

응용 자료

Mass-Directed Isolation of a Pharmaceutical Compound Using AutoPurify with an ACQUITY QDa Detector

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Abstract

In this application note, we demonstrate the feasibility of successfully isolating a synthetic pharmaceutical compound from a crude mixture using AutoPurify on an AutoPurification System configured with an ACQUITY QDa Detector.

Benefits

- The ACQUITY QDa Detector can be seamlessly integrated into the Waters AutoPurification System, making massdirected purification more readily accessible to chromatographers and is ideal for upgrading existing UV-directed systems.
- Pre-optimization and automatic calibration routines make the ACQUITY QDa Detector easy to use and reduce the time required for operator training.
- The Waters AutoPurification System with ACQUITY QDa Detector provides flexible automated compound isolation options that simplify the purification workflow and increase process efficiency by empowering scientists to perform other tasks.
- Mass-directed purification of compounds improves productivity by collecting only target compounds, thereby reducing fraction handling and analysis that would be necessary for UV-directed systems.

Introduction

Pharmaceutical compounds synthesized in chemistry laboratories almost always require purification before they can be used in experimental studies. Preparative chromatography is often the technique used to clean up synthetic mixtures, but the multi-step purification process can be cumbersome and time-consuming. AutoPurify, a feature provided within the FractionLynx Application Manager of MassLynx Software, streamlines the purification process with flexible, automated strategies that reduce or eliminate user intervention between isolation steps. While the benefits of AutoPurify have been discussed in detail previously,¹ the integration of the ACQUITY QDa Detector into the AutoPurification System makes mass-directed purification more readily accessible and provides the added assurance that mass spectral data brings to chromatographic separations. With its pre-optimized hardware and automated calibration routine, the ACQUITY QDa Detector can be easily added to purification systems. In this application note, we

demonstrate the feasibility of successfully isolating a synthetic pharmaceutical compound from a crude mixture using AutoPurify on an AutoPurification System configured with an ACQUITY QDa Detector.

Experimental

Sample description

Dry pharmaceutical intermediate was dissolved in dimethylsulfoxide to a concentration of 50 mg/mL.

LC conditions

LC system:	Waters AutoPurification System
Detectors:	ACQUITY QDa (mass); 2998 Photodiode Array
Analytical column:	XSelect CSH C ₁₈ , 4.6 x 50 mm, 5 μm (p/n 186005287)
Preparative column:	XSelect CSH C ₁₈ OBD Prep, 19 x 50 mm, 5 μm (p/n 186005420)
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Methanol, neat
Column temperature:	Room
Sample temperature:	Room
Injection volume:	Reported in figures
Flow rate:	Reported in figures
Gradient:	Reported in figures

MS conditions

Ionization mode:	Electrospray +
Data:	Centroid
Mass range:	100-850 amu
Scan time:	10 min
Cone voltage:	15
Sampling frequency:	5 Hz
Capillary voltage:	0.8
Probe temperature:	600
Detector gain:	1
Makeup solution:	90:10 water:acetonitrile with 0.01% formic acid

Note: Makeup solution is only used for preparative separations

Data management

MassLynx v4.1

FractionLynx Application Manager

Results and Discussion

Before beginning the isolation process, the AutoPurify method was configured in FractionLynx. The AutoPurify method defines the set of parameters that will be used for analyzing the crude sample, selecting the purification method, running the isolation protocol, and evaluating the collected fractions. In high

throughput laboratories where there is broad sample diversity, the system administrator may choose to define multiple AutoPurify methods to adequately address the different types of molecules that require purification. For example, there may be a specific method for acids, bases, or hydrophobic molecules. While AutoPurify can be configured to run automatically from analytical crude sample screening and prep isolation to fraction analysis, the three stages may also be executed in a semi-automated fashion. The software can be programmed to run the analytical screening and generate the prep sample list. The user can then review the FractionLynx browser report, make changes to the purification strategy if desired, and then manually start the system to perform the compound isolation. In the same manner, the user may review the newly generated purification results in the browser report and edit the sample list before fraction analysis occurs. Thus, the chemist can interact with AutoPurify as much or as little as he deems necessary for a given set of samples. Detailed descriptions of the AutoPurify process have been communicated previously.^{2,3}

Once the AutoPurify method was defined to perform the completely automatic purification process from analytical screening through fraction analysis, the synthetic crude mixture of the pharmaceutical intermediate was placed in the sample manager and the system was started. After completing the analysis of the crude material, the FractionLynx browser report showed that the sample was approximately 67% pure. According to the parameters defined in the AutoPurify method, the sample required purification. The software selected an appropriate gradient for purification (the focused gradient named Narrow B in Figures 1 and 2), and immediately started the isolation.

