Waters™

Application Note

Mass Directed SFC Purification Using ACQUITY QDa: Torus 2-PIC Separation of Goldenseal Alkaloids

Kenneth D. Berthelette, Thomas Swann

Waters Corporation

Abstract

In this study, we employed a Torus 2-PIC Column to purify components from a plant extract via Prep SFC-MS using ACQUITY QDa-based mass-directed purification.

Benefits

- Prep SFC-MS method for mass-directed purification of natural product alkaloids using the Prep 100q SFC System equipped with an ACQUITY QDa Mass Detector.
- Demonstrate how to scale UPC^2 to Prep SFC separations based on matching the average column pressure.

Introduction

Natural products from plants have a long history of medicinal value. To characterize bioactive constituents and determine their properties, it is necessary to extract and purify the compounds. Purification can be achieved using preparative liquid chromatography (Prep LC). Reversed phase (RP) Prep LC is commonly used for this purpose. It employs safer aqueous mobile phases and allows mass-directed purification (Prep LC-MS), features that are lacking in normal phase (NP) Prep LC.

Preparative supercritical fluid chromatography (Prep SFC) is a purification mode that shares some of the benefits of RP Prep LC, but has unique advantages of its own. Both technologies use a non-toxic weak solvent (water vs. carbon dioxide fluid). With an appropriate splitter and solvent makeup pump, Prep SFC, like RP Prep LC, can easily be equipped with a mass detector such as the ACQUITY QDa for mass-directed purification (Prep SFC-MS). However, Prep SFC benefits from much faster fraction dry down since the collected fractions are only small volumes of volatile organic solvent (usually methanol) when the carbon dioxide flash evaporates at the fraction collector. Also, higher flow rates are accessible for Prep SFC, due to the lower viscosity of mobile phases containing carbon dioxide fluid. These result in faster separations compared to RP Prep LC. Prep SFC separations are based on differences in polar interactions, unlike RP Prep LC, which separates analytes primarily by differences in hydrophobicity. Prep SFC can therefore exploit a wider range of column chemistries, such as the Torus stationary phases, to achieve unique selectivities.

In this study, we employed a Torus 2-PIC Column to purify components from a plant extract via Prep SFC-

MS using ACQUITY QDa-based mass-directed purification. We selected goldenseal (*Hydrastis canadensis*) as the plant source because it is a relatively abundant source of a number of alkaloids including some, such as berberine, that have shown medicinal benefits.[1]

Experimental

UPC2 method conditions

System configuration:	ACQUITY UPC^2 with ACQUITY Column Manager 30-S, ACQUITY PDA Detector, ACQUITY ISM (with QDa splitter) and ACQUITY QDa Mass Detector
Columns:	Torus 2-PIC, 1.7 µm, 3.0 x 50 mm (p/n: 186007600) Torus 2-PIC, 5 µm, 3.0 x 50 mm (p/n: 186008544)
Mobile phase A:	CO_2
Mobile phase B:	Methanol with 20 mM ammonium hydroxide
Gradient:	Linear gradient from 5–50% B in 2.75 min, hold at 50% for 0.63 min, return to 5% B in 0.01 min, and hold at 5% for 0.89 min.
Total run time:	4.38 min.
Flow rate:	1.2 mL/min
ABPR setting:	1625 psi (Torus 1.7 µm)

	2250 psi (Torus 5 µm)
Column temp.:	30 °C
ISM solvent:	95:5 methanol:water with 0.1% formic acid
ISM flow rate:	0.5 mL/min
Detection (UV):	220 nm (compensated from 400–500 nm)
Injection volume:	2.0 µL of the 1.68 mg/mL working solution
Data management:	Empower 3 CDS

Prep 100q SFC method conditions

System configuration:	Prep 100q SFC System with SFC Flow Splitter-100, ACQUITY QDa Detector, and 2998 PDA Detector
Column:	Torus 2-PIC OBD Prep, 5 µm, 19 x 150 mm (p/n: 186008587)
Mobile phase A:	CO_2
Mobile phase B:	Methanol with 20 mM ammonium hydroxide
Gradient:	Linear gradient from 5–50% B in 5.14 min, hold at 50% for 1.14 min, return to 5% B in 0.01 min, and hold at 5% for 1.61 min. Total run time: 7.90 min.
Flow rate:	100 mL/min
Flow splitter setting:	MS 0.6 mL/min

