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# Small Scale Purification of Fractions from a Complex Pharmaceutical Formulation Using the Waters Fraction Manager-Analytical and an ACQUITY Arc System

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## Abstract

This application note demonstrates the use of the Waters Fraction Manager - Analytical (W-FMA) in conjunction with an ACQUITY Arc Ultra-High Performance Liquid Chromatography (UHPLC) System to separate and isolate several components present in a model complex pharmaceutical formulation (DayQuil).

## Benefits

- Mass directed purification allows for the collection of compounds that lack chromophores
- The Waters Fraction Manager - Analytical (WFM-A) is designed to accurately and reproducibly operate at sub microliter flow rates to collect very small volume peaks
- Dual detection capability with ACQUITY QDa Mass Detector and ACQUITY UPLC Photodiode Array (PDA) Detector provides the operator with the ability to detect analytes that would not normally be visible in a single detector technology

- Fast and simple method for collecting and isolating active and inactive components in a common cold and cough medicine

## Introduction

In recent years, the drug discovery process has made great advances due to the progression of technology in analytical instrumentation. Isolating, purifying, and identifying active pharmaceutical ingredients in pharmaceutical formulations are essential steps in the drug development process. These steps can be challenging, especially in complex pharmaceutical formulations.

Although mass spectrometry is a destructive method of detection, it still can be used for triggering fraction collection, when configured appropriately. At low flow rates, typically used in analytical scale applications, every drop counts, and the use of a completely optimized and characterized collection system is imperative. Two modes of fraction collection including mass-directed and the time-based approaches will both be demonstrated and discussed in this application note.

Sometimes, the requirement may not be component isolation, but identification. In these instances, online multidimensional chromatographic systems are often used.[1] While the use of such systems is normally sufficient, the setup may not always be available or practical. In this application note, we demonstrate the use of the Waters Fraction Manager - Analytical (W-FMA) in conjunction with an ACQUITY Arc Ultra-High Performance Liquid Chromatography (UHPLC) System to separate and isolate several components present in a model complex pharmaceutical formulation (DayQuil). We will also discuss how the WFM-A can be used effectively in an “off-line multidimensional” approach, to aid in the identification of components from such complex mixtures.


*Figure 1. Analytical purification system with ACQUITY Arc UHPLC System and Waters Fraction Manager - Analytical (WFM-A).*

## Experimental

### Sample preparation

DayQuil was purchased from a local drug store. A sample equivalent to 162.5 mg Acetaminophen, 5 mg

Dextromethorphan HBr, and 2.5 mg Phenylephrine HCl was diluted in 2 mL acetonitrile. Ten µL of this sample was further diluted in 1 mL of acetonitrile giving final concentrations of 812.5 µg $mL^{-1}$, 25 µg $mL^{-1}$, and 12.5 µg $mL^{-1}$, of the three compounds respectively. Ten µL samples of the final solution were injected onto the LC-MS system for analysis. Potential peaks of interest were identified and collected using the WFM-A. Collected fractions were next reanalyzed under optimized gradient conditions.

## Separations

All separations were performed on an ACQUITY Arc UHPLC System equipped with an ACQUITY UPLC Photodiode Array (PDA) Detector and an ACQUITY QDa Mass Detector. The system was coupled with a WFM-A and controlled by MassLynx Software and the FractionLynx Application Manager.

## LC conditions

| | |
|---|---|
| LC system: | ACQUITY Arc |
| Detector: | ACQUITY UPLC Photodiode Array (PDA) Detector |
| Column: | SunFire $C_{18}$ 3.5 µm, 4.6 x 100 mm (p/n 186002553) |
| Column temp.: | 40 °C |
| Injection volume: | 10 µL |
| Flow rate: | 1.5 mL/min |
| Mobile phase A: | $H_2O$ with 0.1% formic acid |
| Mobile phase B: | Acetonitrile with 0.1% formic acid |
| Gradient: | As noted in figures |
| ISM configuration: | 10:1 splitter, Flow rate 0.5 mL/min, 50% acetronitrile/water, 0.1% formic acid |

## MS conditions

| | |
|---|---|
| MS system: | ACQUITY QDa Mass Detector |
| Ionization mode: | ESI+ |
| Capillary voltage: | 0.8 kV |
| Con voltage: | 10 V |
| Source temp.: | 550 °C |

# Results and Discussion

The WFM-A is an optimized low flow rate fraction collector, specifically designed to collect the narrow peaks normally associated with analytical scale applications. Multiple modes of fraction collection can be performed using this WFM-A.[2] In this application note a new mode of collection (mass-directed) along with the time-based fraction collection modes were both explored.

