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Streamlining Compound Isolation Automatically with UPLC to Prep Chromatography using Mass-Directed AutoPurification

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Abstract

This study illustrates how dedicating the UPLC is for compound screening and fraction analysis, reserving the preparative LC system for compound isolation, and using AutoPurify to coordinate and execute the workflow effectively manages the purification process, increasing efficiency and throughput.

Benefits

- Effective streamlining of the compound isolation workflow is easily accomplished with UPLC to prep chromatography by mass-directed autopurification with AutoPurify, a FractionLynx feature which saves instrument and process time.
- Fast UPLC gradients on sub-2 µm columns combined with narrow gradients at the prep scale reduce analysis and isolation time.
- The purification protocol is more cost-effective with early run termination, which saves time and solvent.

Introduction

Drug discovery laboratories that process large numbers of samples must develop efficient purification protocols, thereby reducing the solvent, time, and effort necessary to make critical strategic decisions about therapeutic candidates and their progression to the next phase of testing. Combining UPLC driven compound screening and fraction analysis with mass-directed purification effectively streamlines the compound isolation process. Sub-2 µm particle size columns, combined with the fluidically-optimized flow path[1,2] of the UPLC, increase sensitivity and resolution, as well as reduce chromatographic run times, making UPLC ideal for fast sample analysis. AutoPurify, a feature included in FractionLynx Software, assesses the purity of the target compound in the crude mixture, suggests a purification strategy, and generates the sample list for the isolation on the preparative LC system. The sample list is imported and run on the preparative LC system. Once the isolation is completed, AutoPurify reports the purity of the fractions and creates a sample list for fraction analysis on the UPLC system. In this study, we illustrate how dedicating the UPLC for compound screening and fraction analysis, reserving the preparative LC system for compound isolation, and using AutoPurify to coordinate and execute the workflow effectively manages the purification process, increasing efficiency and throughput.

Experimental

Conditions

Analytical column:	ACQUITY UPLC BEH C_{18} Column, 130Å, 1.7 µm, 2.1 x 30 mm (p/n 186002349)
Analytical flow rate:	1.0 mL/min
Prep column:	XBridge BEH C_{18}, OBD Prep Column, 130Å, 5 µm, 19 x 100 mm (p/n 186002978)
Prep flow rate:	25 mL/min

Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Makeup solvent:	50:50 water acetonitrile with 0.01% formic acid
Cone voltage:	10 V
Probe temperature:	500 °C
Ionization mode:	ES+, centroid
Sampling frequency:	2 Hz
Scan range:	150–650 amu
Wavelength:	254 nm
Gradients and injection volumes:	as noted in figures
Samples:	Dissolved in dimethyl sulfoxide Acetaminophen, 19.2 mg/mL Hydrocortisone, 14.6 mg/mL Warfarin, 16.4 mg/mL

Instrumentation

UPLC System:	ACQUITY UPLC H-Class, ACQUITY Sample Manager, ACQUITY PDA Detector, ACQUITY QDa Detector

AutoPurification System:	2545 Binary Gradient Module, 2767 Sample Manager, System Fluidics Organizer, 8–30 mL Flow Splitter, two 515 HPLC pumps, 2998 Photodiode Array Detector, ACQUITY QDa Detector Both systems were controlled by MassLynx Software

Figure 1. ACQUITY UPLC H-Class System and AutoPurification System.

Results and Discussion

Efficiently isolating compounds from large chemical libraries requires predictable strategies with fast analysis pre- and post-purification. Quick and sensitive analyses are easily realized with the fluidically-optimized flow path of the UPLC and short, sub-2 µm particle columns, with the preparative LC system being preserved solely for purification. The decision-making process can be simplified by employing AutoPurify, a standard feature included with FractionLynx Software. The user creates a method which specifies a set of instructions for the

screening, isolation, and sample analysis on both the UPLC system and the preparative LC system. AutoPurify processes the data, creates sample lists, and sends the files to a user-specified location. The sample lists are then imported to the system for the next step in the process, i.e., analysis lists go to the UPLC, while sample lists for compound isolation go to the preparative system.

Preserving the length of the column to the diameter of the particle ratio (L/dp) is critical to the success of maintaining predictability when scaling from UPLC to prep. For this reason, a 2.1 x 30 mm, 1.7 µm column for UPLC was paired with a 19 x 100 mm, 5 µm column for prep. The user specifies the target, required purity, and purification strategy in the AutoPurify method (Figure 2).

