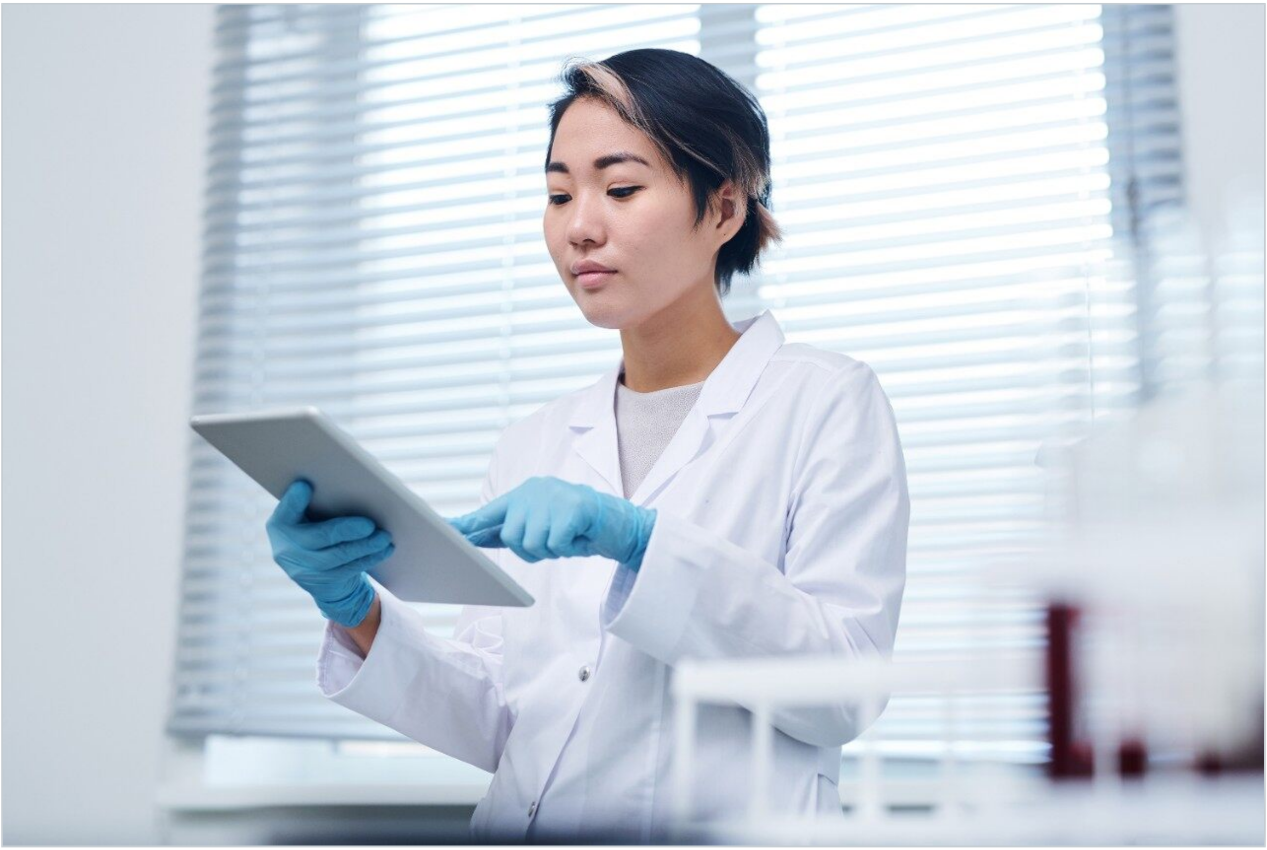
Making a Purification System More Rugged and Reliable

Ronan Cleary, Paul Lefebvre, Warren B. Potts

Waters Corporation



Abstract

This application note highlights how the Waters AutoPurification System hardware and software can be utilized to alleviate theses concerns.

Introduction

The demand for the number of samples requiring purification continues to grow. This increase requires purifications systems to be able to run more efficiently and with less user intervention. However, there are a number of serious, corporate concerns with running unattended purification. These include losing samples due to system failure, solvent leaks, overflowing waste containers, and solvent reservoirs running dry. Another concern is the verification that the system is actually running properly and collecting fractions as expected.