ABPR setting:	120 bar (1740 psi)
Column temp.:	30 °C
Detection (UV):	220 nm (compensated from 400–500 nm)
Fraction makeup solvent:	Methanol
Injection volume:	0.8 mL of the 10 mg/mL stock solution
Data management:	MassLynx v4.1

QDa settings (used for all MS analyses)

Ionization mode:	Positive
Capillary voltage:	1.5 kV
Cone voltage:	15 V
Mass range:	135–1000 Da

Sample preparation

Goldenseal capsules (containing ground goldenseal rhizome and root) were purchased from a food and herbal supplement store. The samples were prepared as follows.

1. Open and remove 400 mg of material from the goldenseal capsules, and sonicate with 5 mL aliquot of ethyl acetate: methanol (1:1).
2. Centrifuge and remove supernatant.
3. Repeat steps 1 and 2 three times.
4. Combine and filter the supernatants through a 0.2 µm PTFE filter.
5. Evaporate the solution and weigh the extracted solid (82 mg).

6. Reconstitute in methanol to a stock concentration of 10 mg/mL (prep sample).

7. Dilute an aliquot of stock to final working solution of 1.68 mg/mL (analytical sample).

Results and Discussion

Goldenseal (*Hydrastis canadensis*) is believed to have many medicinal benefits due to the alkaloids present in the plant. Some of these compounds are shown in Figure 1. Before a natural product can be analyzed, an extraction step is usually required. This step can use various solvents and techniques in order to extract all of the target compounds present in the sample. In this case, the sample was extracted using sonication and 1:1 ethyl acetate:methanol to generate the analytical and prep samples.

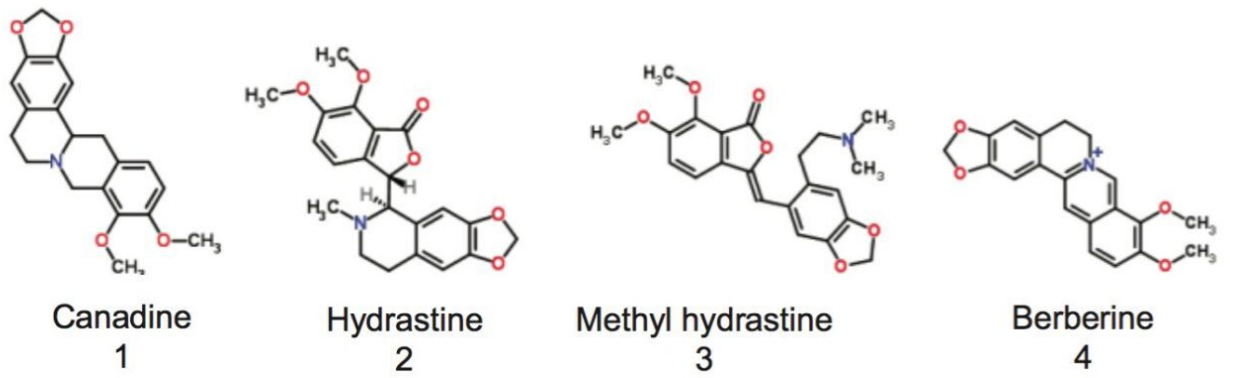

Figure 1. Structures of alkaloids that can be found in goldenseal extracts.

The next step after extraction is to develop an analytical SFC-MS method that can then be scaled to Prep SFC-MS. Typically a method development strategy is employed in order to get ample separation to scale to the prep column. For the analysis of the goldenseal extract, the Waters achiral method development strategy was employed.[2] The first step in this strategy is to try a Torus 2-PIC Column for the analytical sample. This was done and we found that the basic alkaloids in the sample benefited from the use of 20 mM ammonium hydroxide as an additive. Specifically, a gradient of 5–50% methanol with 20 mM ammonium hydroxide over 2.85 minutes was used on a Torus 2-PIC, 1.7 µm, 3.0 x 50 mm Column (p/n:186007600). The resulting chromatogram is shown in Figure 2. Good separation of the major and minor goldenseal alkaloids is obtained on the 1.7 µm particle analytical column.