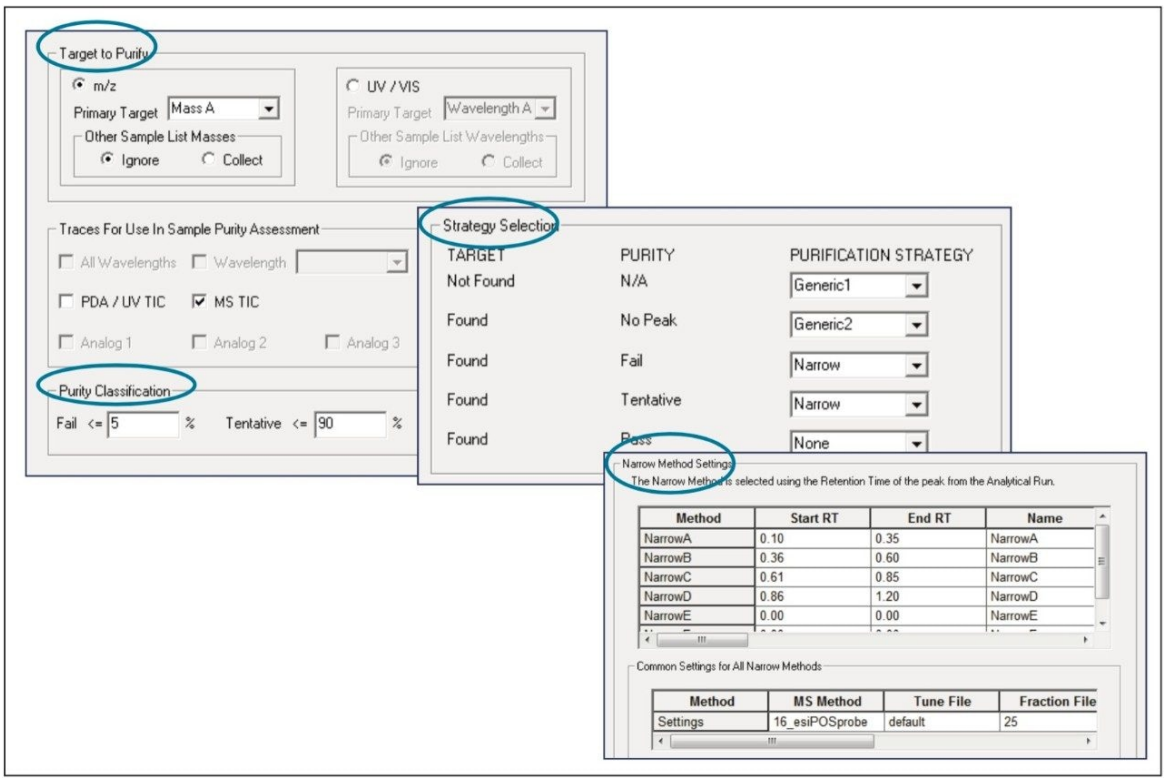

Figure 2. The target, required purity, strategy, and narrow methods are specified in the AutoPurify method.

Figure 3 shows the UPLC screening profile for each of the three compounds. The chromatographic method started at 5%B and increased to 95%B over 1 minute, a fast gradient with a total run time of 2.6 minutes. The FractionLynx browser reports the retention time, sample purity, and suggested purification strategy for each

compound in the sample list, as shown in Figure 4. The highlighted sample, as well as the last entry in the purity pane of the report, correspond to hydrocortisone and warfarin, and suggest the Narrow B and Narrow C purification methods, respectively. The retention time of the target compound in the analytical profile determines which pre-made narrow gradient will be used for prep. Four narrow prep methods were written to correlate with the full range (5–95%B) of the UPLC gradient: Narrow A (0.10–0.35 min, 0–25%B), Narrow B (0.36–0.60 min, 25–50%B), Narrow C (0.61 0.85 min, 50–75%B, Narrow D (0.86–1.20 min, 75–100%B). Using Narrow B as an example, Figure 5 shows the method template used for all of the narrow methods, where the injection occurs at 5%B and then increases to the low end of the narrow gradient in 1 minute, resulting in improved retention of the compound at initial conditions. Narrow A is slightly different with the injection occurring at 0%B and then progressing to 25%B in 7.5 minutes. A sample list for purification is automatically generated and sent to the user-specified location, accessible by both the UPLC and prep LC systems.