This application note highlights how the Waters AutoPurification System hardware and software can be utilized to alleviate theses concerns. Examples include software tools for monitoring solvent usage and that can monitor the number of injections without fraction collection. We also show how the system can be efficiently shut down in case of error to minimize the risk of sample loss.

Finally, we demonstrate how a new splitter can increase recovery rates and how a post-fraction collector detector can be used as a quality control monitoring tool.

Figure 1. The mass-directed AutoPurification System consists of the 2545 Solvent Manager, 2767 Sample Manager, System Fluidics Organizer, and PDA Detector.

Results and Discussion

System Configuration

System configurations can vary depending on customer applications and requirements. Waters has developed a purification system based on input from our customers.

The requirement for chemists to be able to make analytical injections to evaluate a sample before purification led to the development of the Waters 2767 Sample Manager, which has two separate flow paths – one analytical, and one for preparative. A separate and additional flow path allows for fractions to be collected onto the instrument bed for further analysis. This injector/collector requires a solvent delivery system that is capable of delivering reproducible and accurate analytical and preparative flow rates. Additional pumps are regularly added to the system for other purposes, such as post-column splitter make-up, at-column dilution (US Patent #6,790,361), off-line column regeneration, and pre-column modifier solvent addition. Mass spectrometry was added to further increase the selectivity and efficiency of the systems. These components comprise the Waters AutoPurification System.

Solvent Monitoring

The various pumps and vessels configured in a purification system can be defined in the monitoring software. The volume of solvent pumped from a solvent reservoir or into a waste container is monitored using the solvent monitor software.

Graphical solvent level indicators allow for easy viewing of the system status. Each solvent reservoir has information specific to that container, maximum volume, and various warning levels.

The status of the vessels is indicated by symbols, indicating that the system is either OK , or in Warning or an Acute Warning state. The response to the warning level is determined by the administrator.

