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Purification Workflow Management

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

Abstract

This application brief illustrates how a sample is efficiently taken through a three-step purification process utilizing the AutoPurify capabilities within the Waters FractionLynx Application Manager for MassLynx Software, and the AutoPurification System for MS-directed analysis.

Introduction

A standard requirement for drug discovery screening of synthetic libraries is that the test compounds must have a minimum purity. Purity is based on the area percent of an LC chromatogram from a detector such as UV, evaporative light scattering (ELS), MS with a total ion chromatogram (TIC), or a combination of multiple detectors. If the screening compounds do not meet this standard, purification is required. Managing the flow of samples, subsequent fractions, and all the associated data through this process can often be difficult and time consuming.

This application brief illustrates how a sample is efficiently taken through a three-step purification process utilizing the AutoPurify capabilities within the Waters FractionLynx Application Manager for MassLynx Software, and the AutoPurification System for MS-directed analysis. This comprehensive informatics solution enables automation from the initial evaluation, through the purification, to analysis of the collected fraction.

Results and Discussion

The AutoPurify functionality uses the results of the analytical analysis to determine the purification process. By performing an analytical evaluation of the sample, the presence of the target compound is confirmed and its purity measured (Figure 1).

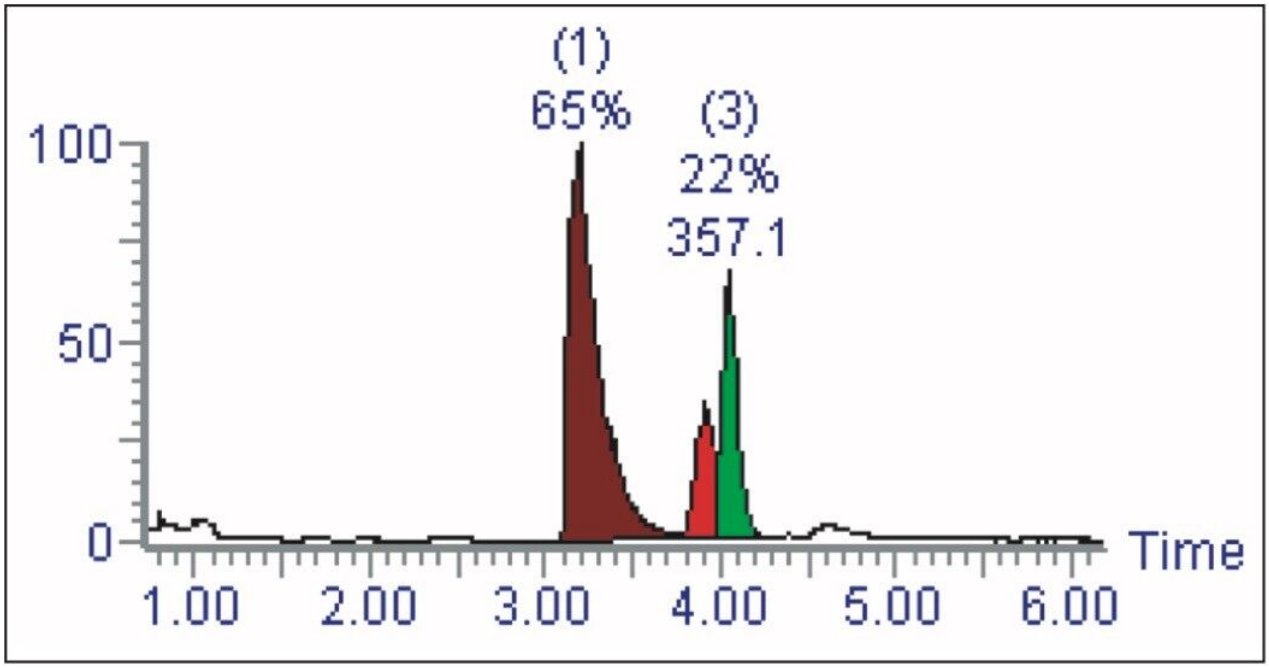


Figure 1. TIC chromatogram of the analytical-scale analysis of the crude sample.

The software will decide which shallow gradient should be used to perform the purification (Figure 2).