## Mass-directed fraction collection

In mass-directed mode, collection is triggered when the molecular mass of interest passes through the ACQUITY QDa Mass Detector. In this experiment, 10 µL of a DayQuil sample, prepared as previously described, was injected onto the column and two masses were targeted for collection. The two targets, 261 *m/z* and 272 *m/z* were successfully collected, as can be seen in Figure 2. FractionLynx identifies the collected fraction by color in a real time display on the chromatogram. The target value and the vial location are also noted on the chromatogram in real time. The inset in Figure 2 shows the mass spectrum of the collected fraction 272 *m/z* and as can be seen, a pure fraction of the desired mass (272 *m/z*) was collected. This indicates that the system is capable of targeting and collecting a pure compound, when the mass of interest is well resolved from other peaks. The mass spectrum information can be obtained from the actual collection data, without the need for additional fraction analysis.

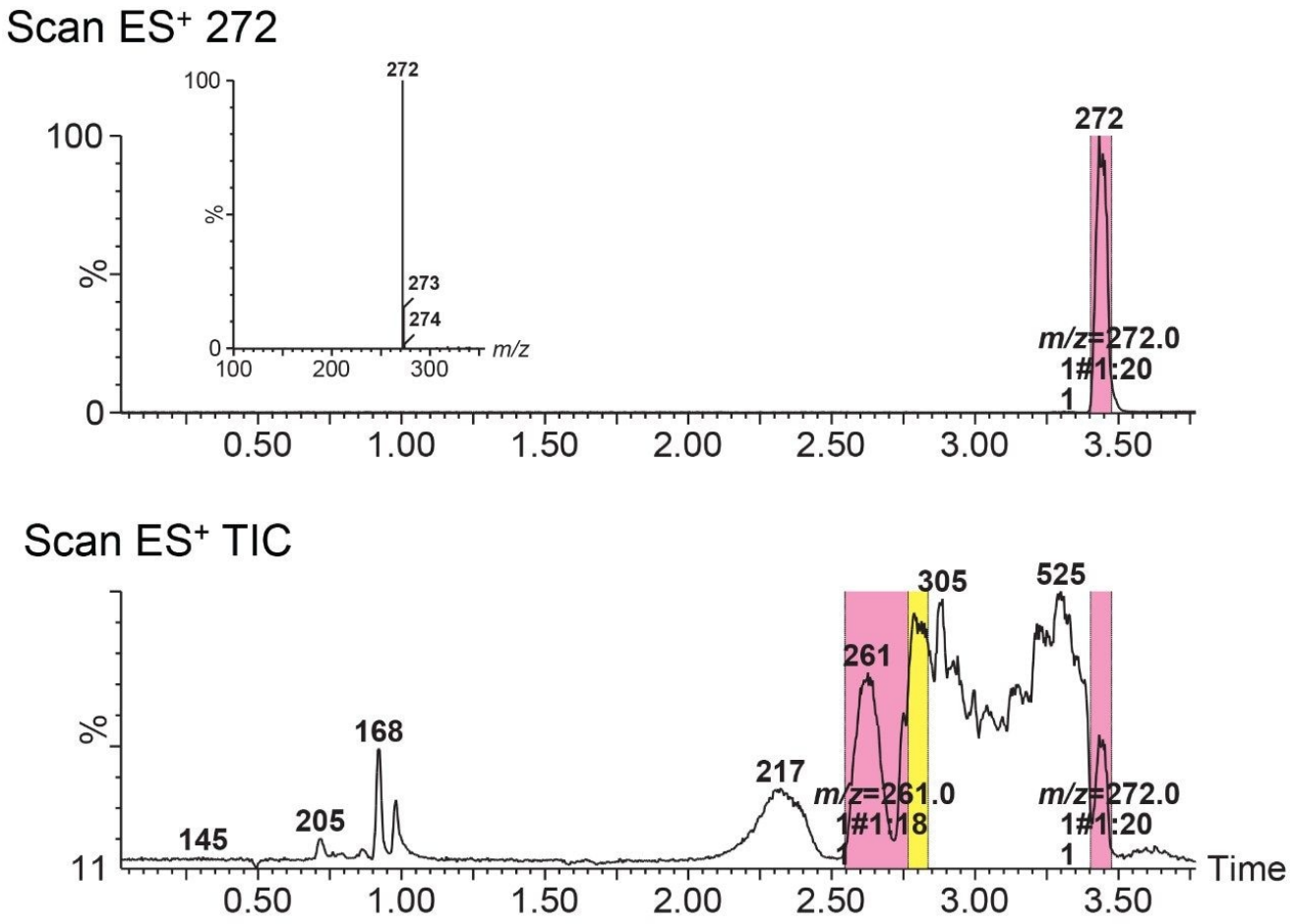


*Figure 2. Representative separation of a DayQuil sample with mass-based collection. Gradient, one to 70% B over five minutes, masses of 261 Da and 272 Da were collected. The inset region in the upper chromatogram shows the mass spectrum of 272 m/z.*

However, if the separation method is not able to resolve the individual components, then the collector is unable to separate those components during the collection process. The system will collect the masses that have been indicated in the sample list, and the overall level of success is now dependent on the chromatographic method. For example, in Figure 3, even though 261 *m/z* was targeted, the spectral analysis indicates that 217 *m/z* and 305 *m/z* was also collected. When 217 *m/z* is extracted, the late elution of a small portion of the compound can be seen overlapping with the beginning of the 261 *m/z* fraction collection, confirming where the contamination came from.