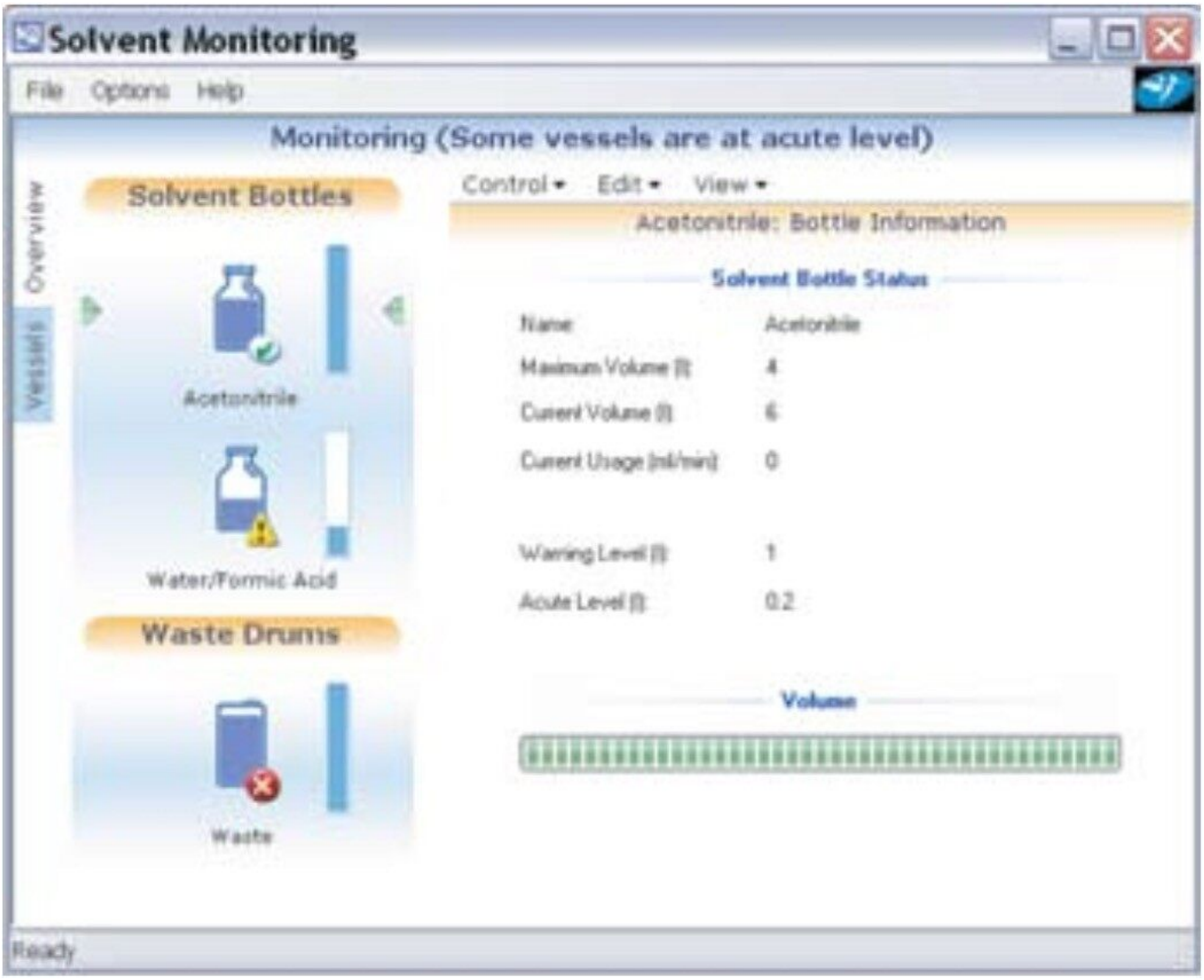


Figure 2. Solvent monitoring interface with both graphical and numerical reporting of system status.

A color-coded status page is also available, and can be accessed remotely through the remote status monitor component of the software.

Once all the solvents are defined, monitoring occurs in the background without any user interaction. Any volume of solvent pumped, either during an acquisition or while idle, will be accounted for. Even the amount of solvent used to prime the pump is monitored. When the software monitoring the solvent vessels identifies a solvent level that has generated a warning condition, multiple notifications and responses can occur, such as:

- Warning notification on the instrument page
- Color-coded notification on the remote monitoring software

- Email condition report sent to primary responsible party
- Terminate the analysis or batch
- Secondary emails can be sent to different individuals, notifying them of the condition of the particular system

