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Mass-Directed Isolation of Sulfa Compounds from an Antibiotic Mixture with an ACQUITY QDa Detector

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief illustrates the utility of mass-directed purification on preparative chromatography systems configured with an ACQUITY QDa Detector.

Benefits

Mass-directed purification with an ACQUITY QDa Detector configured in the system selectively isolates compounds of interest from complex mixtures and reduces the number of fractions for subsequent evaporation and analysis, leading to a more efficient workflow.

Introduction

Although traditional compound isolation is usually performed with UV detection, introducing a mass detector to the preparative chromatography system provides an increased level of confidence in elucidating target compounds and collecting them. With the specificity that mass detection brings to purification, fewer fractions are collected (reducing evaporation time and analysis), compounds without chromophores are more easily targeted, and, when combined with UV detection, higher product purity and yield may be realized. Streamlining the purification process ultimately leads to improved efficiency with both time and cost savings. In this application brief, we illustrate how mass-directed purification with an ACQUITY QDa Detector configured in the Waters AutoPurification System decreases postcollection fraction analysis and workup.

The ACQUITY QDa Detector, with its small size, automatic calibration and optimization routines, and easy system integration, makes mass-directed isolation more readily accessible to preparative chromatographers.

Results and Discussion

While the isolation of compounds from sample mixtures can be accomplished with UV detection, mass-directed isolation effectively simplifies the purification process by only targeting specific compounds whose masses are known. As shown in Figure 1, the UV-directed collection is nonspecific and all compounds are collected because the collection threshold is satisfied in the fraction method. Without prior knowledge of the

elution order, the mass-directed purification identifies all of the compounds and yields only two fractions, those corresponding to the two specific masses requested by the user. With only the desired compounds collected, valuable space is saved in the fraction collection bed, fewer fractions require evaporation and analysis, and the overall purification process efficiency is improved. Faster turnaround time leads to increased productivity with savings in both time and cost. Figure 2 shows the total ion chromatogram and the extracted ion chromatograms for each of the two desired drug compounds, with the triggering mass as well as the tube location in the fraction collector bed.