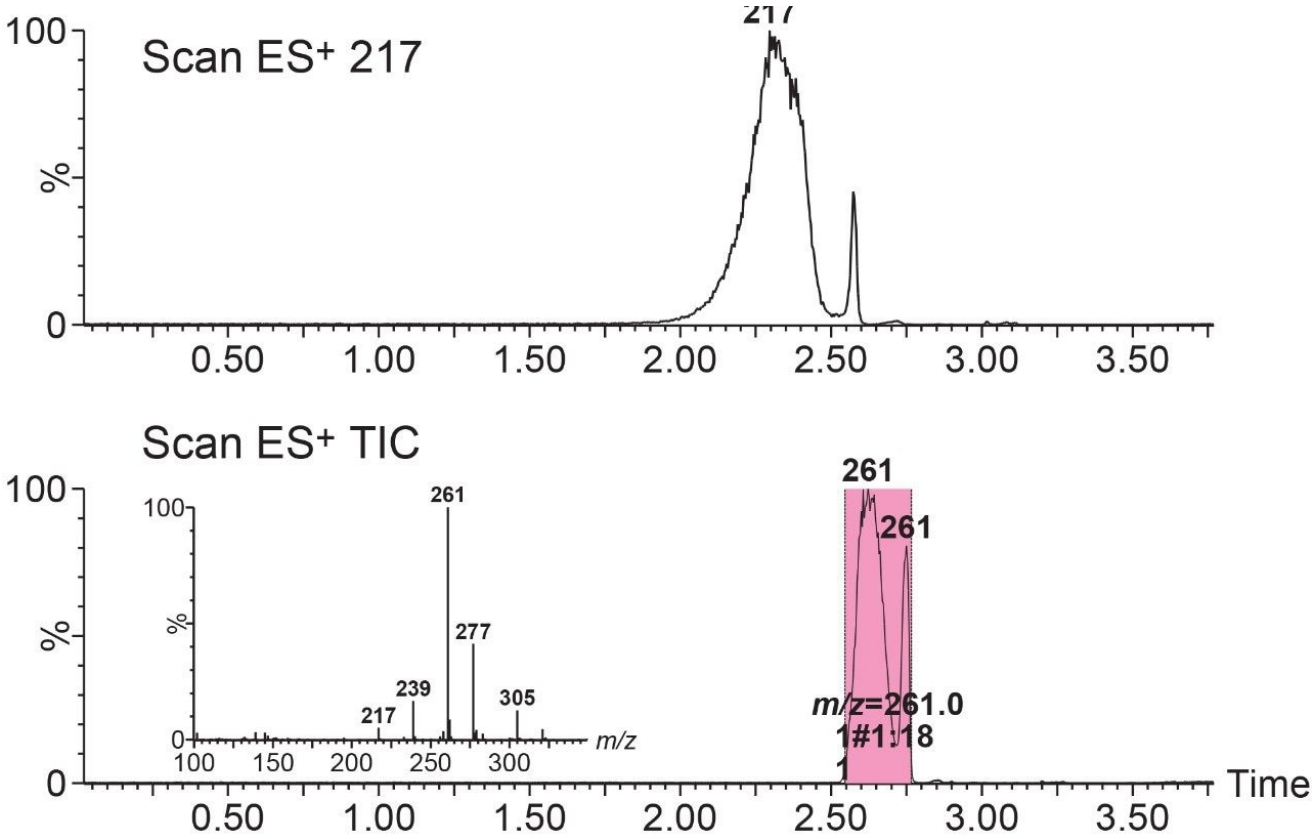


*Figure 3. Representative separation of a DayQuil sample with mass-based collection. Gradient, one to 70% B over five minutes, masses of 261 Da and 272 Da were collected. The inset region in the bottom chromatogram shows the mass spectrum of 261 m/z.*

## Fraction analysis

Post purification analysis of the collected fraction of 261 *m/z* confirmed this co-elution, and indicated that the three components reported in the purification spectrum were confirmed, and contributed to a 9.6% impurity level (Figure 4). The impurities were collected in the front and the tail of the fraction.

In this case, we were purposely overloading the column and expecting situations like this. With this information, subsequent collections could have made use of additional software tools to avoid this situation, such as the Boolean logic NOT function. Using this function allows for collecting only the desired mass and not collecting any other co-eluting masses.