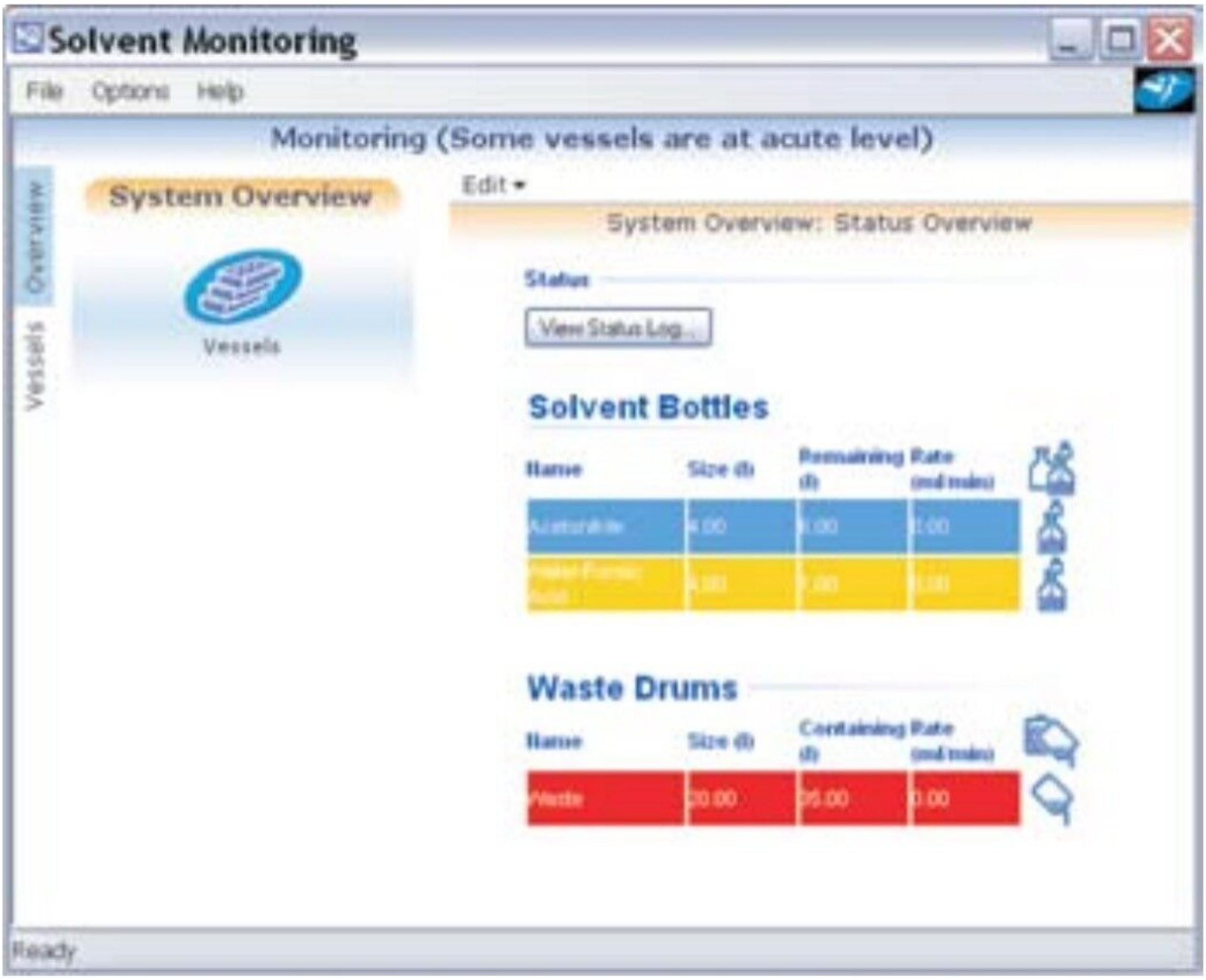


Figure 3. Color-coded system status page, with icons that indicate the need to refill or empty the containers.