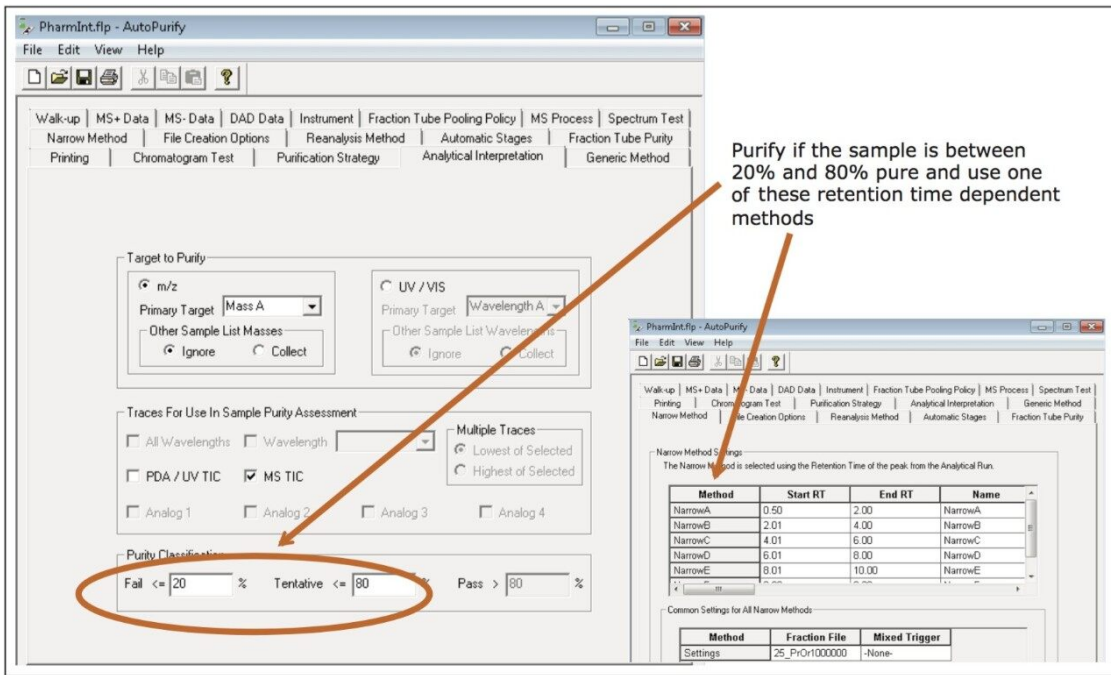


Figure 1. The Analytical Interpretation tab in the AutoPurify method specifies the primary target and its required purity. The Narrow Method tab defines the retention time ranges for each focused gradient method.