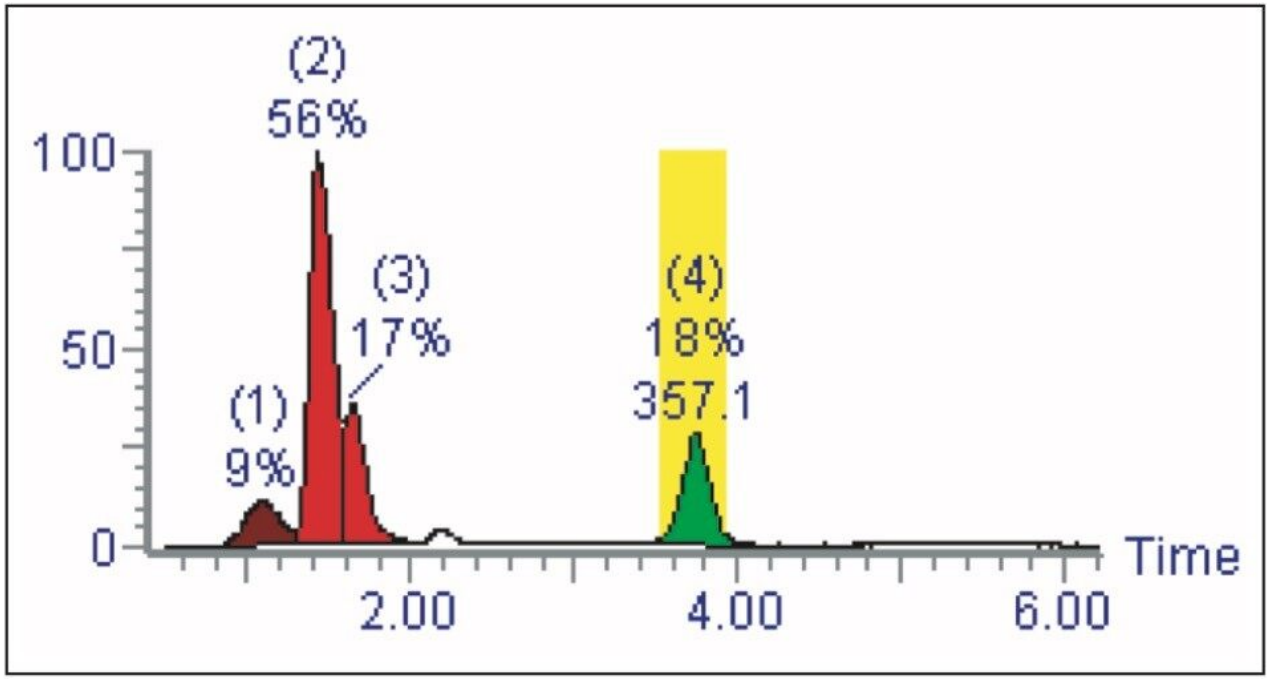


Figure 2. TIC chromatogram after purification, with fraction collection indicated by the shaded area.

Then, it automatically performs analysis of the collected fractions (Figure 3).