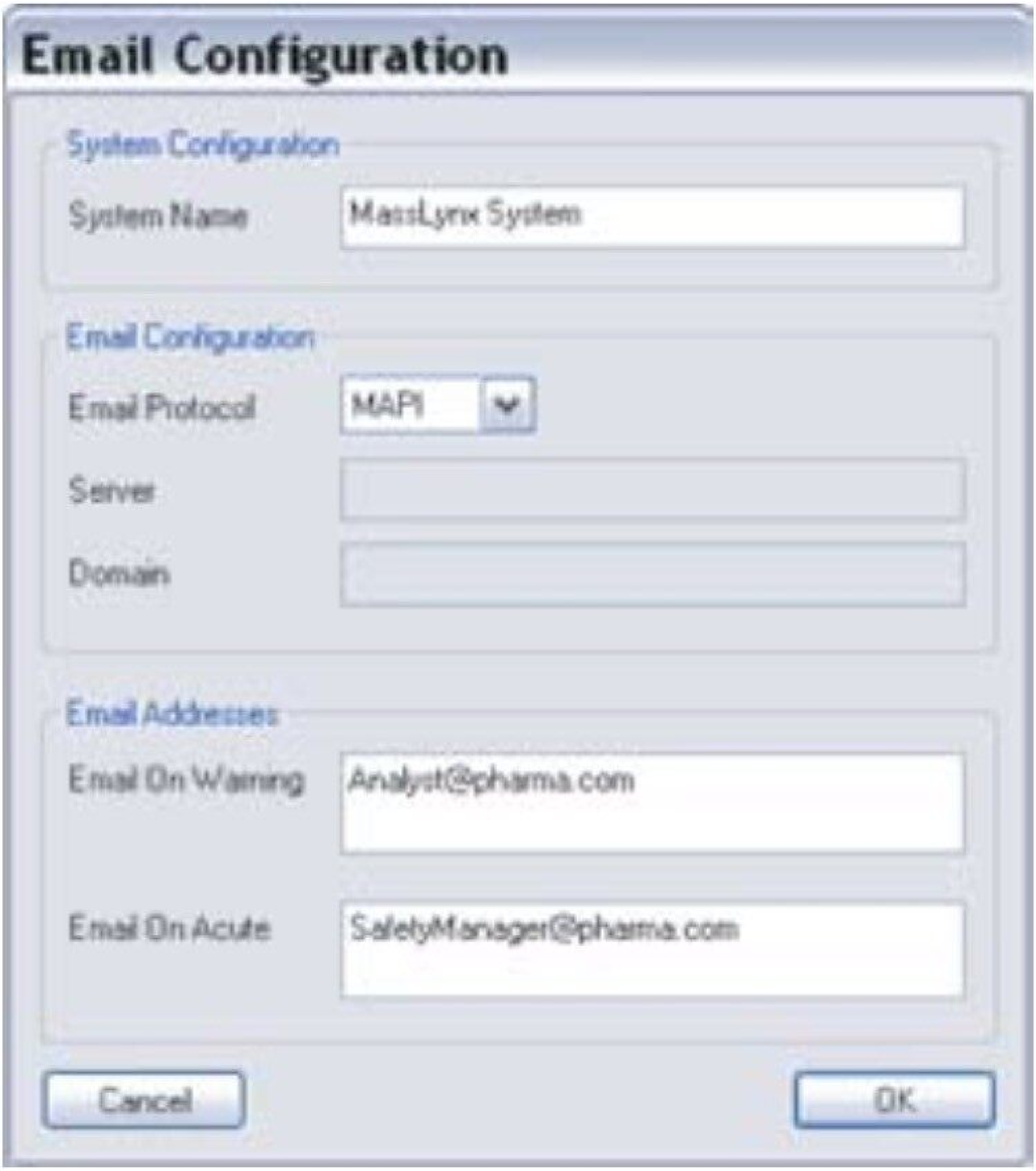
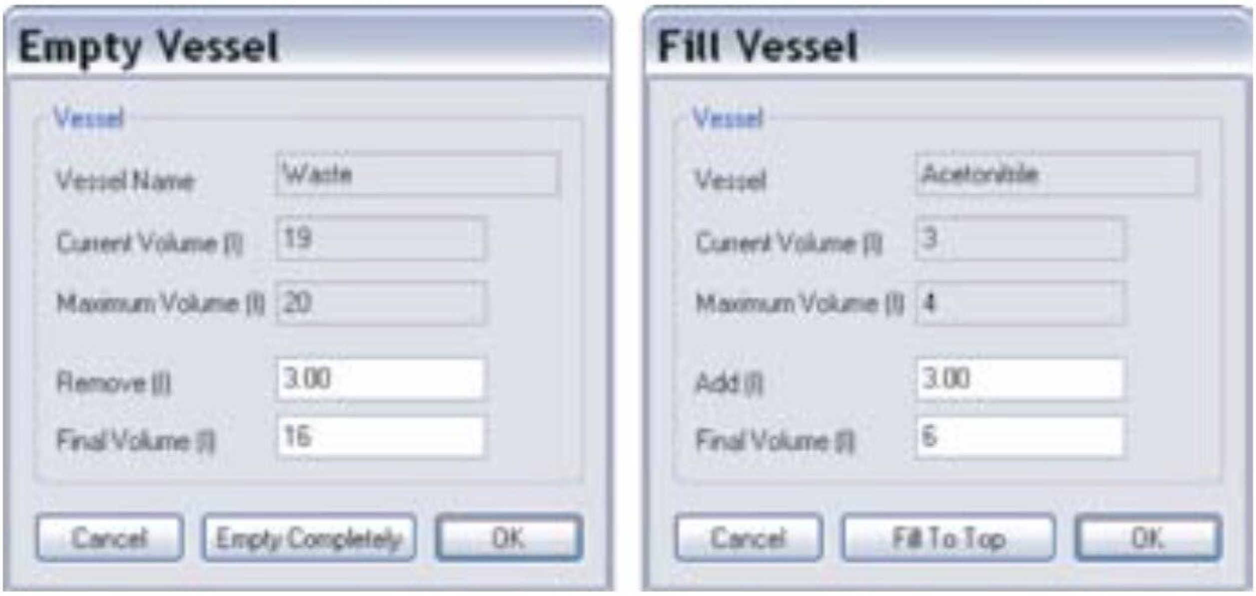


Figure 4. Email configuration with primary and secondary email contacts.

Once the administrator has been notified, they can choose to manage the condition by emptying or refilling the containers as necessary, or allow the software to deal with the error condition and shut the system down safely.



Figures 5 and 6. The user can partially add or remove solvents as necessary.