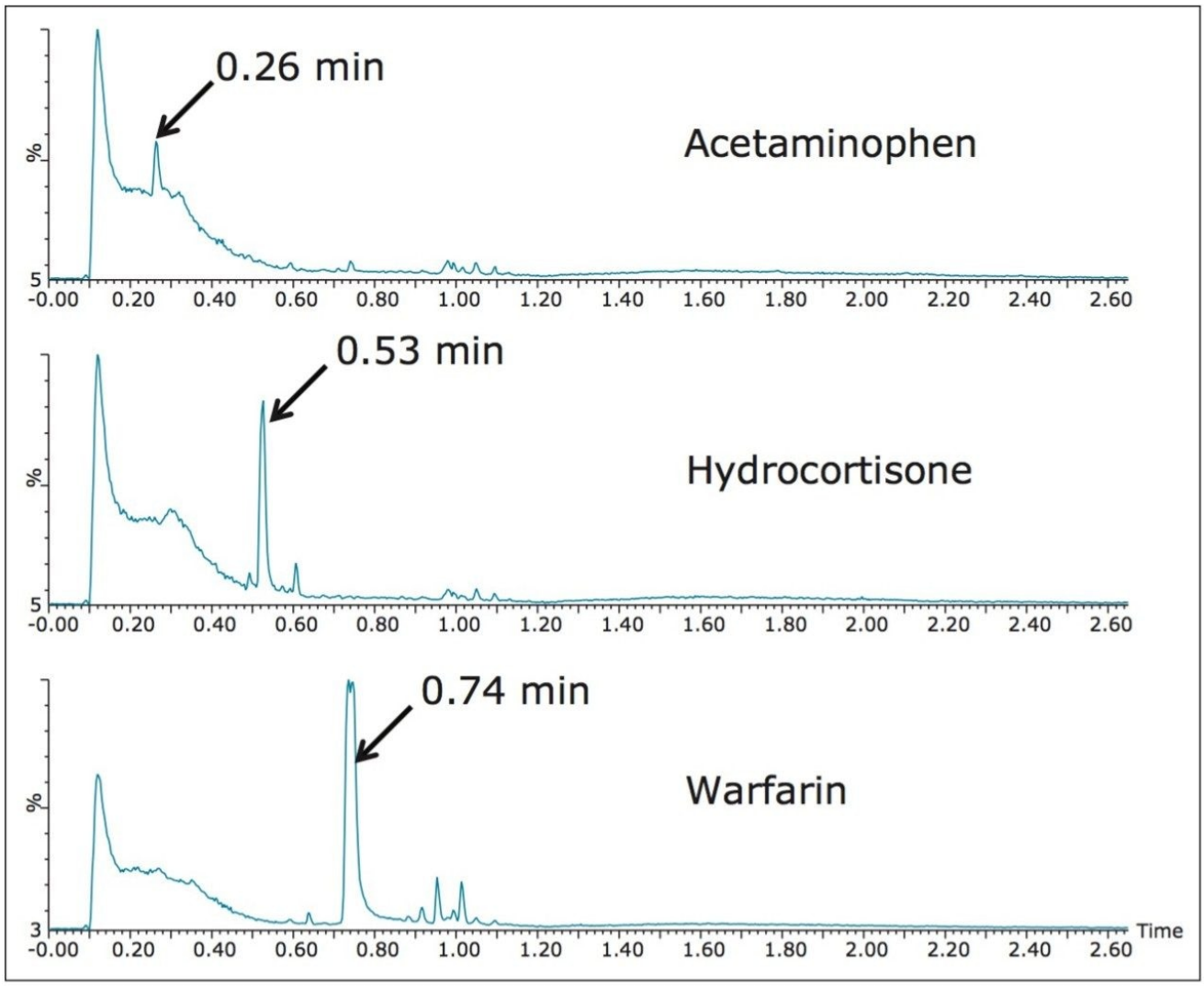

Figure 3. UPLC screening of 3 compounds, TICs; 5–95%B in 1 min, total run time 2.6 min; 2.1 x 30 mm, 1.7 µm *column, 0.2* µL.