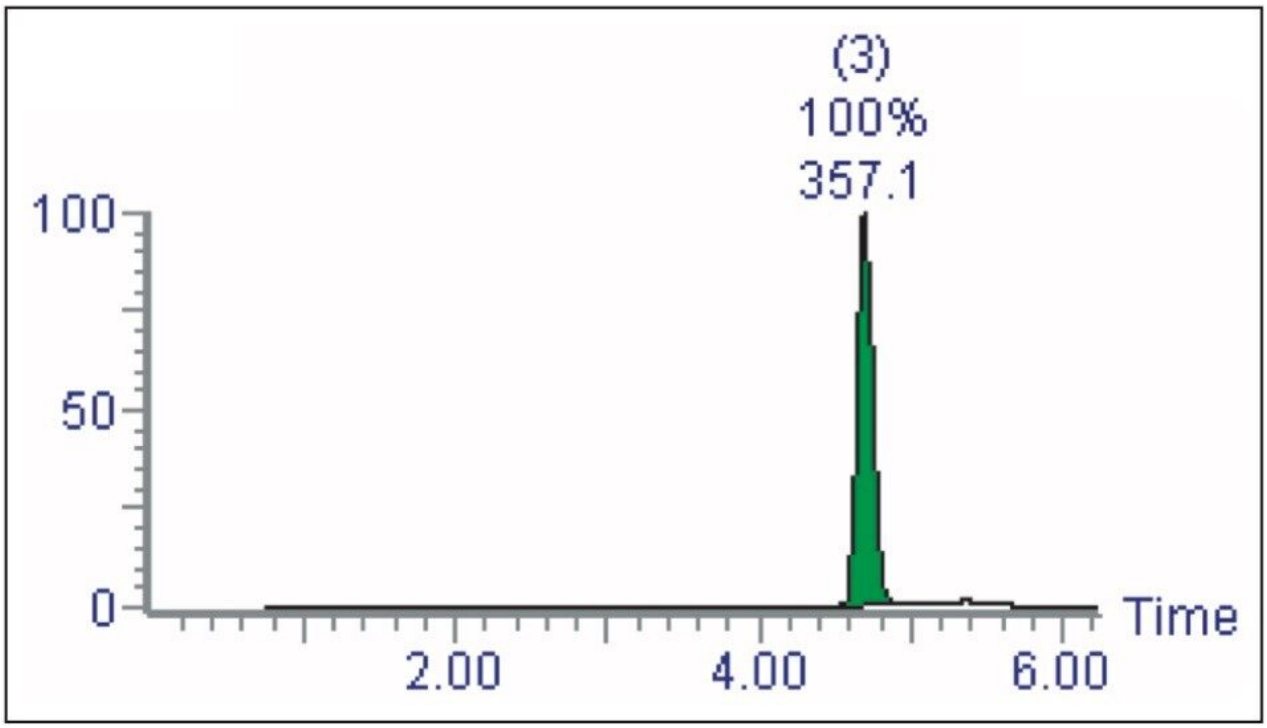


Figure 3. TIC chromatogram of the analysis of the collected fraction.

Information determined from analysis of the fractions can be used to help with post-purification handling such as fraction pooling and transfer to an evaporator. A report can be exported in different file formats such as .xml, .csv, and .tab, to easily interface with other sample handling software packages.

Step 1: Analytical interpretation

In the first of the three-step process, the purity of the target mass is identified by integrating the chromatogram. In the example shown in Figure 4, the area percent of the target determined from the TIC (22%) is then used to calculate the sample purity.

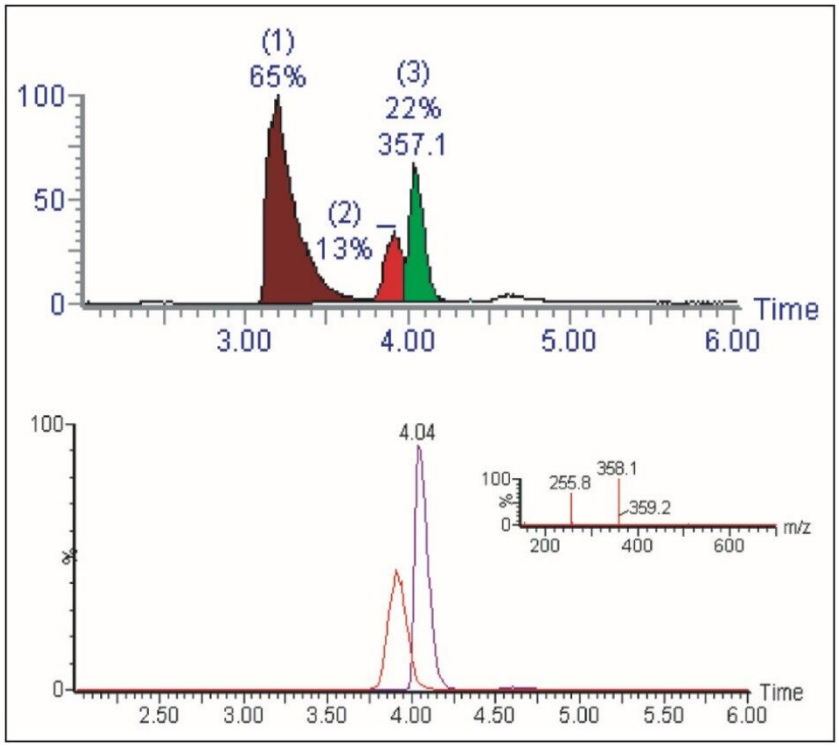


Figure 4. Analytical evaluation of mass 357.1 is 22% of the TIC, and the target sample is co-eluting with peak 2. An overlay chromatogram of the two co-eluting peaks, with the spectrum, indicates the potential fraction contamination that could occur.