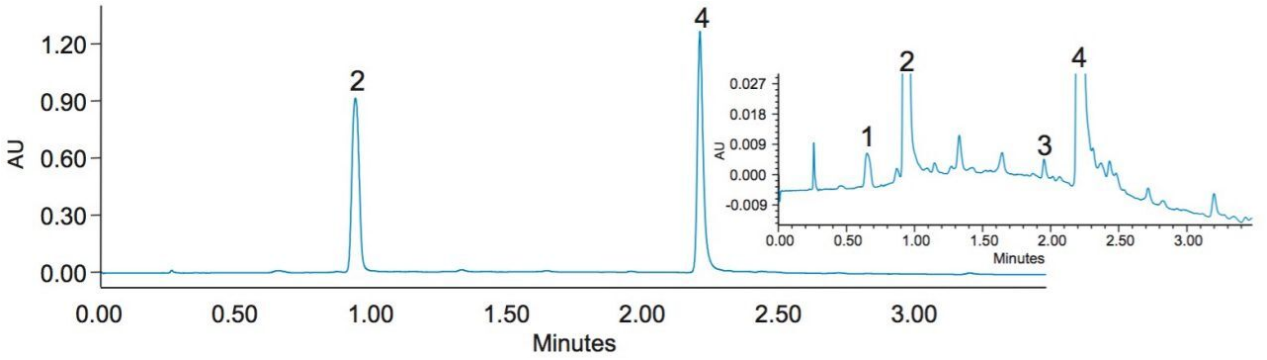

Figure 2. Separation of the goldenseal extract on a Torus 2-PIC, 1.7 µm, 3.0 x 50 mm Column. Inset is a 50x magnification of the baseline. Refer to figure 3 for peak assignments.

Additionally, good MS signal intensities were obtained using an ACQUITY QDa Detector on the ACQUITY UPC^2 System, as demonstrated in Figure 3. This gives confidence that a mass directed SFC-MS purification will be successful.

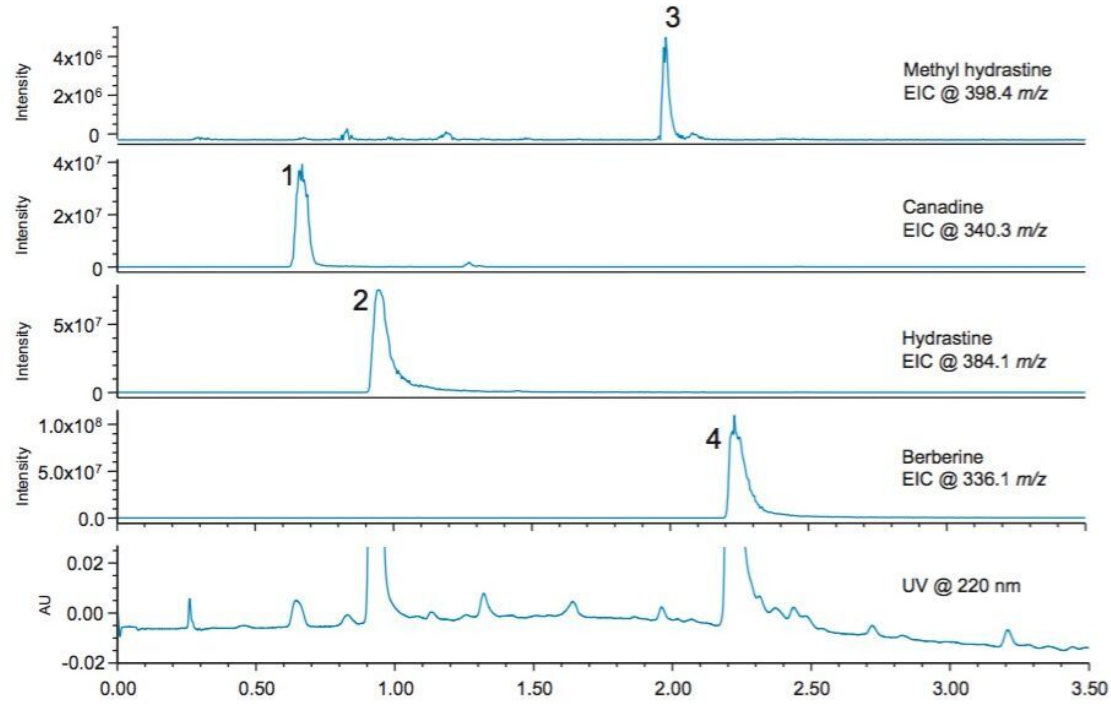

Figure 3. Overlay of a chromatogram obtained using UV detection at 220 nm and the extracted ion chromatograms of the four peaks of interest obtained using a ACQUITY QDa Detector.

