

Typical Conditions for Analyzing and Isolating the Compounds in the Preparative Chromatography Mixture Standard with an ACQUITY QDa Detector

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GOAL

To provide chromatographic method conditions for preparative systems configured with an ACQUITY® QDa® Detector using the Preparative Chromatography Mix Standard as a system test prior to injecting samples for isolation.

BACKGROUND

Compound isolation requires careful chromatographic system preparation to ensure that the instrumentation is properly setup prior to injecting valuable samples. The Preparative Chromatography Mix Standard is specially formulated with an acid, a base, and a neutral compound and can be used daily to evaluate the condition of the column and to benchmark system performance. Changes in column efficiency or collection parameters are apparent and can be addressed before sample loss occurs. In this technology brief, we document the use of this standard mixture in the Waters® AutoPurification™ System configured with an ACQUITY QDa Detector.

- Preparative Chromatography Mix Standard, [Part Number 186006703](#)
- Preparative Column: XBridge® BEH C₁₈ OBD Prep, 19 x 50 mm, 5 μm, [Part Number 186002977](#)
- Analytical Column: XBridge BEH C₁₈, 4.6 x 50 mm, 5 μm, [Part Number 186003113](#)

The Preparative Chromatography Mix Standard assesses the suitability of the preparative LC system for isolating target compounds, ensuring proper system setup and column performance before purifying valuable samples.

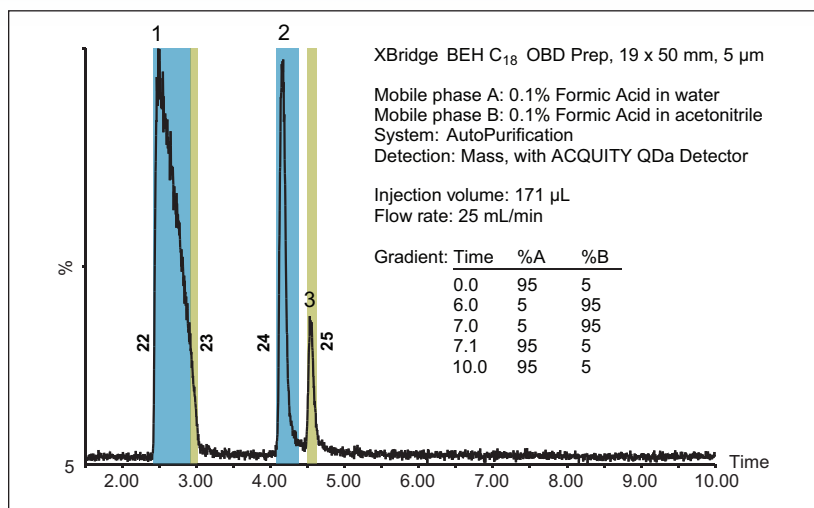


Figure 1. TIC of the mass-directed collection of the three compounds (5 mg/mL each in DMSO) in the Preparative Chromatography Mix Standard; 1 Diphenhydramine ($m/z = 256.2$, Fractions 22 and 23), 2 Flavone ($m/z = 223.2$, Fraction 24), and 3 Diclofenac ($m/z = 296.0$, Fraction 25).

RESULTS

The separation shown in the TIC chromatogram in Figure 1 is typical for injections of the prep standard on the XBridge BEH C₁₈ Column. While diphenhydramine, a base, elutes first at about 2.5 minutes, flavone and diclofenac, a neutral compound and an acidic compound, respectively, elute later but with good resolution between them. Different method conditions and column chemistries may change the chromatographic profile, but routine use of the prep standard should show consistent results when the parameters are held constant.

Analysis of the collected fractions, shown in Figure 2, indicates that the isolated compounds are reasonably pure. These results suggest that the chromatographic system is properly set up and is performing as expected, making it acceptable for isolating and purifying compounds. The ACQUITY QDa Detector method parameters are the same at both the analytical and preparative scales, and are listed in Figure 3.

SUMMARY

- The Preparative Chromatography Mix Standard is suitable for assessing the chromatographic performance and system suitability before injecting samples for isolation and purification on prep systems configured with an ACQUITY QDa Detector.
- While different column chemistries and method conditions may alter the chromatographic profile of the Preparative Chromatography Mix Standard, repeatability of results from day to day is a good indication of the prep system's suitability for isolating compounds.
- The mass spectrometer method parameters for the ACQUITY QDa Detector illustrated here are acceptable for this sample mixture, but should be optimized for other isolations.

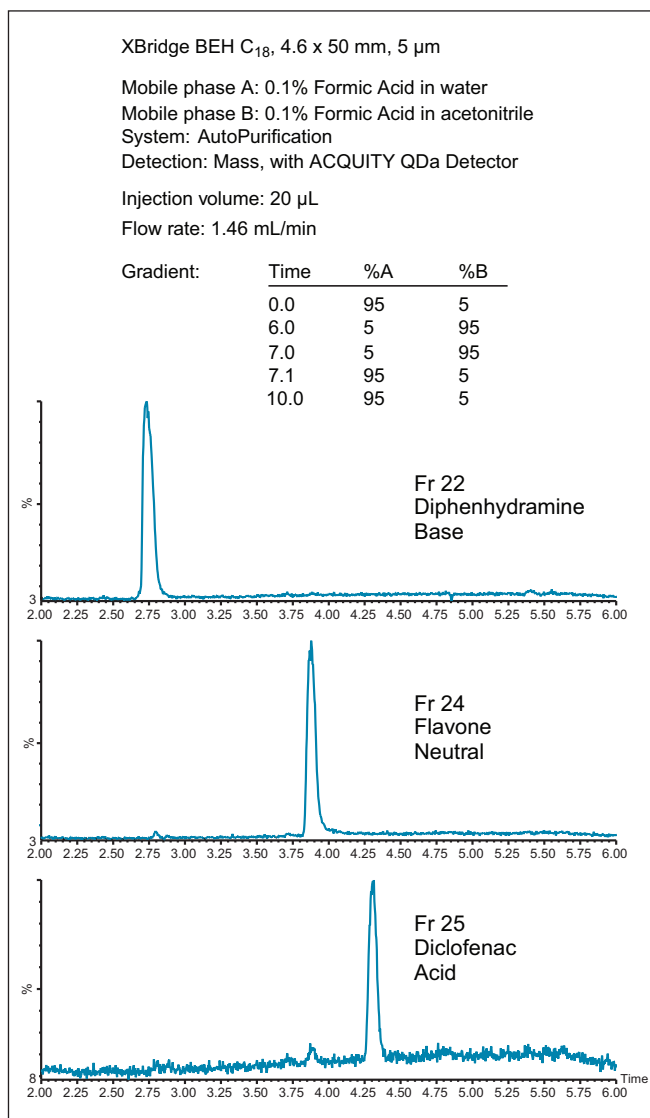


Figure 2. TICs of the fraction analysis of the three collected compounds in the Preparative Chromatography Mix Standard. Fraction 23 was not analyzed due to the very low fraction volume.

ACQUITY QDa Detector	
Ionization mode: ES+	Capillary voltage: 0.8
Data: Centroid	Probe temperature: 600
Mass range: 100–650	Detector gain: 1
Cone voltage: 15	Makeup: 90% water/ 10% acetonitrile/0.01% formic acid
Sampling frequency: 5 Hz	

Figure 3. ACQUITY QDa Detector method parameters.

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