The area percent can also be determined by total absorbance current, wavelength, or analog signal. The purity of the target is then classified as “pass,” “tentative,” or “fail,” based on user-defined limits. In this example, less than 10% pure means purification will not occur, 10% to 80% purity requires purification, greater than 80% is pure enough, and does not require further purification.

In a manual process, the analyst would evaluate the separation, and adjust the gradient to achieve the best results. However, in an open access environment or where large numbers of samples are being handled, automation is necessary.

Step 2: The purification process

In the second step of the process, purification occurs. The software will determine the purification method best suited to improving the separation by choosing one of six different shallow gradients. Using the analytical retention time of the target, the appropriate shallow gradient-based method will be chosen.

Shallow gradients, also referred to as narrow gradients, allow for optimal target separation from closely eluting impurities, thus improving the purity of the resulting fraction. Each narrow gradient, whose time window is indicated by the colored lines (Figure 5), is created to cover a different timed section of the analytical gradient.

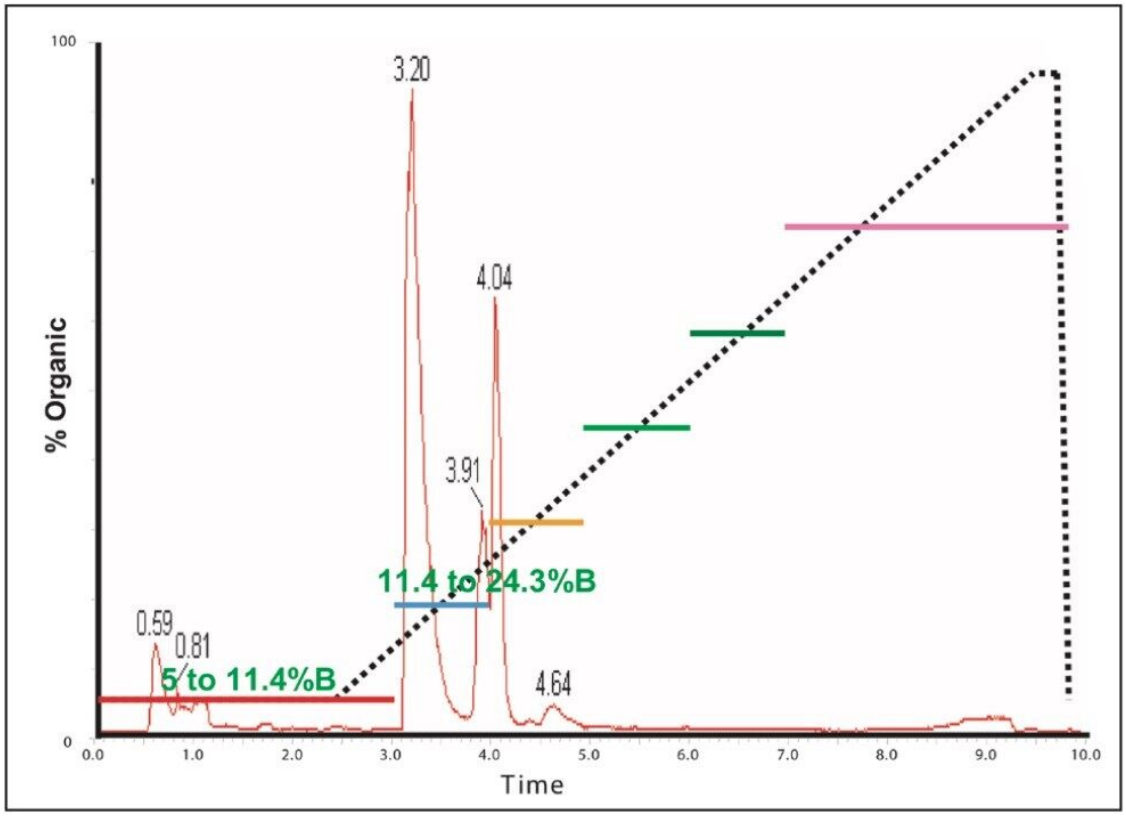


Figure 5. Graphical representation of analytical and prep gradients.