Shutdown software allows the user to configure a response produced when either the warning or acute level is reached:

- Shut down immediately
- Shut down after delay
- Shut down after sample
- Shut down after batch
- Ignore the warning

The shutdown procedure configured is linked to a particular shutdown method. This allows for an orderly shut down of the system to occur, allowing for columns to be flushed and returned to the correct conditions for storage, thus reducing the risk of damage.

Tracking Failures

A critical component to ensure rugged and reliable unattended operation is to have the system be able to stop after a defined number of consecutive samples without fraction collection. There are various reasons why a system may not have collected fractions, and yet not be in an error state, such as a blocked splitter or MS sample cone that prevents detection, or a blocked injection port that keeps the sample from being loaded onto the column. User error can also be a contributing factor. Incorrect information such as mass or wavelength can also contribute to fractions not being collected.

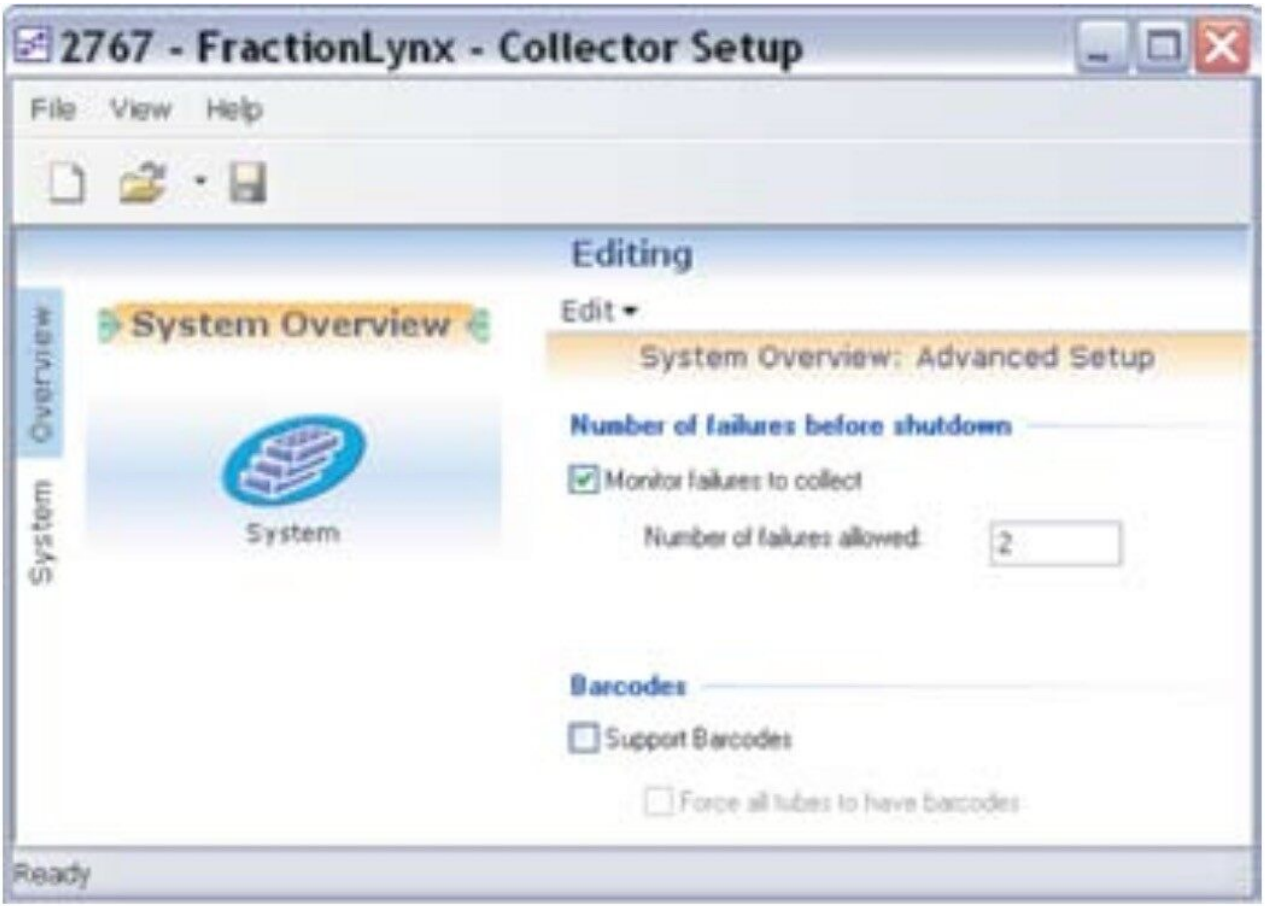


Figure 7. The user can define the number of injections that can occur without fraction collection before the run is ended.