The next action is to scale the method to a 5 µm particle preparative column. While this is often done in one

step, it is sometimes useful to first scale the method to a 5 µm particle analytical column. This allows the analyst to check column loading on a small scale and to assess the decreased resolution due to the larger particle size that will be used in the Prep SFC purification.

Transferring methods in SFC is similar to transferring methods in LC, however the compressibility of the mobile phase must be accounted for. In SFC, the carbon dioxide fluid is compressible, and its elution strength is based on density. Matching the mobile phase density when changing columns dimensions and/or particle sizes is very important to achieve similar chromatographic results and a successful method transfer.[3,4] A simple and effective way to match mobile phase density is to match the average column pressure between methods. The average column pressure is the average of the pressure measured by the system and the active backpressure regulator (ABPR) setting.[5]

The successful transfer of the goldenseal extract analysis to an analytical Torus 2-PIC, 5 µm, 3.0 x 50 mm Column (p/n: 186008544) was performed as depicted in Figure 4. This was done by adjusting the ACQUITY UPC^2 ABPR method setting to 2250 psi, thereby achieving the same average column pressure as in the 1.7 µm analytical column run. As expected, there are slight differences in the chromatographic results, due to the lower efficiency of the 5 µm particle, such as wider peaks and lower signal intensity. However, the separation selectivity is maintained with the 5 µm particle column.

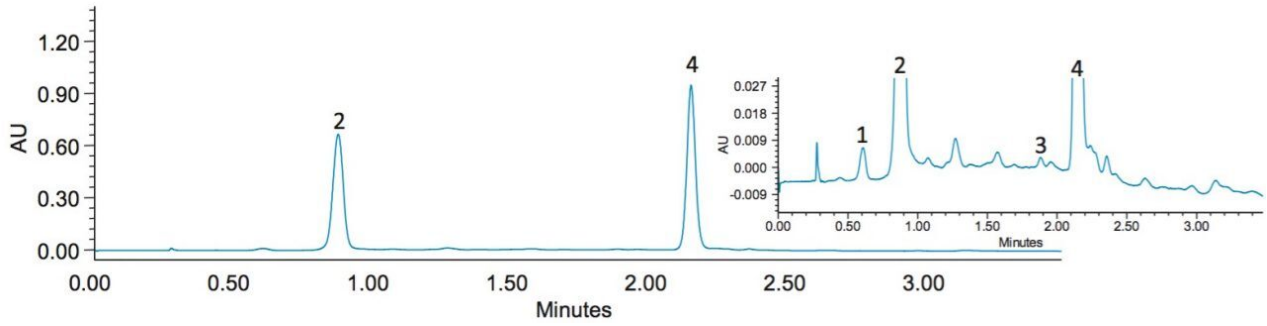

Figure 4. Separation of the goldenseal extract on a Torus 2-PIC, 5 µm, 3.0 x 50 mm Column. Inset is a 50x magnification of the baseline to visualize the minor constituents.

After confirming that the separation can be performed on a 5 µm particle column at the analytical scale, we next transferred the method to a Prep SFC-MS system. Again, it is important to maintain mobile phase density when scaling an SFC method. Matching the density of the separation obtained with the 1.7 µm column was achieved on the Prep 100q SFC System by using an ABPR setting of 120 bar (~1740 psi) at a flow rate of 100 mL/min with a Torus 2-PIC, 5 µm, 19 x 150 mm OBD Prep Column (p/n: 186008587). Fractions of the main constituents (berberine and hydrastine) were collected based on mass and UV absorbance as both

compounds had good signal under both forms of detection. The other constituents were collected based on mass only due to the low UV absorbance of the compounds. The chromatograms of the resulting fractions are shown in Figure 5.