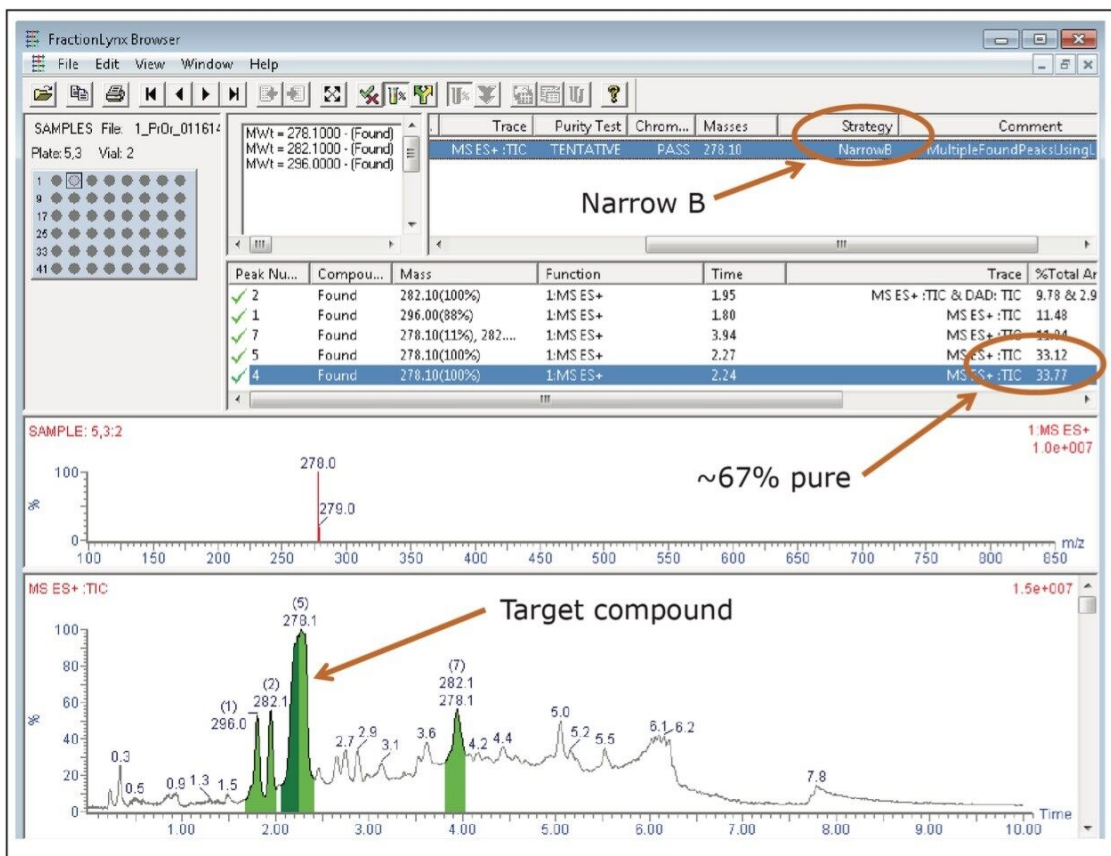


Figure 2. The analytical browser report shows the chromatogram of the crude sample mixture, the purity of the target compound, and the purification method that will be run. The analytical gradient ran from -95%B in 6 minutes. The injection volume was 5 μ L.

AutoPurify uses the retention time of the target peak to select one of the predefined narrow gradients. Narrow gradients, also known as focused gradients,⁴ are useful in preparative chromatography because they effectively increase the resolution between the target peak and its closely eluting neighbors without increasing run time. Increased resolution ultimately leads to higher loading and greater product purity. Figure 3 shows the preparative chromatography of the isolation using the Narrow B gradient method described in Table 1.

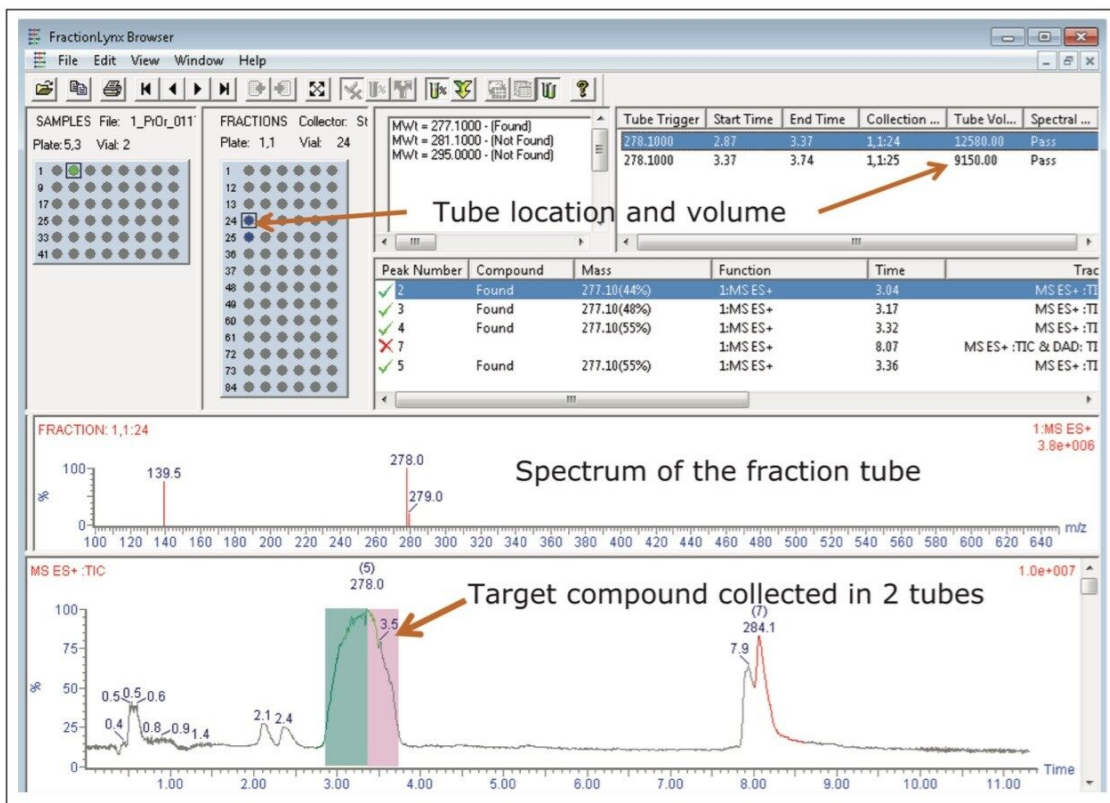


Figure 3. Browser report for the preparative chromatography with Narrow Method B. The focused gradient increased the resolution between the contaminant peak eluting at 2.4 minutes and the target peak. The spectrum of the fraction tube showed excellent purity. The mass at 139.5 was most likely due to in-source fragmentation. The injection volume was 85 μ L, geometrically scaled from the 5- μ L injection on the analytical column.