The analytical gradient is indicated by the dotted black line, and shows the solvent change over the course of the gradient to be from 5% to 95% B. With the relationship between the analytical retention time and the elution organic composition known, the software can choose which of the narrow gradients will be used to automatically purify the samples during the purification stage of the process.

When the software evaluates the analytical sample, it creates a browser report defining the recommended strategy. The user has the opportunity to change the strategy if necessary. The part of the report that refers to the strategy is the results pane (Figure 6). In this example, there are several other samples analyzed, but the one that is of interest is that last one on the list, A123008.

Sample ID	Ti...	Sample Purity	Purity Test	Masses	Strategy
A123004	4.61	46.13	TENTATIVE	375.00	NarrowC
A123005	-	-	N/A	-	None
A123006	3.42	68.15	TENTATIVE	226.90	NarrowB
A123007	-	-	N/A	-	None
A123008	4.04	21.96	TENTATIVE	357.10	NarrowC

Figure 6. Browser results pane with sample purity and prep strategy displayed.

The sample in this case eluted at 4.04 min (Figure 7), so the narrow gradient chosen for the purification was “Narrow Gradient C,” the one that targeted the solvent change that occurred between 4 and 5 minutes. This gradient is denoted by the green line, which changes from 24% to 37% organic over 6.5 min, and is defined graphically as below.