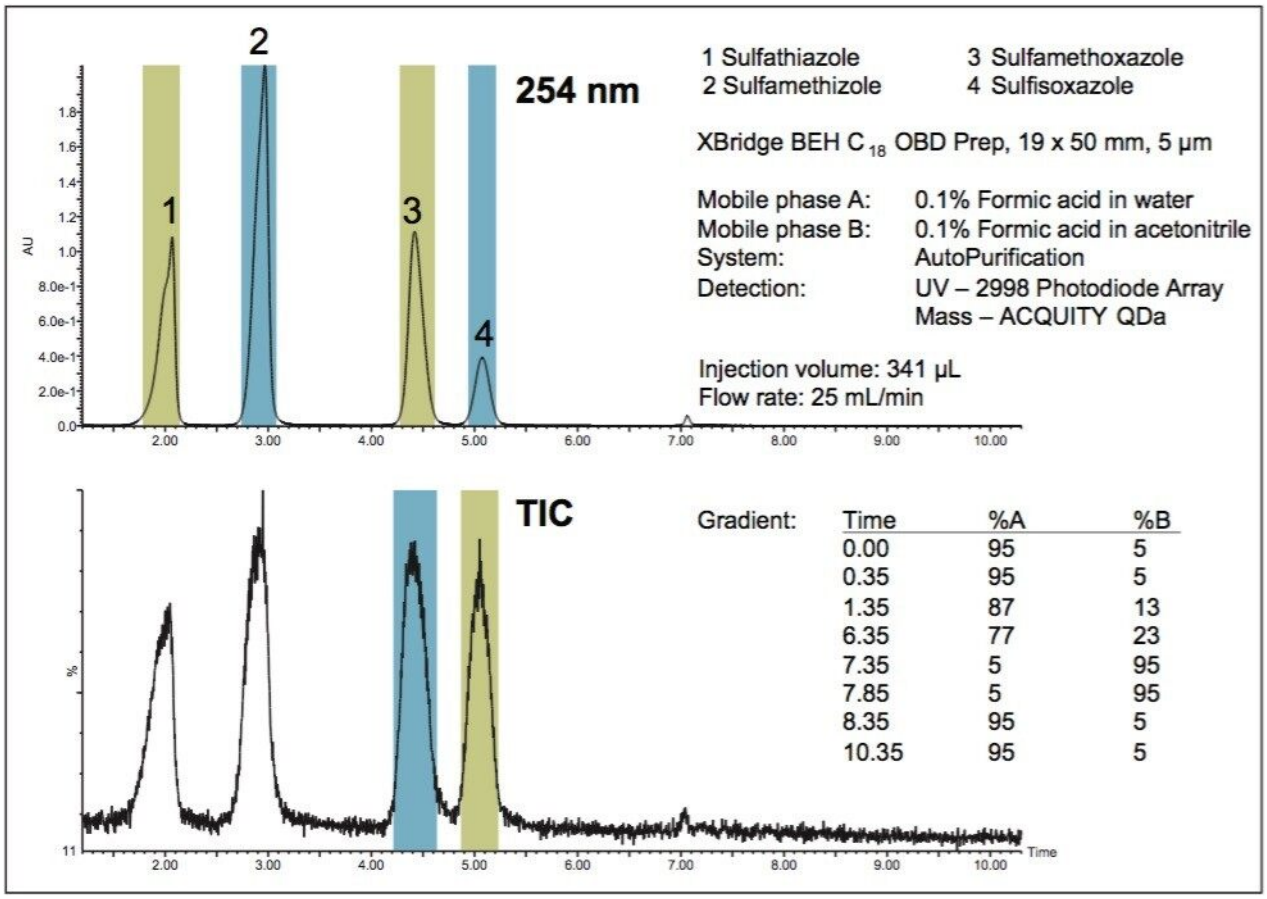

Figure 1. Top chromatogram: UV-directed isolation of compounds in the sulfa antibiotic mixture at 254 nm. Bottom chromatogram: Mass-directed isolation of the two desired compounds in the sulfa antibiotic mixture reduces the number of collected fractions.

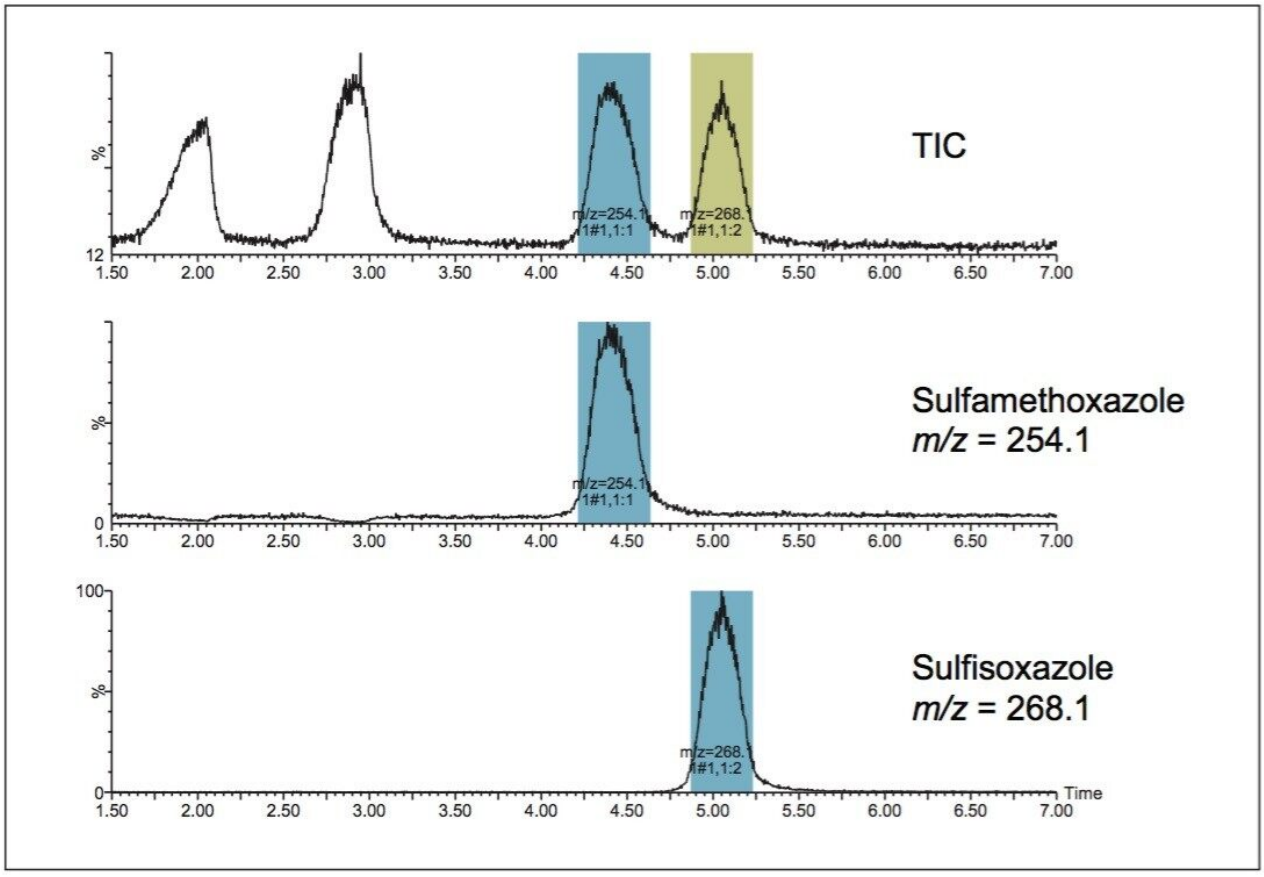

Figure 2. Mass-directed isolation only collects the two desired compounds. Their identification and location in the collection bed are readily available on the chromatogram.