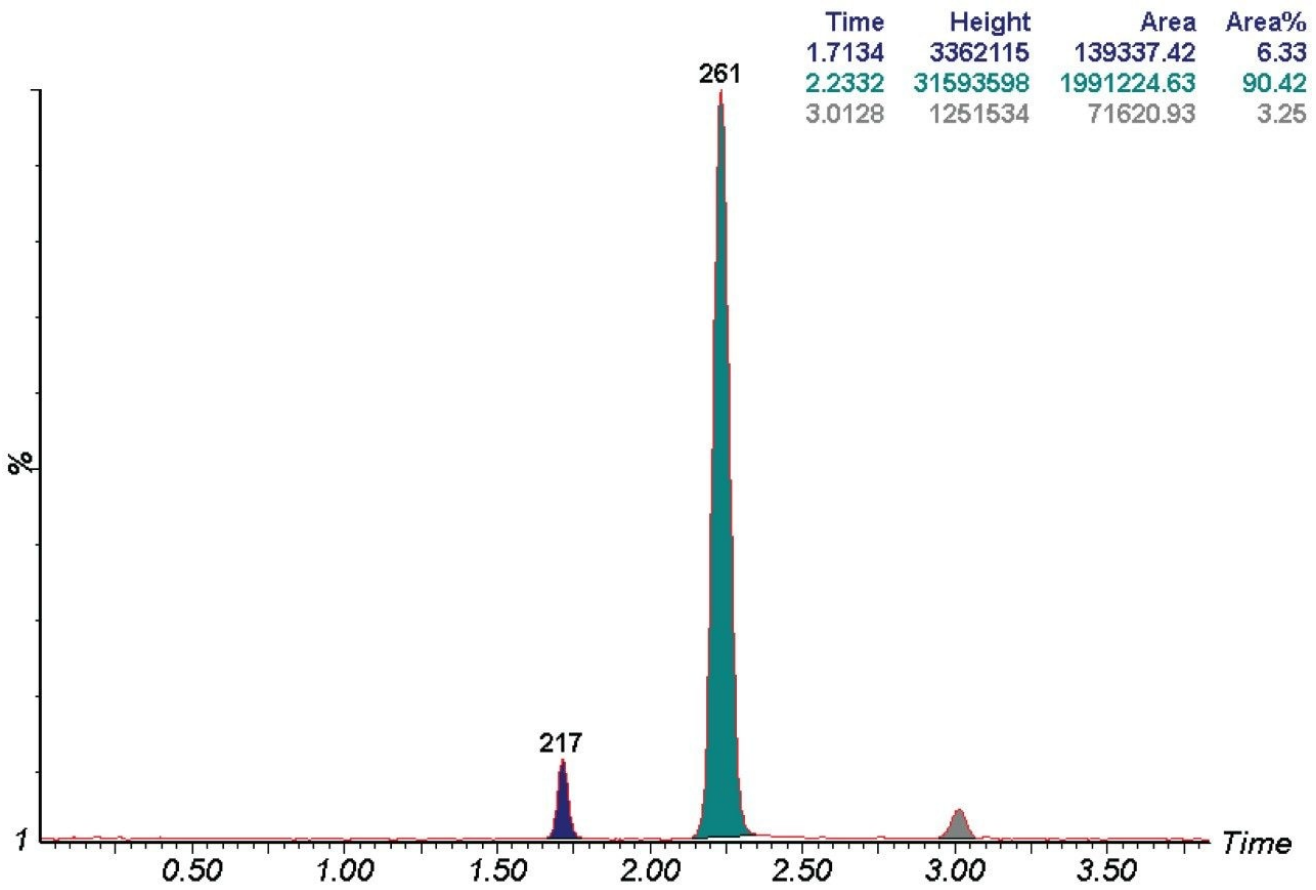


*Figure 4. Representative separation of the post purification analysis of the 261 m/z that was collected as described in Figure 2. Gradient, ten to 30% B over five minutes.*

## Fraction collection based on time

The goal of the experiment was to isolate and identify several components present in a complex pharmaceutical sample. This sample is reasonably complex, and separating all of its components in a single separation method can be challenging and time consuming. Therefore, a two-step approach that uses a time-based fraction collection (Figure 5) and reanalysis of these collected fractions was employed. In this approach, this complex sample was sliced into smaller, more manageable fractions that were then collected and analyzed again.

The fractions were analyzed using more focused gradients (Figure 6) which provided fast and efficient separations for these collected fractions. It is worth mentioning here that Fraction 3 showed polyethylene glycol (PEG) interference. This interference can be seen in the bottom trace, showing the typical PEG mass

distributions with adjacent peaks separated by 44 Da, corresponding to the mass of the repeat unit, ethylene oxide.

The re-analysis of the fractions in this case was performed on the same column as the purification. However, to gain even additional separation efficiency, the re-analysis could also be carried out using orthogonal chemistry, or even an orthogonal chromatographic technique such as Supercritical Fluid Chromatography (SFC) or gas chromatography (GC).