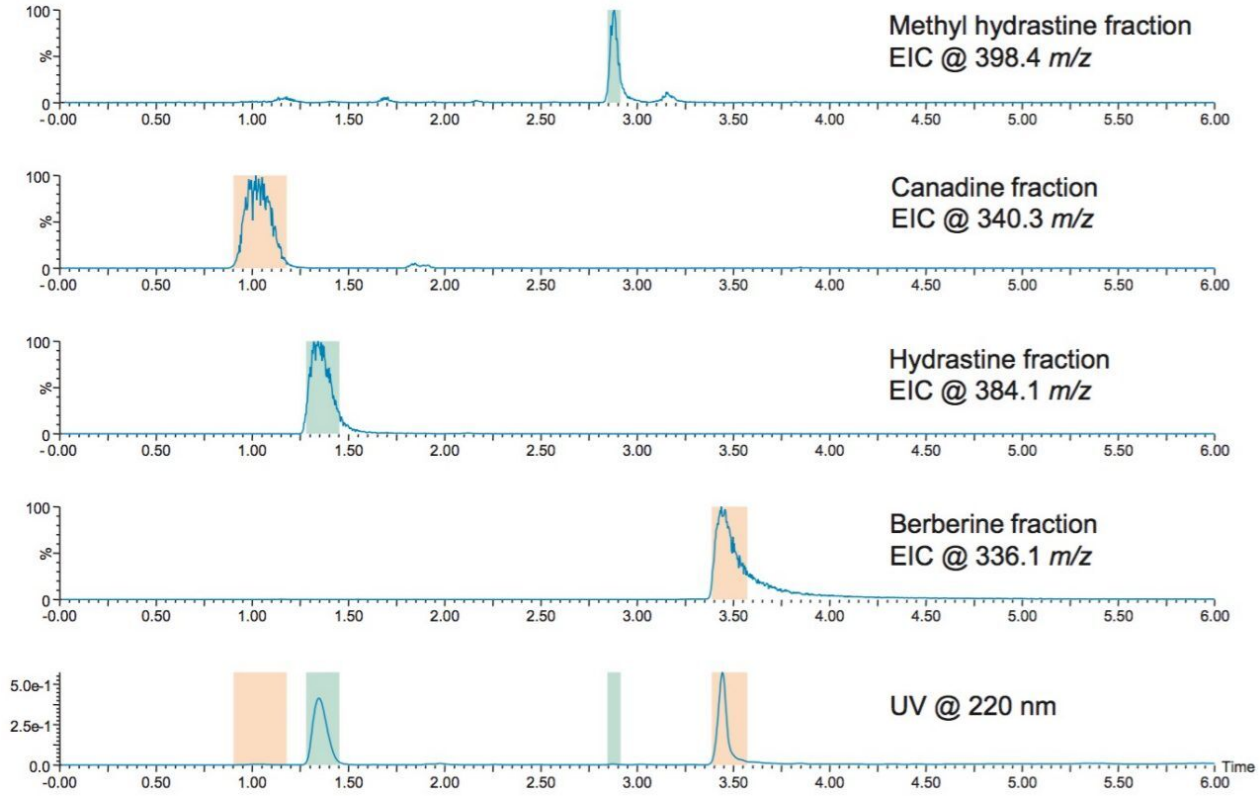

Figure 5. Chromatograms of the fractions collected from the goldenseal extract using a Torus 2-PIC, 5 µm, 19 x 150 mm OBD Prep Column on a Prep 100q SFC System with UV and mass detection.

The methanol fractions (6 to 20 mL each) were quickly dried (<10 min total) using a Savant SpeedVac vacuum concentrator. The resulting isolates were analyzed for fraction purity. The canadine, hydrastine, and berberine fractions were found to have a purity of 99% or greater when analyzed on the Torus 2-PIC 1.7 µm Column. An example of a confirmation run is shown in Figure 6. The fraction of methyl hydrastine had insufficient material to analyze from this single injection, though additional injections of the extract could have allowed its isolation.