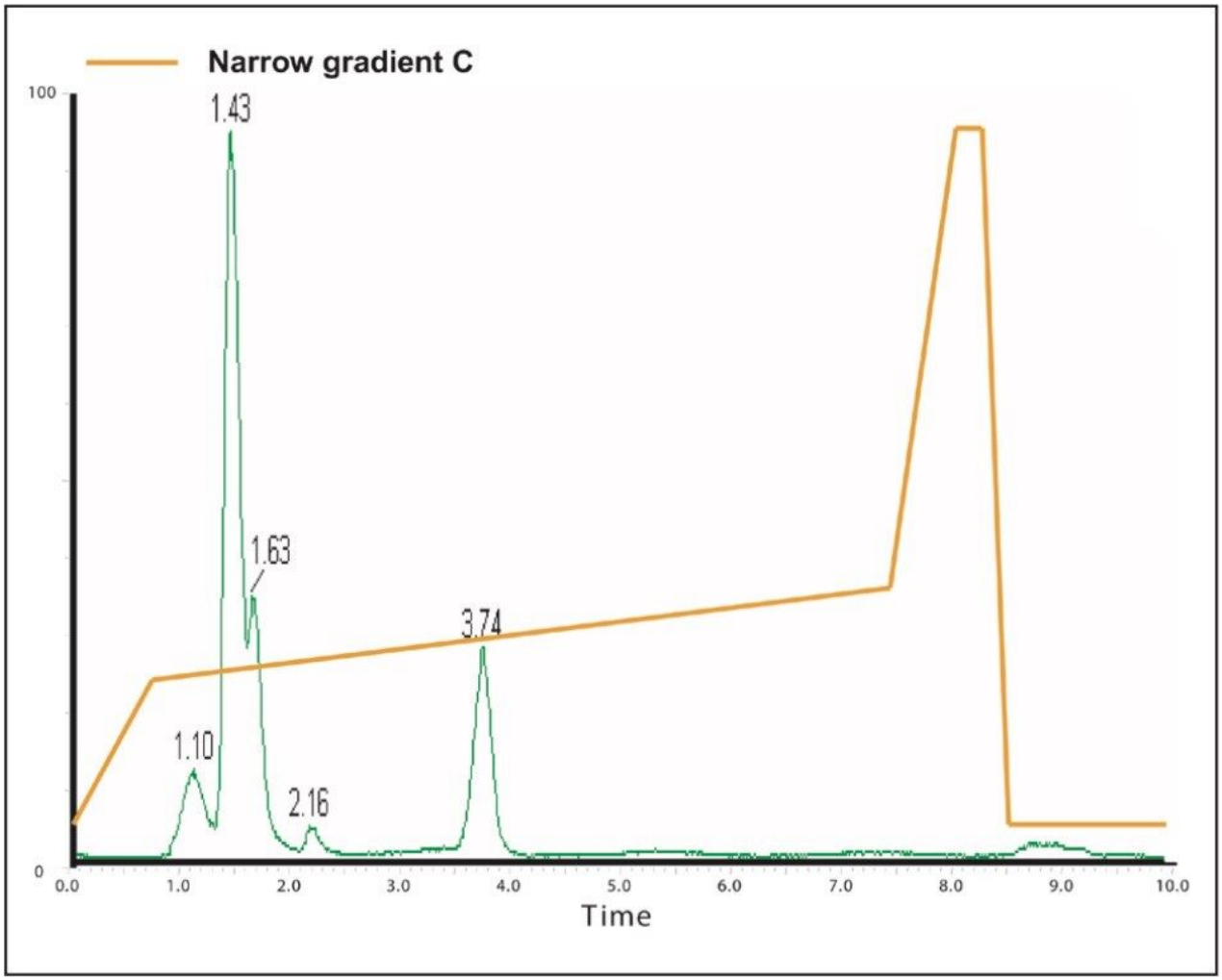


Figure 7. Representation of the narrow prep gradient chosen for the purification of the compound eluting at 4.06 min, with improved separation showing the isolated peak at 3.74 min collected.

The improved separation is more clearly displayed when the chromatograms of the two co-eluting compounds, as seen in Figure 4, are extracted and their chromatograms reviewed. Figure 8 shows the two chromatograms of masses 255 and 358, overlaid, and the improved separation achieved.

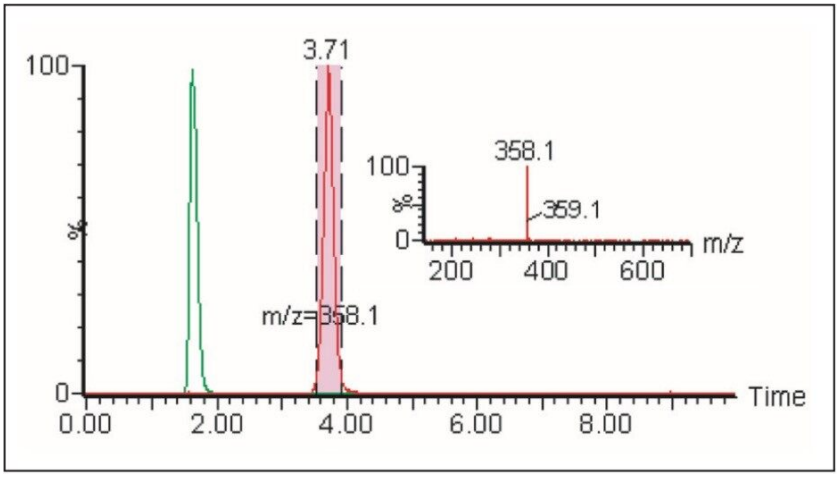


Figure 8. Overlay of the chromatograms of the two masses that were co-eluting earlier, showing the improved separation that was achieved. Spectra highlight the success also.

Step 3: Fraction analysis

With the first two steps of the process complete, the user can also decide to analyze the fractions (Figure 9). AutoPurify creates a sample list containing the fractions required for analysis and automatically runs them.