Additional Collectors

Frequently, analysts find that compounds other than the primary compound of interest are of importance, so it may be necessary to capture them in a separate collector. Examples include collection of a starting material or impurities along with the primary target. Another example is collecting all the other major peaks in addition to the primary target. This is shown in Figure 8 with a complex natural product separation.

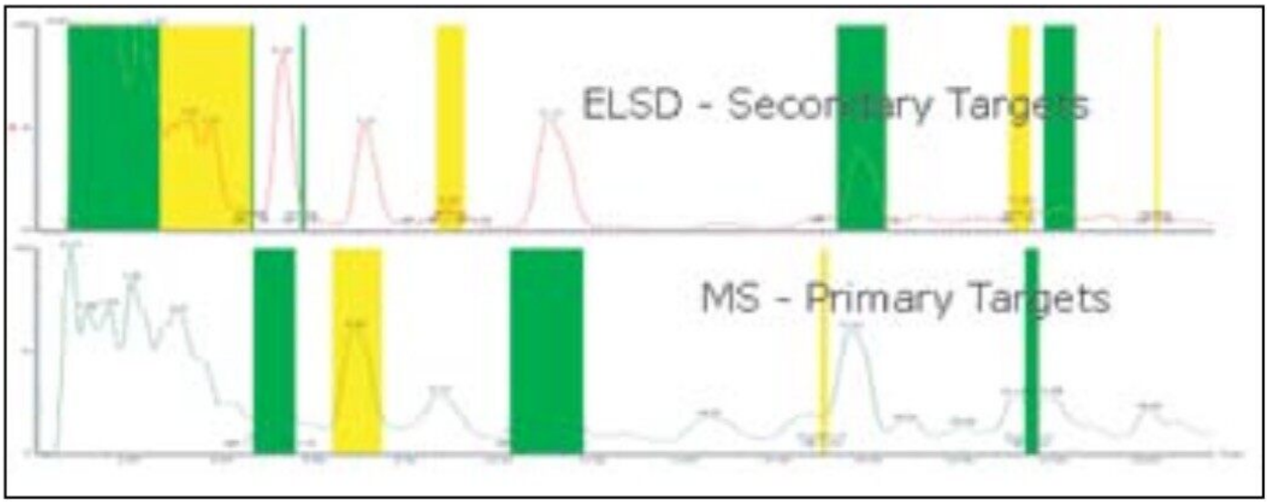


Figure 8. The top chromatogram shows collection of peaks detected by ELS detection. The lower chromatogram shows the peaks detected by the MS and collected by mass trigger.

There is no such thing as a universal detector, so it is possible that some compounds may not be detected. A waste collector can be added to the system, enabling all column eluent not diverted for collection earlier to be collected separately. In Figure 8, any of the sample not collected by either the primary or the secondary collectors was captured in a separate waste collector, thus minimizing the possibility of any sample loss.

Splitter Performance

On any purification system where a destructive detector is being used, a splitter is necessary to isolate a portion of the primary flow for analysis, allowing the rest of the sample to be directed to the fraction collector. The flow to the collector must also go through a delay coil to prevent this much faster flow from reaching the collector before the triggering detector has identified the peaks to collect.

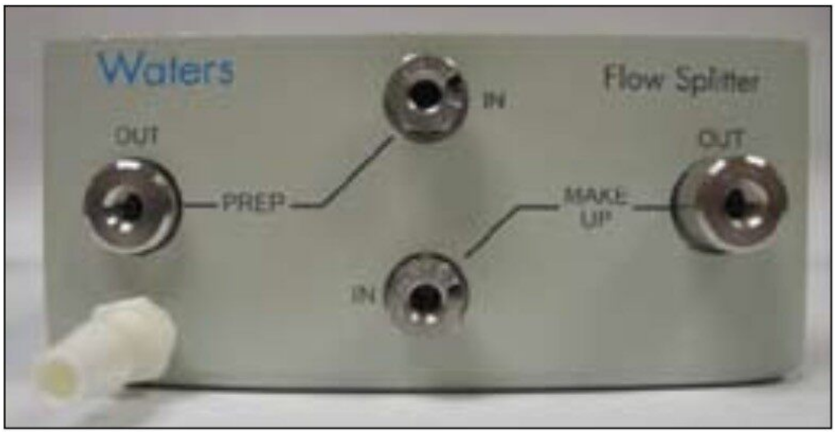


Figure 9. The Waters splitter is matched to column dimensions for optimized performance.

