

응용 자료

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

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This is an Application Brief and does not contain a detailed

Experimental section.

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## Abstract

This application brief demonstrates 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.

### Benefits

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.

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## Introduction

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 C<sub>18</sub> OBD Prep, 19 x 50 mm, 5 µm, Part Number 186002977
  - Analytical Column: XBridge BEH C<sub>18</sub>, 4.6 x 50 mm, 5 µm, Part Number 186003113
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## Results and Discussion

The separation shown in the TIC chromatogram in Figure 1 is typical for injections of the prep standard on the XBridge BEH C<sub>18</sub> 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.

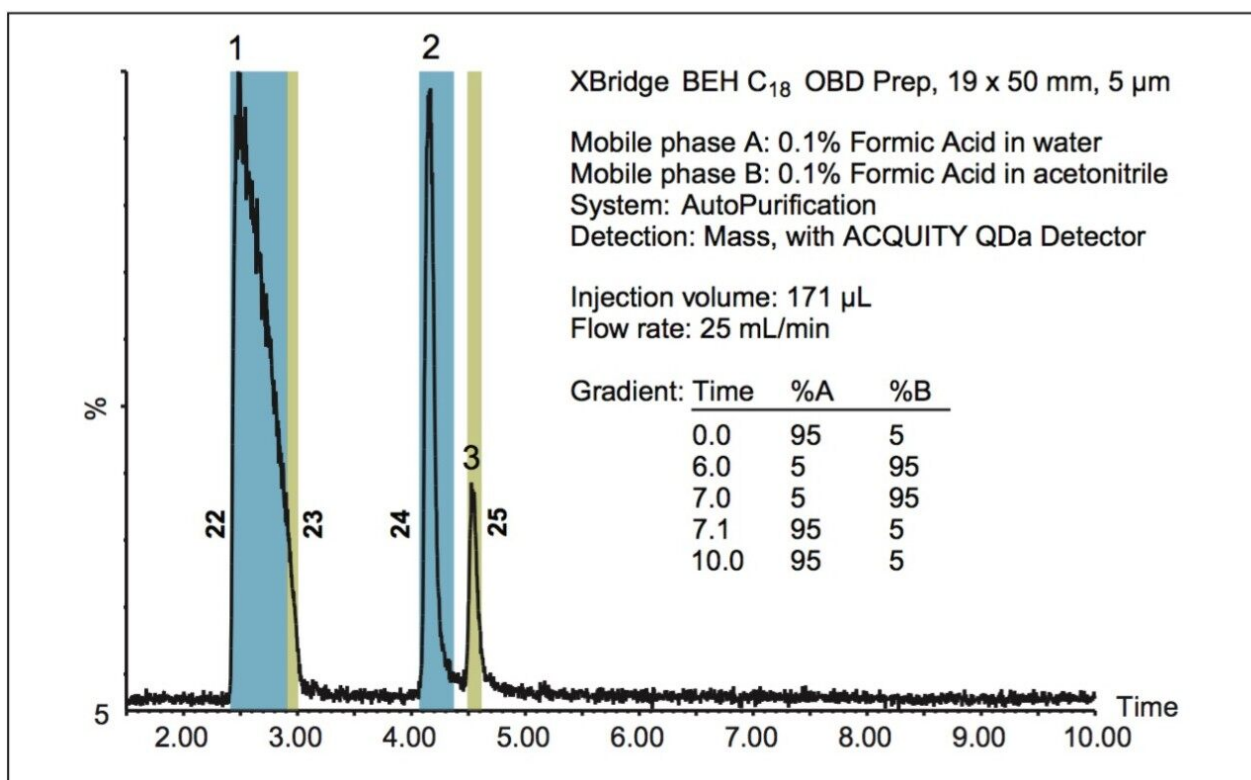


Figure 1. TIC of the mass-directed collection of the three compounds (5 mg/mL each in DMSO) in the Preparative Chromatography Mix Standard; 1 Diphenhydramine ( $m/z = 256.2$ , Fractions 22 and 23), 2 Flavone ( $m/z = 223.2$ , Fraction 24), and 3 Diclofenac ( $m/z = 296.0$ , Fraction 25).

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.