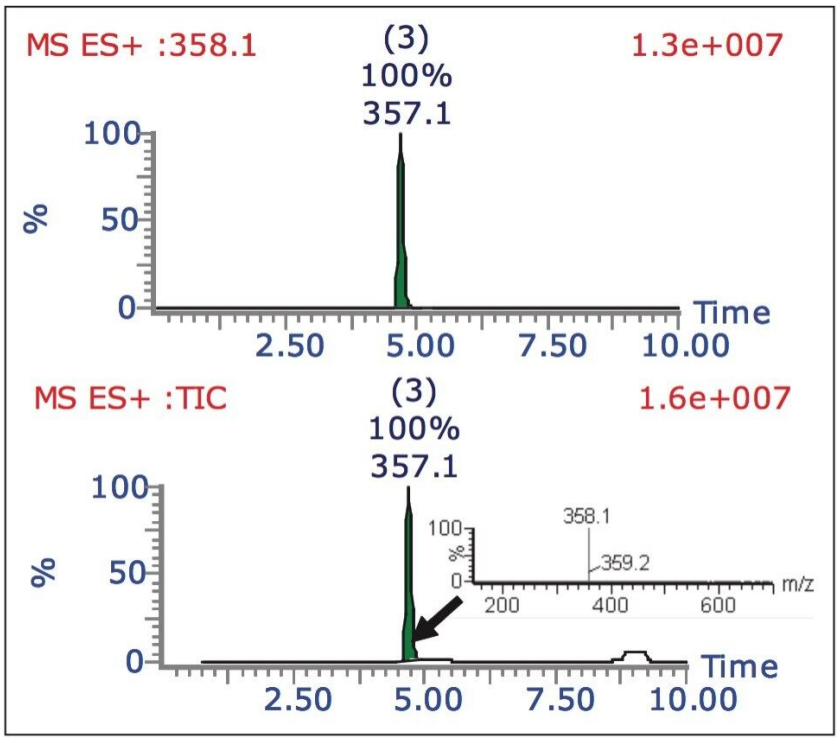


Figure 9. Fraction analysis post-collection, and post-fraction mixing by the injector/collector. TIC shows no other compounds present in the collection vessel.

To ensure that the portion of the sample taken for analytical analysis is representative of the entire collected fraction, it may be necessary to pre-mix fractions prior to injection (done with the injector/collector). Once homogenized, analysis can be performed on an analytical scale.

Automating the process

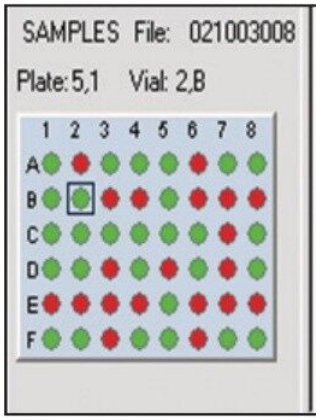
Automation of the three-step purification process is accomplished through AutoPurify.

A FractionLynx browser is created after each of the three stages to display results of the analysis and to report the recommended strategy for the next stage in the process. The software can automatically create and run the list of samples that are to continue to the next step. The user has a choice whether to allow the three stages to run unattended, or to manually review the results of each stage and edit the software's decision.

The determined strategy can be adjusted as necessary by the user through the interactive browsers that are produced. By automating the process, decisions can be made after regular work hours, allowing the work to

continue unattended, saving time and resources.

The root name of the data, the sample ID, sample list, and the FractionLynx browser, A123, as shown in Figure 10, are edited by the software and carried through the purification process to make sample and results tracking easier.



Sample ID	Time	Sample Purity	Purity Test	Masses	Strategy
A123001	4.53	70.42	TENTATIVE	282.00	NarrowC
A123002	5.97	75.15	TENTATIVE	308.00	NarrowD
A123003	3.73	19.85	TENTATIVE	217.90	NarrowB
A123004	4.61	46.13	TENTATIVE	375.00	NarrowC
A123005	-	-	N/A	-	None
A123006	3.42	68.15	TENTATIVE	226.90	NarrowB
A123007	-	-	N/A	-	None
A123008	4.04	21.96	TENTATIVE	357.10	NarrowC
A123009	4.10	29.50	TENTATIVE	268.00	NarrowC

Figure 10. Browser report created after the analytical evaluation. The resulting strategy is displayed using different colors for the injection plate. Green = mass is found, purity level between 20% and 80%, and sample requires purification; and red = mass is either not found or sample is already pure enough, and purification will not be performed.

Analytical interpretation

FractionLynx browsers also include chromatograms and spectral information that are not shown in this application brief. The portion of the browser file in Figure 10 shows sample purity and the prep strategy decision that was determined after the samples were analyzed on an analytical scale.

The preparative sample list is automatically created and run after the analytical analysis. Once the purifications are complete, the results are processed and a new FractionLynx browser report is generated (Figure 11).