Time	Flow	%A	%B
0.00	25	95.0	5.0
0.35	25	95.0	5.0
1.31	25	86.3	13.7
7.31	25	75.0	25.0
7.41	25	5.0	95.0
8.41	25	5.0	95.0
8.51	25	95.0	5.0
11.31	25	95.0	5.0

Table 1. Narrow Method B. The 0.35 minute hold at the beginning of the gradient is to account for the difference in system volume between the analytical and preparative system flow paths.

Without user intervention, analysis of the collected fractions was performed immediately following the completion of the preparative isolation. The analysis method used was the same one as the original screening gradient, running from 5–95%B in 6 minutes. The estimated purity of the fraction shown is 97%, as the peak in red is actually part of the column washout. The results of the fraction analysis are shown in Figure 4.

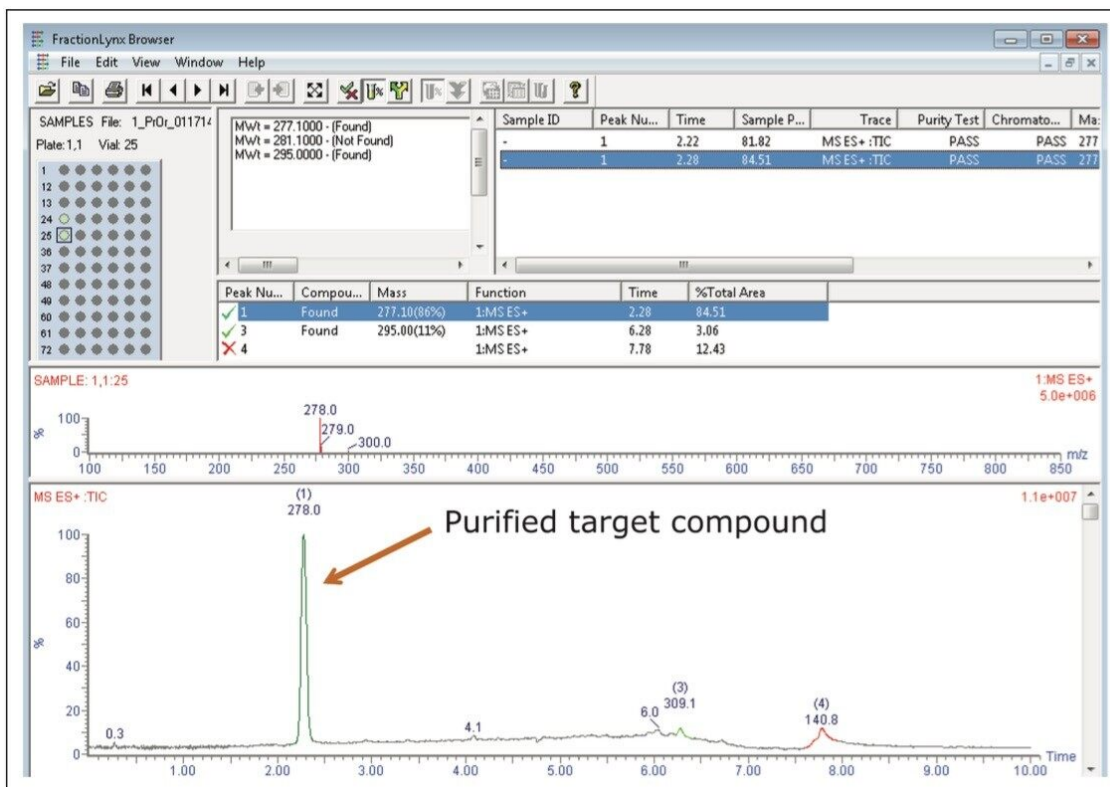


Figure 4. Browser report for the analysis of a fraction. The injection volume was 20 μ L, taken directly from the collection tube immediately after purification.

Conclusion

- The ACQUITY QDa Detector is suitable for integration into purification systems, making mass-directed purification more accessible to all isolation chemists.
- Mass-directed purification of compounds reduces cost and saves time by collecting only specific target compounds, thereby reducing the number of fractions requiring analysis and handling.
- The ACQUITY QDa Detector is compatible with AutoPurify, the software feature in FractionLynx that executes all phases of the purification process from crude sample analysis to final fraction evaluation.
- AutoPurify effectively improves productivity by increasing sample throughput with minimal user intervention at each step of the isolation process.

References

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