XBridge BEH C<sub>18</sub>, 4.6 x 50 mm, 5 μm

Mobile phase A: 0.1% Formic Acid in water

Mobile phase B: 0.1% Formic Acid in acetonitrile

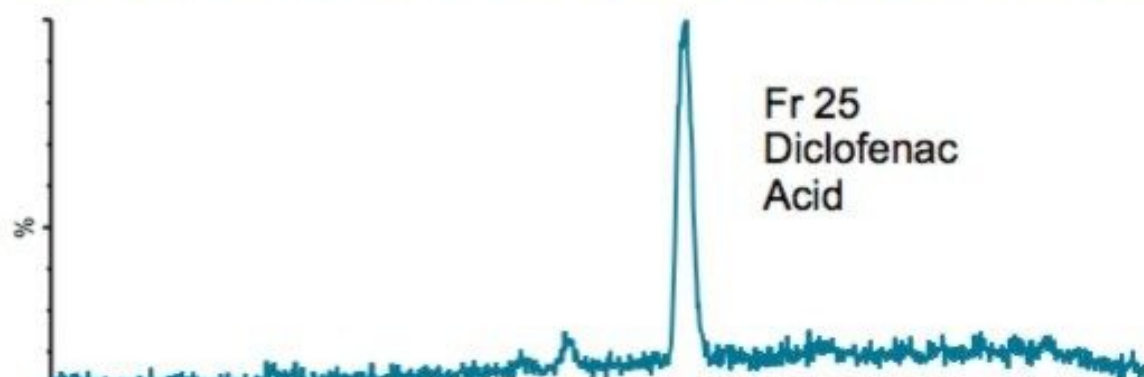
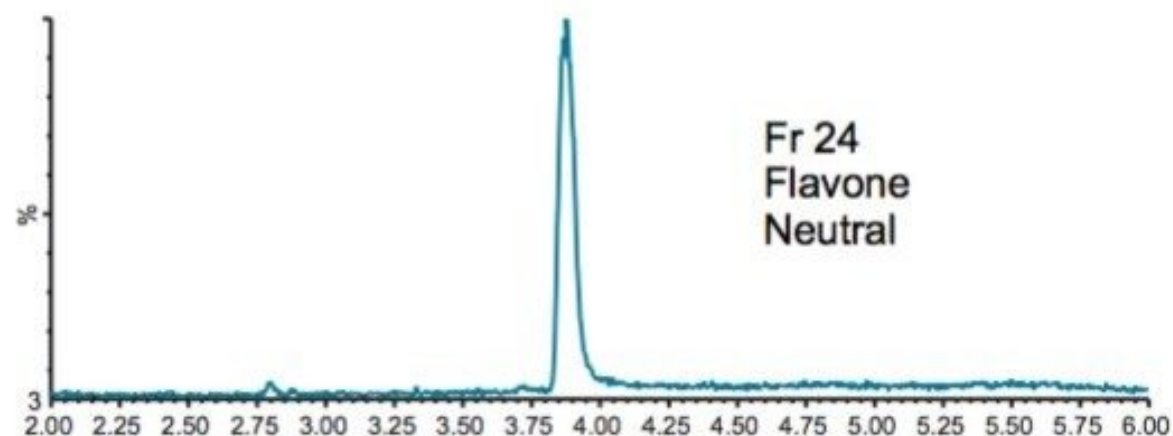
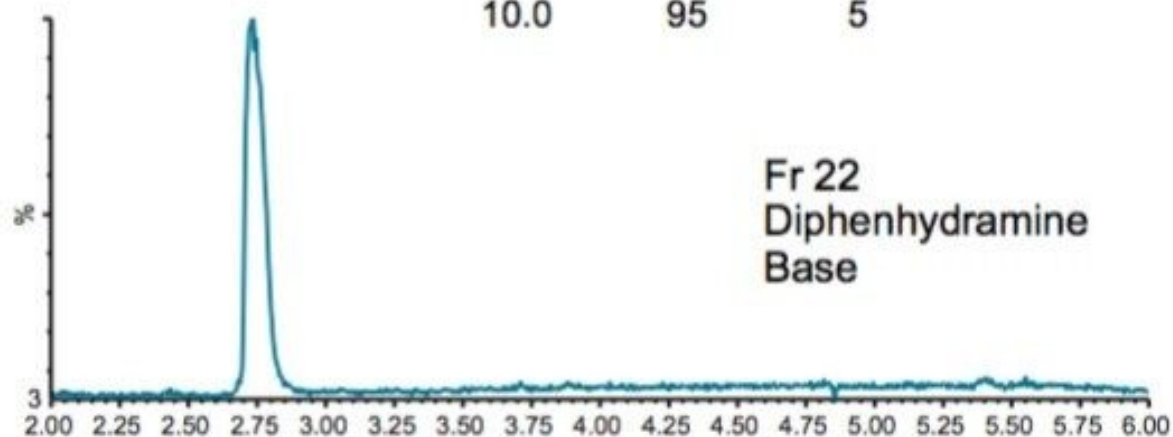
System: AutoPurification

Detection: Mass, with ACQUITY QDa Detector

Injection volume: 20 μL

Flow rate: 1.46 mL/min

Gradient:	Time	%A	%B
	0.0	95	5
	6.0	5	95
	7.0	5	95
	7.1	95	5
	10.0	95	5



- 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.

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## Featured Products

[AutoPurification System <https://www.waters.com/10007147>](https://www.waters.com/10007147)

[ACQUITY QDa Mass Detector <https://www.waters.com/134761404>](https://www.waters.com/134761404)

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