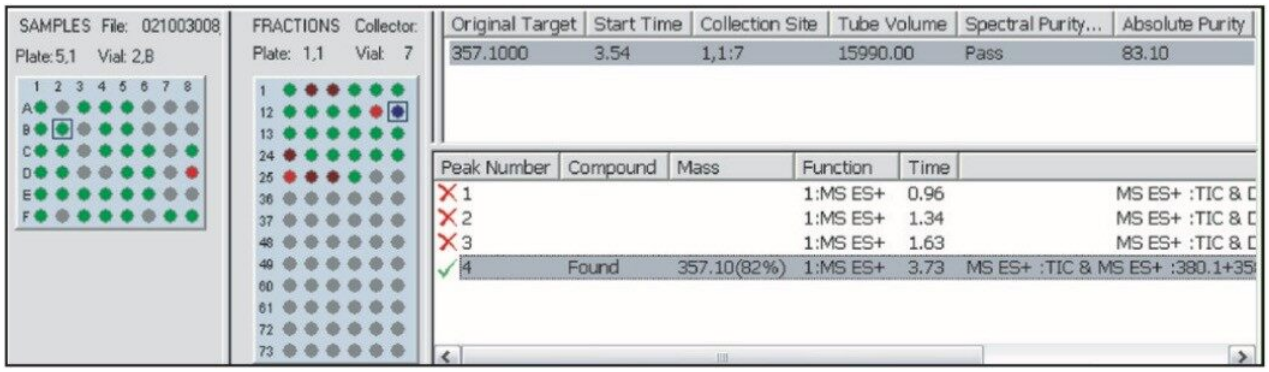


Figure 11. Purification results, indicating where the fractions were collected, including fraction volume and spectral purity. Blue = collected fraction of the sample highlighted in the injector plate, green = passed spectral purity assessment, burgundy = review required, and red = failed purity assessment.

Purification process

Upon completion of the processing of the purification results, a sample list is generated and automatic analysis of the fractions generated is performed (Figure 12).

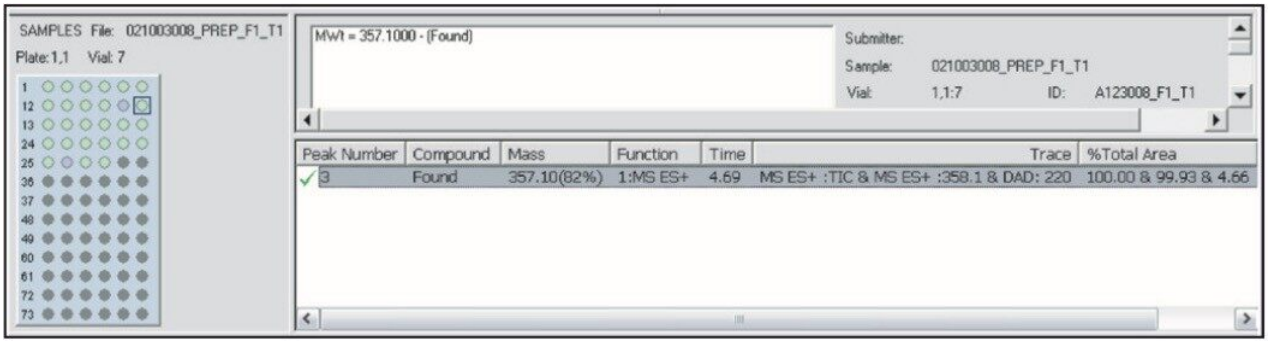


Figure 12. Fraction analysis results, indicating the sample purity of the collected fractions.

Fraction analysis

The final report shows the locations of the fractions, chromatograms, and spectra. The information in the reports can then be easily exported in different file formats such as .xml, .csv, and .tab, to easily interface with sample handling software packages such as liquid handlers or weighing devices.

Conclusion

This application brief shows how a library of compounds can easily and efficiently be purified using the AutoPurify capabilities within the FractionLynx Application Manager. The software is capable of automating the entire purification process, from the original analytical purity assessment, to purification, and finally to the analysis of the fractions.

AutoPurify allows the process to be performed intelligently. Analytical results are used to determine if the target is present and its purity. Based on these criteria, only samples that truly require purification continue on through the process. Samples that do not contain the target compound, not enough of the target, or are already pure enough can simply be excluded from purification.

The benefits of using AutoPurify can be measured in time savings, reduced solvent consumption, and overall productivity gains. This is noticeable in several main areas:

- Automated evaluation of samples before purification prevents unnecessary purification from being performed by removing samples that do not require purification.
- Computerized evaluation of samples throughout the entire process saves analysts from having to manually review batches between stages of the process, and enables the subsequent analysis to be performed immediately – without waiting for the analyst to be present.
- Computerized determination of methods required during the process saves analysts from having to make or decide which gradients should be used to improve separations.
- The use of narrow gradients allows for the use of shorter, more focused gradients, saving time and solvents.
- Automation from stage to stage allows for unattended operation, combining all the savings of the process.

Featured Products

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

MassLynx MS Software <https://www.waters.com/513662>

FractionLynx <https://www.waters.com/513795>

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