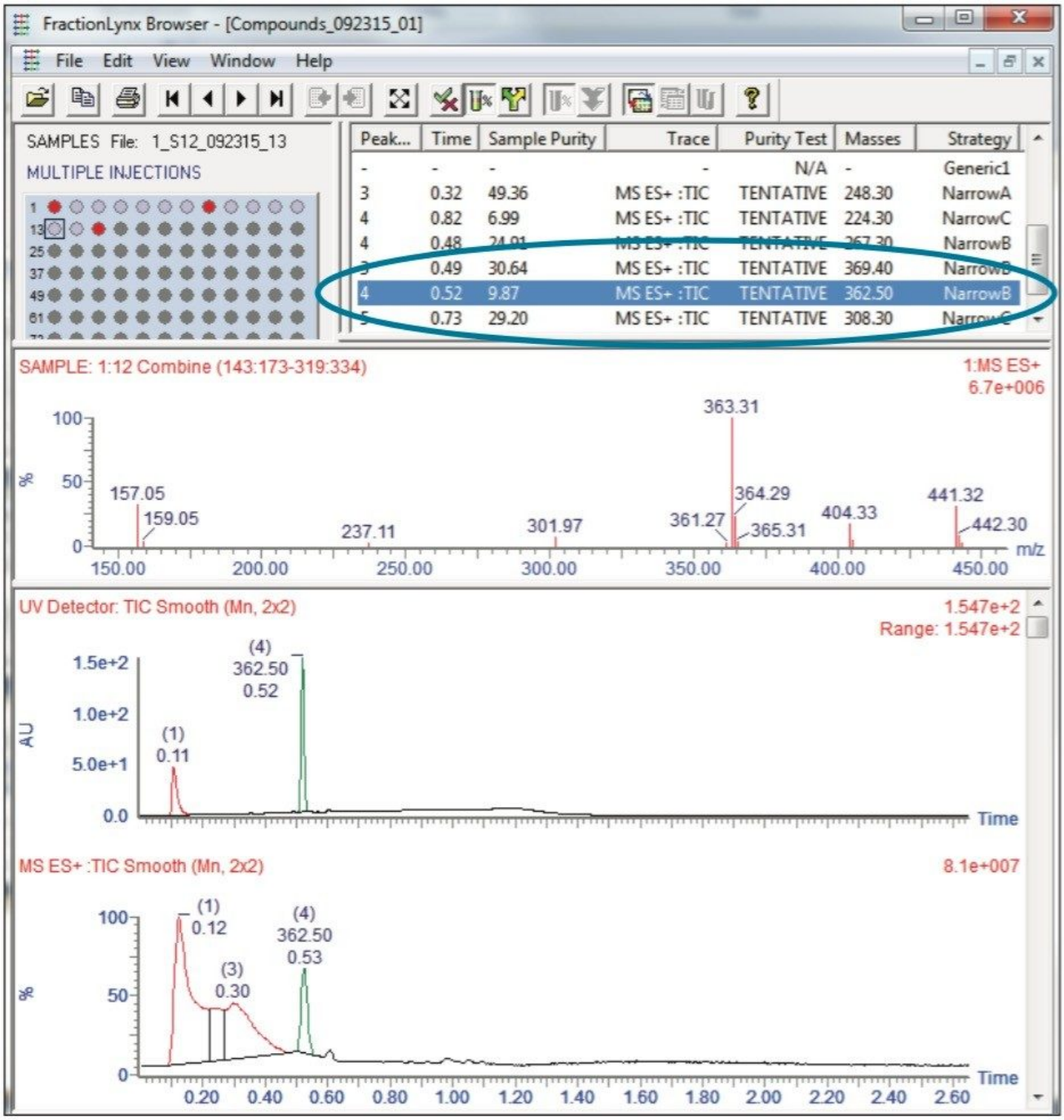

Figure 4. FractionLynx browser report for UPLC compound screening.

	Time	Flow rate	%A	%B
1	0.00	25	95	5
2	1.00	25	75	25
3	8.50	25	50	50
4	8.75	25	5	95
5	10.75	25	5	95
6	11.00	25	95	5
7	16.00	25	95	5

Figure 5. Example LC prep method template showing Narrow method B. Flow rate for all prep methods: 25 mL/min.

The prepared sample list, which the user has the option to edit, is imported from the user-specified directory to the prep LC system (Figure 6). Mass directed purification of each compound is shown in Figure 7. Because the acetaminophen peak was not integrated with the conditions stipulated inthe AutoPurify method, its purity was not calculated, and it was labeled as N/A in the FractionLynx Browser report. For this reason, the purification strategy selected was a generic method (Generic1) directly scaled from the UPLC gradient, as specified in the AutoPurify method (Figure 2). Narrow gradients reduce the time needed for isolation, but column re-equilibration is still required for reproducible results. A good rule of thumb for re-equilibration is to pump 3X system volume plus 5X column volume of the initial mobile phase composition prior to the injection.[3] Each narrow gradient has a 16 minutetotal run time, which include s column washing and equilibration at initial conditions.

