaks of interest were isolated from three complex natural product extracts.
- Multiple modes of collection were demonstrated (time, threshold, and mixed mode).
- Real time collection and collection bed information can quickly be viewed from the ACQUITY console.
- Collections can be simulated using the WFM-A method editor collection simulation which helps the user optimize and understand collection conditions.

- Analysis of collected fractions showed purities of greater than 85% up to 98%, with the exception of schisandra berry peak 1 at 70.6%, which was expected as this was known to be a co-elution.
- The WFM-A is capable of collecting very narrow fractions from sub-2-µm columns without distortion from band broadening making this a useful tool for challenging separations and collections.

Featured Products

Supercritical Fluid Extraction (SFE) Systems <https://www.waters.com/10146521>

ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

Waters Fraction Manager - Analytical <https://www.waters.com/134782878>

Empower 3 Chromatography Data Software <https://www.waters.com/513188>

Available for Purchase Online

ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm X 100 mm, 1/pkg <https://www.waters.com/waters/partDetail.htm?partNumber=186002352>

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