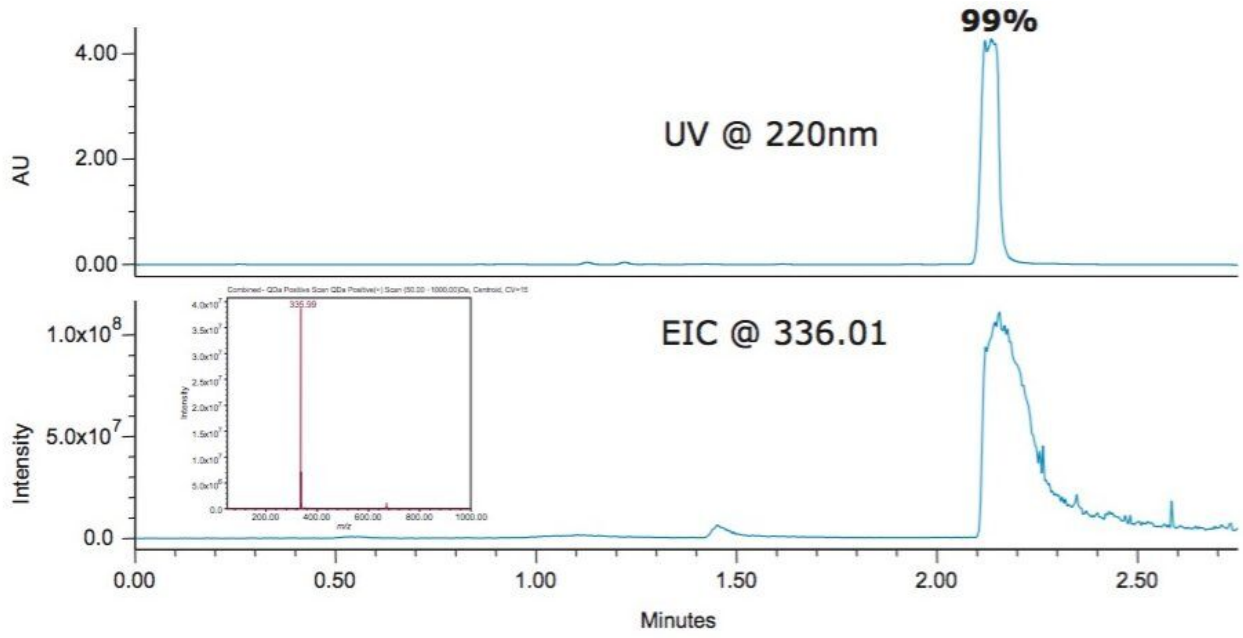

Figure 6. Re-analysis of the berberine fraction using a Torus 2-PIC, 1.7 µm, 3.0 x 50 mm Column on an ACQUITY UPC^2 System. Percentages listed are % area of the given peak.

Conclusion

The purification of natural products from herbal extracts is sometimes necessary to study their properties and preparative chromatography is often employed for this purpose. A goldenseal (*Hydrastis canadensis*) extract was separated using SFC-MS methods. An analytical ACQUITY UPC^2 method was first developed on a 1.7 µm Torus 2-PIC Column. This method was then scaled to a 5 µm analytical Torus 2-PIC Column using matched average column pressure to maintain the same average density and resulting elution strength of the mobile phase. Scaling in the same way to a preparative SFC-MS method using a Prep 100q SFC System with an ACQUITY QDa Detector allowed very quick separation and selective isolation of the individual alkaloids.

References

1. “Berberine: Uses, Side Effects, Interactions and Warnings” WebMD, 2016,

http://www.webmd.com/vitamins-supplements/ingredientmono-1126-berberine.aspx?activeingredientid=1126&activeingredientname=berberine.

2. Hill, J.; Fairchild, J. "Design and Implementation of a Simple Achiral Method Development Strategy for UPC^2 Chemistries" Waters Poster PSTR134833594.

3. Hudalla, C; Tarafder, A; Jablonski J; Fountain K. "UPC^2 Strategy for Scaling from Analytical to Preparative SFC Separations" Waters Application Note 720004818EN.

4. Hudalla, C; Tarafder, A; Jablonski, J; Roshchin R; Fountain K; Patel, M. "UPC^2 Strategy for Scaling SFC Methods: Applications for Preparative Chromatography" Waters Application Note 720005064EN.

5. Tarafder, A., Hill, J., "Scaling rule in SFC. II. A practical rule for isocratic systems" *J. Chromatogr. A*, 1482 (2017) 65–75.

Featured Products

ACQUITY UPC2 System <https://www.waters.com/134658367>

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

Prep 100q SFC System <https://www.waters.com/10145748>

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

720005951, March 2017

©2019 Waters Corporation. All Rights Reserved.