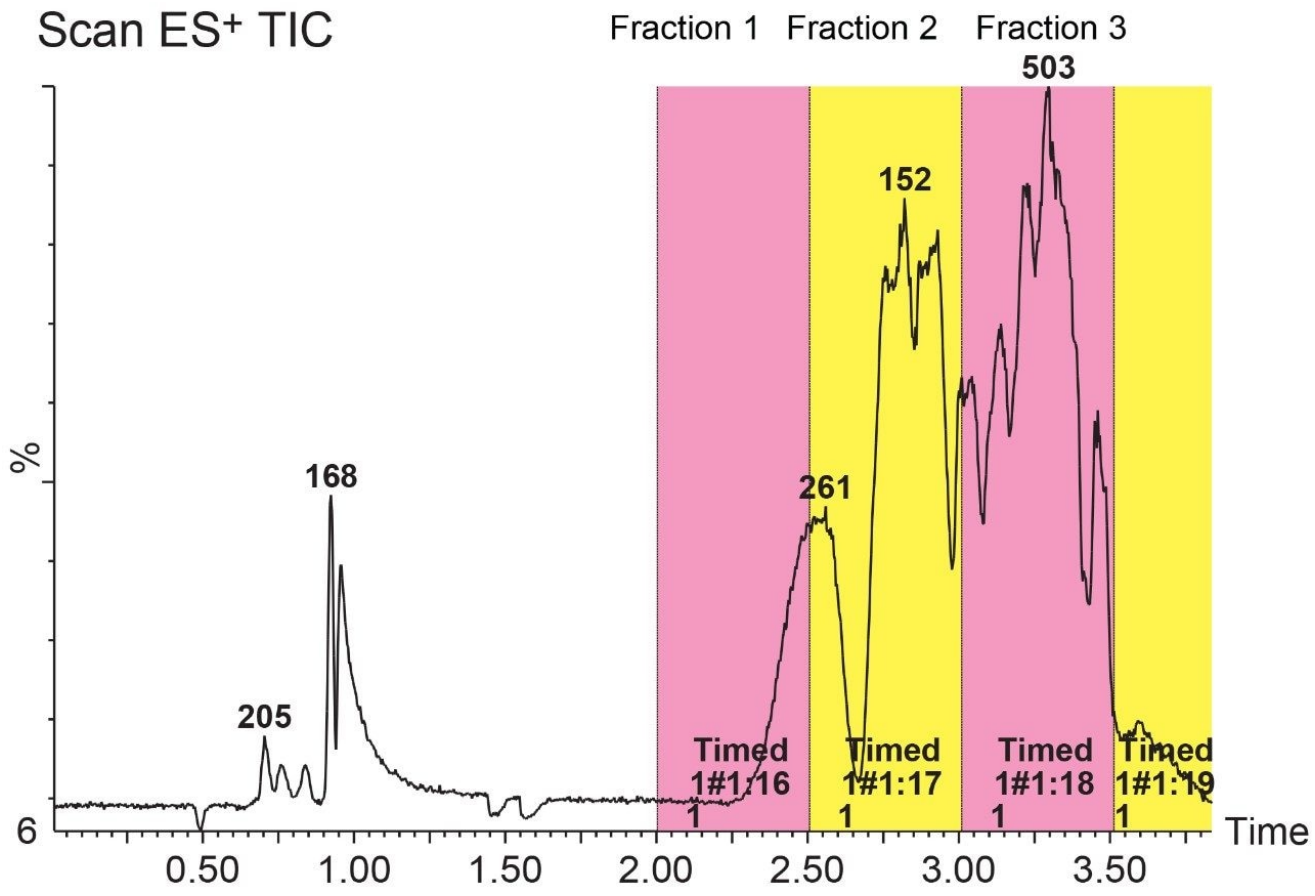


*Figure 5. Representative separation of a crude DayQuil sample with time based collection. Gradient, one to 70% B over five minutes.*