Fraction analysis with injections drawn directly from the collection tubes before evaporation is shown in Figure 3. UV and mass analysis indicate that the target compounds are pure.

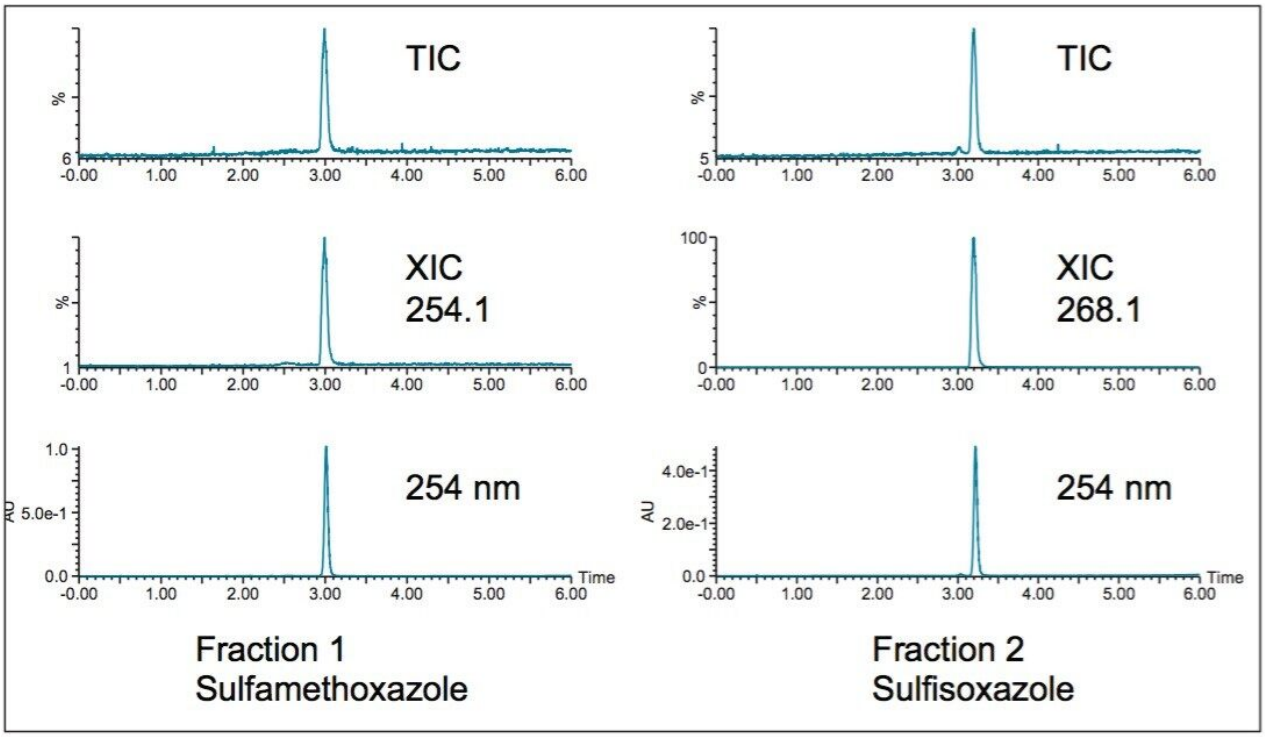

Figure 3. Analysis of the collected fractions showed very good purity by both UV and mass detection. The analytical gradient ran from 5–95% B in 6 minutes on an XBridge BEH C_{18}, 4.6 x 50 mm, 5 µm column with a 20-µL injection of the fraction before evaporation.

Both the analytical and preparative chromatographic methods utilize many common UV and mass spec parameters. These are listed in Figure 4.

2998 PDA Detector	ACQUITY QDa Detector
Resolution: 1.2 nm	Ionization mode: ES+
Range: 210 – 650 nm	Data: Centroid
Sampling rate: 10 points/sec	Mass range: 100 – 650
Filter time constant: Normal	Cone voltage: 15
	Sampling frequency: 5 Hz
	Capillary voltage: 0.8
	Probe temperature: 600
	Detector gain: 1
	Makeup: 90% water/ 10% acetonitrile/0.01% formic acid
	Note: Makeup solution is only used for preparative separations.

Figure 4. PDA and MS method parameters for the analytical and preparative chromatographic methods.

Conclusion

- Purification systems configured with the ACQUITY QDa Detector are suitable for specific mass targeting of compounds for isolation from sample mixtures, thereby reducing the number of collected fractions, saving space on the collector bed, and increasing throughput.
- The ACQUITY QDa Detector identifies non-UV absorbing compounds, making their analysis and isolation easier to accomplish.
- Mass-directed isolation simplifies the purification process and improves efficiency by reducing fraction handling and analysis.
- Streamlined sample handling saves time and cost and ultimately increases productivity.

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

AutoPurification System <https://www.waters.com/10007147>

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

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