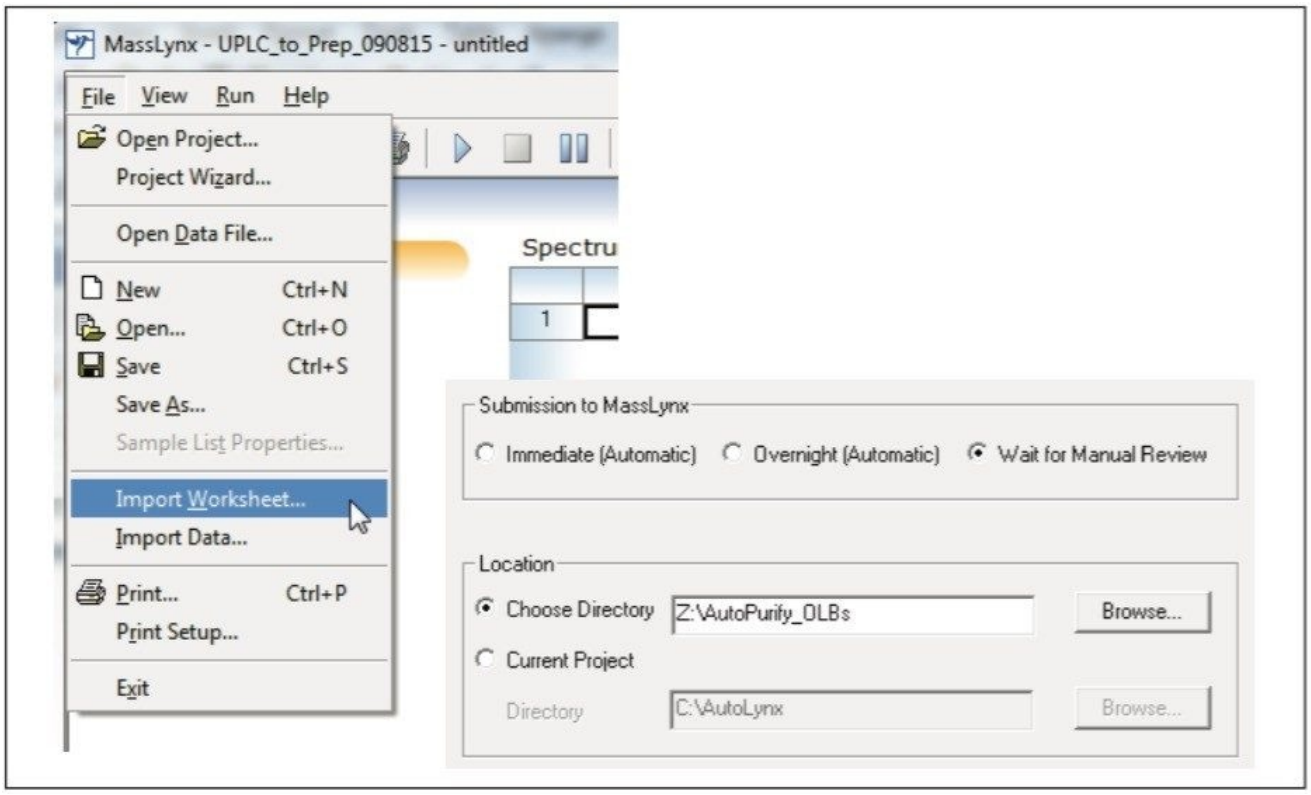

Figure 6. Importing the sample list from the user-specified network location to the LC system.