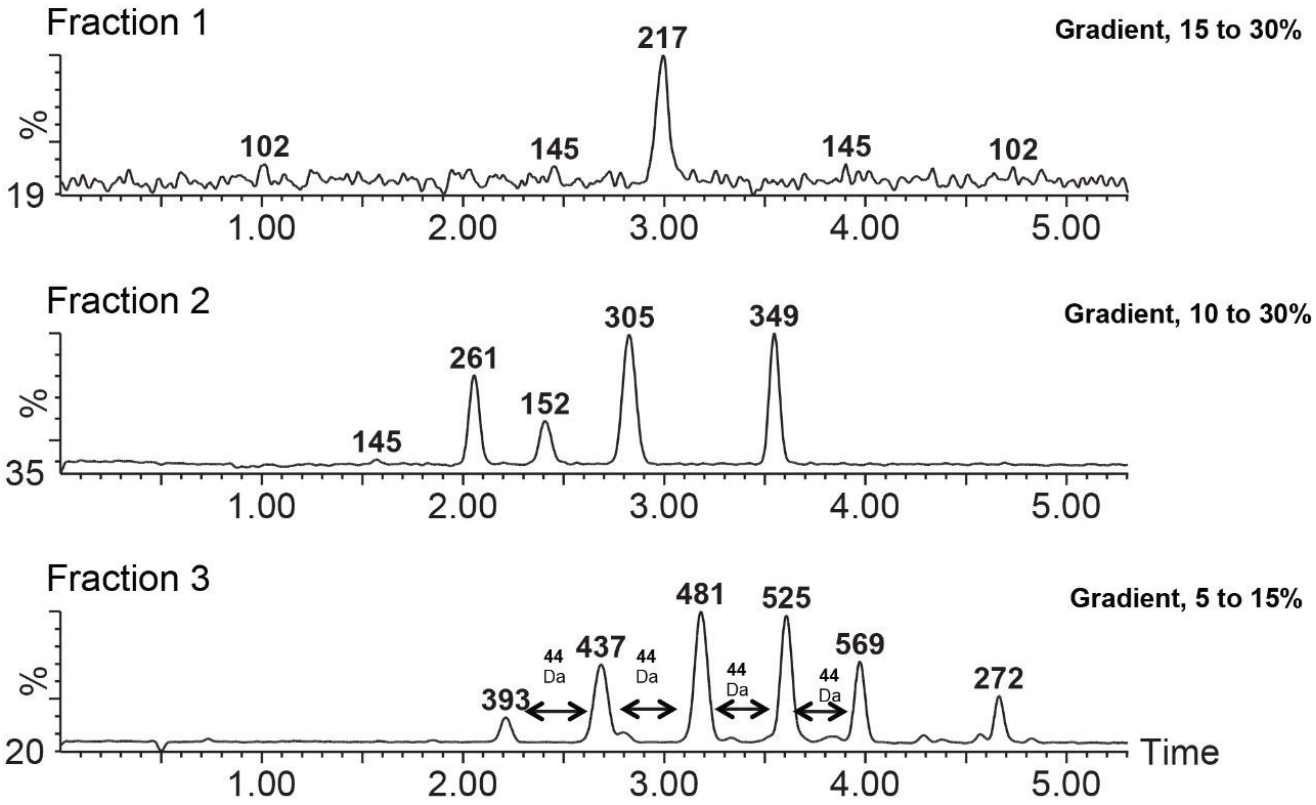


*Figure 6. Re-analysis of time based collected fractions from the DayQuil sample.*

## Dual detector capability

Another interesting feature about the setup we employed in this application note is the dual detection capability. As previously described, our system employs two separate detection modes, ACQUITY QDa and ACQUITY PDA.

The advantage of this setup is that the ACQUITY QDa is capable of detecting analytes that do not have chromophores. This feature is particularly important in improving the collection capabilities for the WFM-A. For example, as shown in Figure 7, multiple components could have been missed and not collected if only ACQUITY PDA detection was employed.