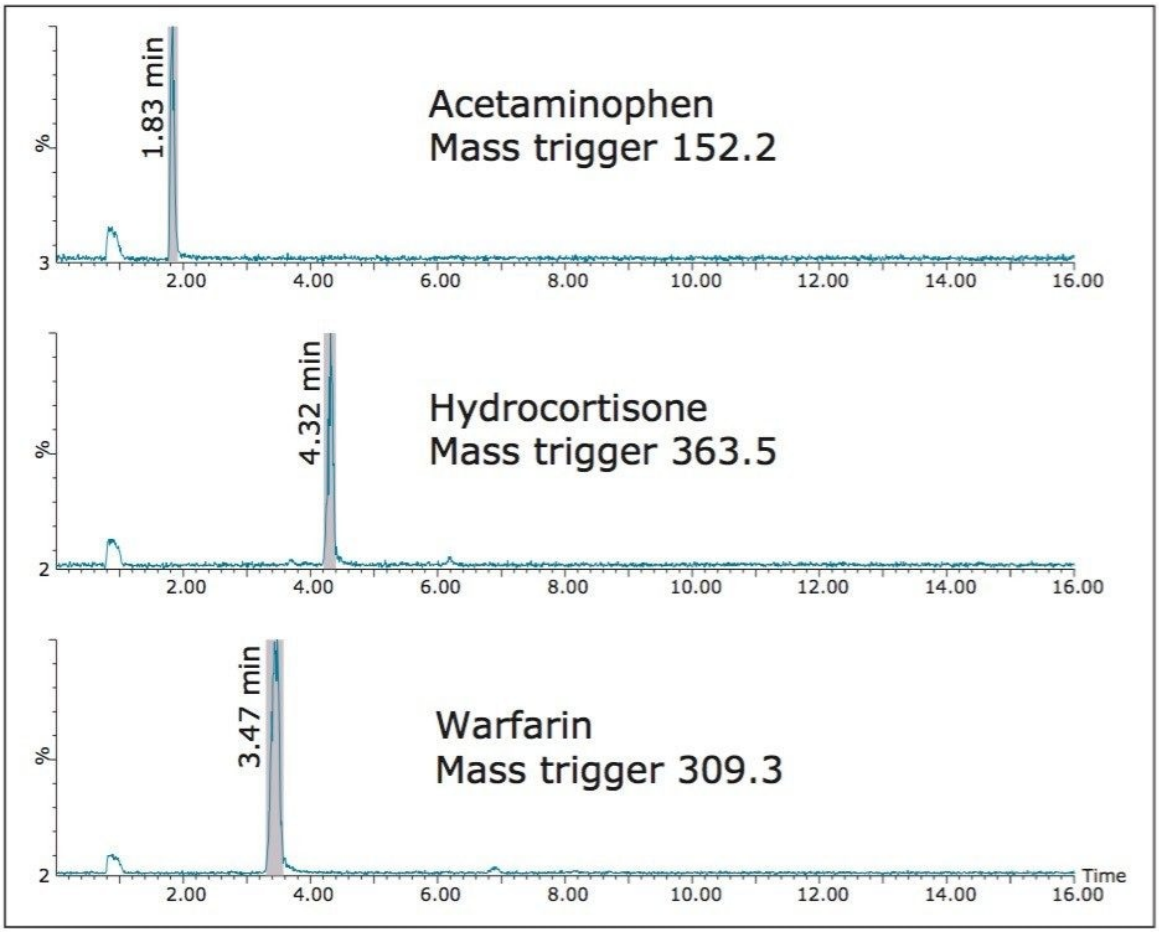

Figure 7. Mass-directed purification of acetaminophen with the generic gradient, hydrocortisone with narrow method B, and warfarin with narrow method C, TICs. Injection volume: 100 µL. Total run time: 16 minutes.

Simply directing the software to stop the run after target peak collection, and then using a post run method to wash and re-equilibrate the column, saves time and solvent (Figures 8, 9, and 10). For the three compounds used in this illustration, early run termination saved about 20 minutes and approximately a half liter of solvent (Figure 12).

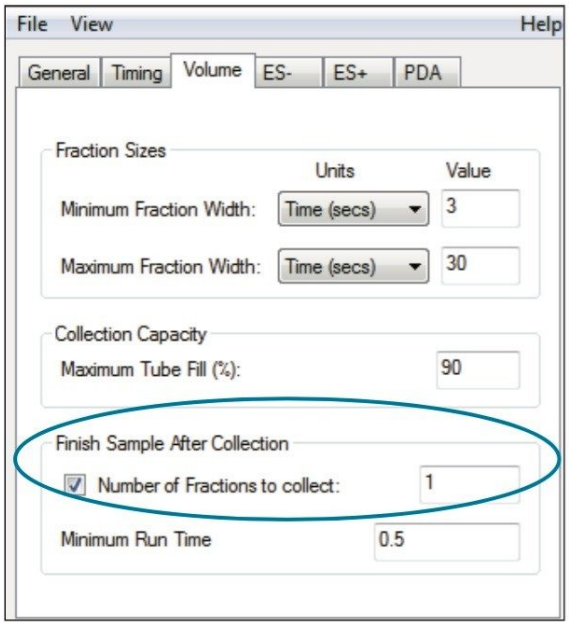

Figure 8. Early run termination is specified in the fraction collection method.

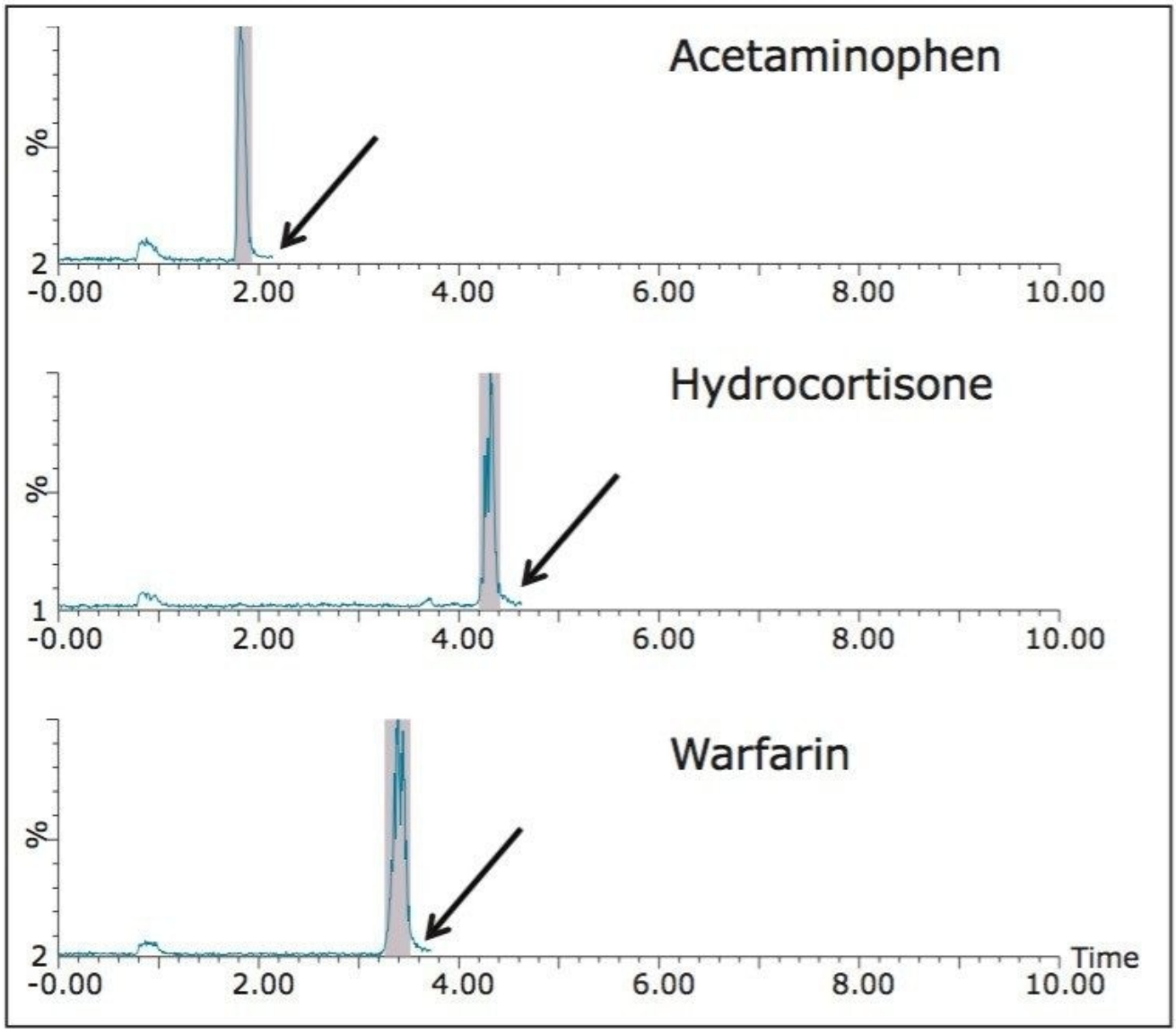

Figure 9. Mass-directed purification with early termination, as indicated by the arrow. The post run method commences at this time point. Injection volume: 100 µL. Total run times: acetaminophen, 8.1 min; hydrocortisone, 10.6 min; warfarin, 9.7 min.