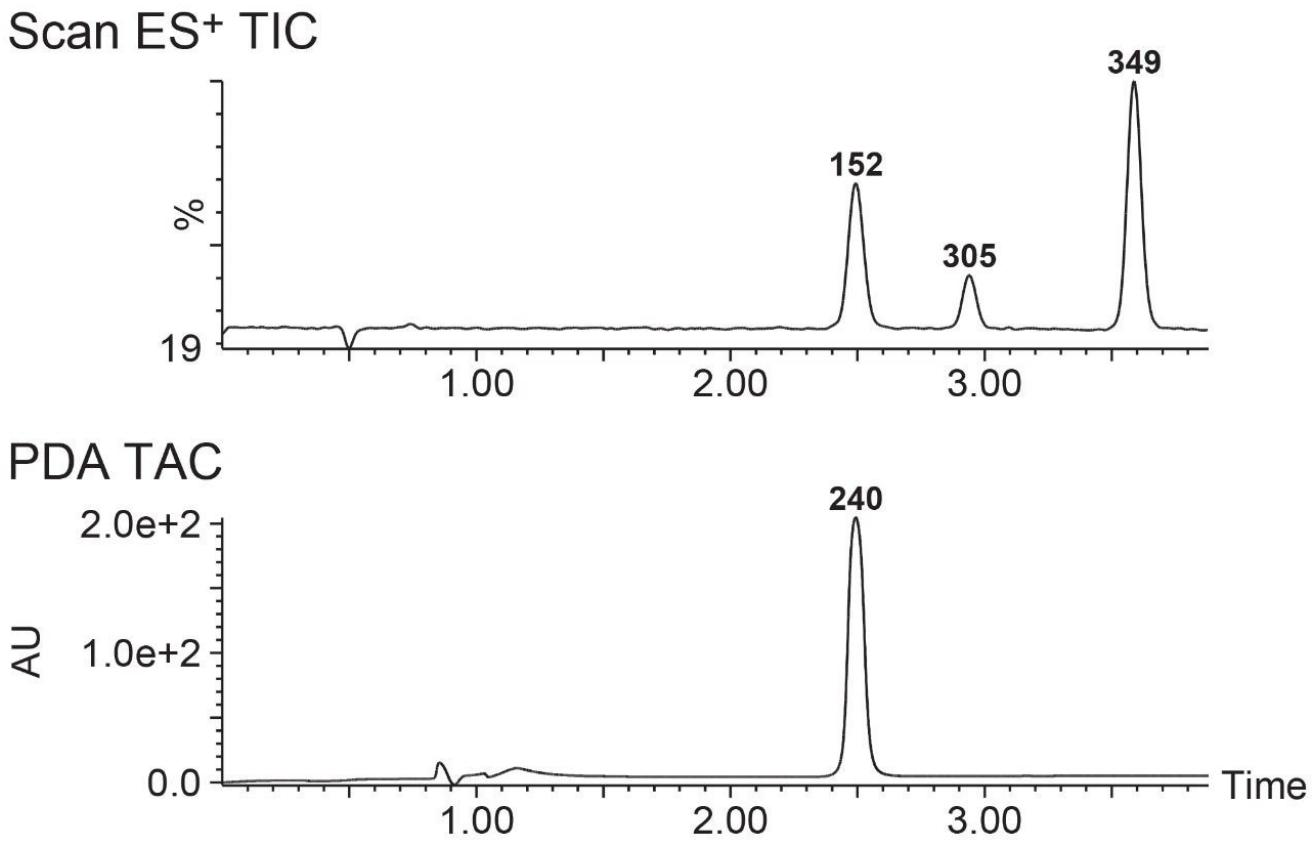


*Figure 7. The ACQUITY QDa (upper trace) and the ACQUITY PDA (lower trace) responses of the fraction containing 152 m/z from Figure 2. Gradient, 10 to 30% change over five minutes.*

## Conclusion

- Both MS directed and other collection techniques using the WFM-A were successfully shown (time based and mass based).
- Dual detection showed to be very beneficial especially when analytes that lack chromophores need to be collected.
- A two-step approach using the ACQUITY Arc UHPLC System in conjunction with the WFM-A was successfully used to isolate and identify several ingredients present in a complex pharmaceutical formulation.

- Using the WFM-A, masses of interest were successfully collected for subsequent analyses.
- Collection efficiency is only as efficient as the separation, and if the compound is not separated on the column, then the fraction will not be pure.

## References


1. Dwight SR., Maloney TD., Recent Advances in Two-Dimensional Liquid Chromatography for Pharmaceutical and Biopharmaceutical Analysis. *LCGC North America* 2017, Volume 35 (9): 680–687.
2. Aubin, A., Jablonski, J. Small Scale Peptide and Impurity Isolation Using the ACQUITY UPLC H-Class and Waters Fraction Manager – Analytical Systems. 720005500EN. 2015.


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