	Time	Flow rate	%A	%B
1	0.00	25	5	95
2	2.00	25	5	95
3	2.25	25	95	5
4	6.00	25	95	5

Figure 10. Post-run method used to wash and equilibrate the column back to initial conditions after early termination prep runs.

As described above for the preparative portion of the isolation workflow, the automatically generated sample list for fraction analysis was sent to the designated network directory and then imported to the UPLC system. All collected fractions were analyzed with the original UPLC screening gradient and determined to be very pure, as shown in Figure 11.

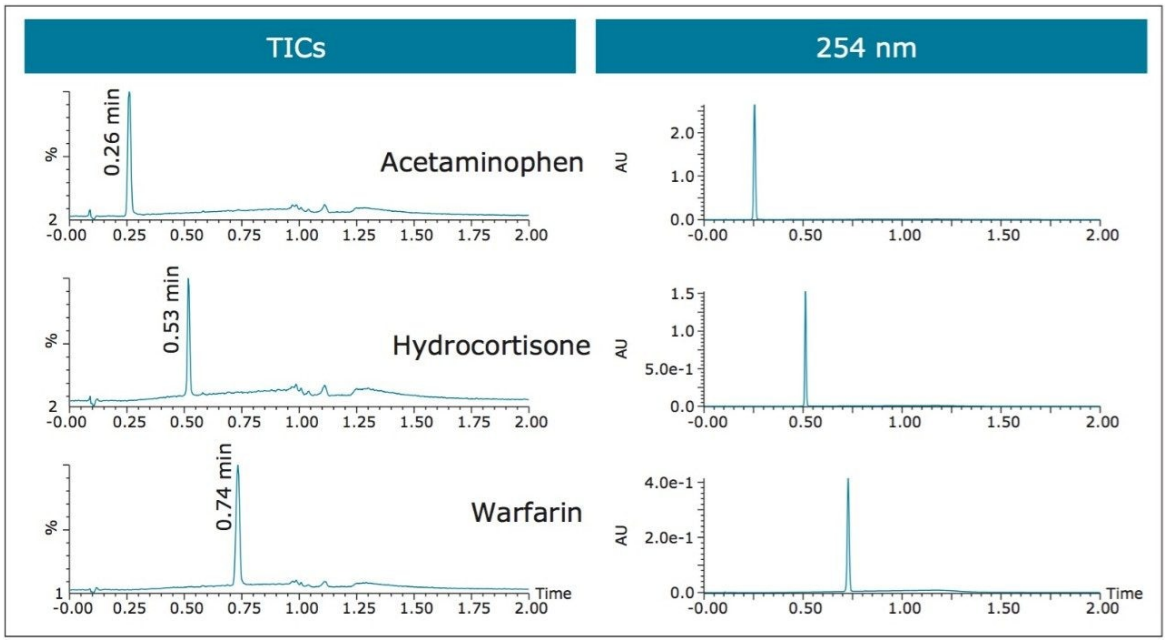

Figure 11. Fraction analysis on the UPLC system using the original screening gradient, 5–95%B in 1 min. Injection volume: 2 µL.

	Prep with Early Termination			
	Termination time (min)	Total run time (min)	Minutes saved	Solvent saved (mL)
Acetaminophen	2.1	8.1	7.9	197.5
Hydrocortisone	4.6	10.6	5.4	135.0
Warfarin	3.7	9.7	6.3	157.5
Totals			19.6	490.0

Figure 12. Time and solvent savings for each compound using prep with early termination.

Conclusion

- Effective streamlining of the compound isolation workflow is easily accomplished with UPLC to prep chromatography using mass-directed autopurification with AutoPurify, which saves time, effort, and solvent.
- Fast UPLC gradients on sub-2 µm columns combined with narrow gradients at the prep scale reduce analysis and isolation time.
- Early run termination saves time and solvent, resulting in a more cost-effective purification protocol.

References

1. Optimized System Dispersion for UPLC Performance in a Versatile LC Design. Waters Technology Brief 720003651EN. 2010.
2. Exact Control of Solvent Composition with the ACQUITY UPLC H-Class Quaternary Solvent Manager. Waters Technology Brief 720004405EN. 2012.
3. Aubin A, Jablonski J. Prep 150 LC System: Considerations for Analytical to Preparative Scaling. Waters Application Note 720005458EN. 2015.

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