The most important requirement of the splitter is that peak shape and resolution achieved from the column be retained in both the low- and high-flow solvent streams. The low-flow stream is sent to the detectors used to trigger fraction collection. If the peaks' shapes differ between the triggering detector and the fraction collector, the collection of the fraction will be less than optimal. Laminar flow can cause the peaks on the high-flow side of the system to be larger than the peaks on the low-flow side of the system. This can contribute to decreased recoveries and impure fractions.

We evaluated a new Waters splitter against another commercially available splitter to highlight the improvements that have been made with the splitter technology.

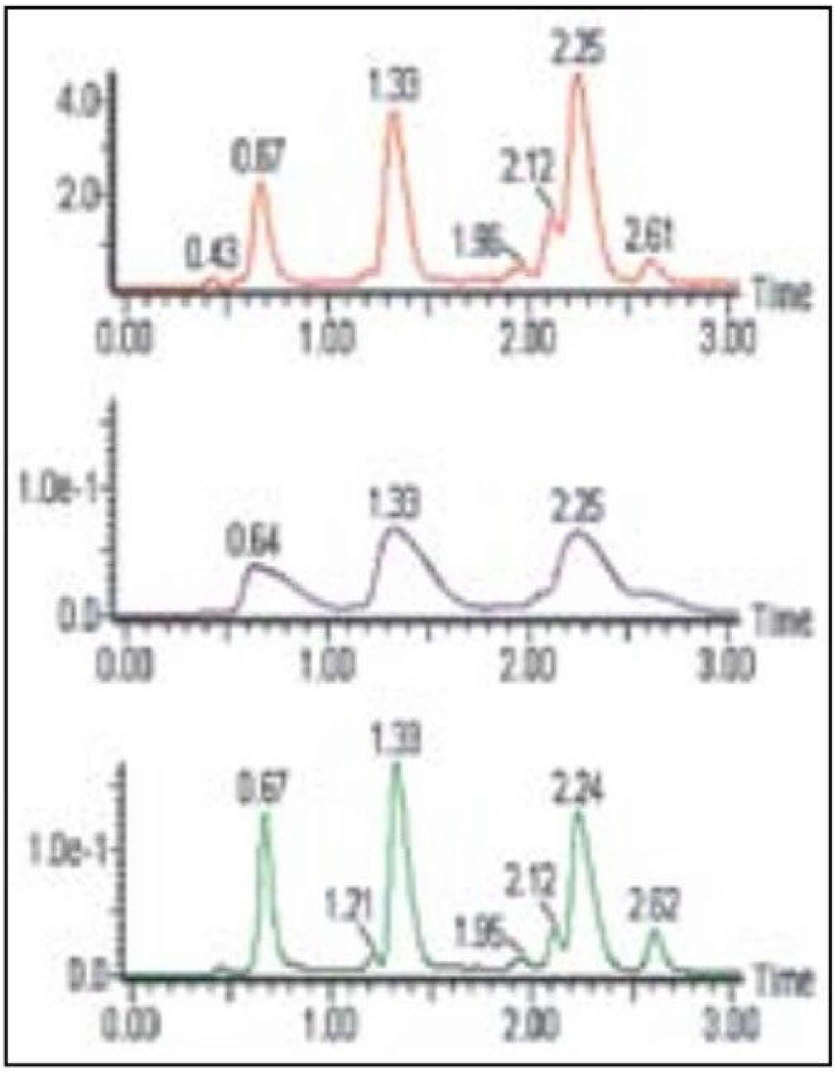


Figure 10. The upper chromatogram shows the low-flow split to the fraction trigger detector. The middle chromatogram shows the high-flow split of the sample after using another commercially available splitter to the waste detector. The lower chromatogram shows the highflow split of the sample using the Waters splitter to the waste detector.

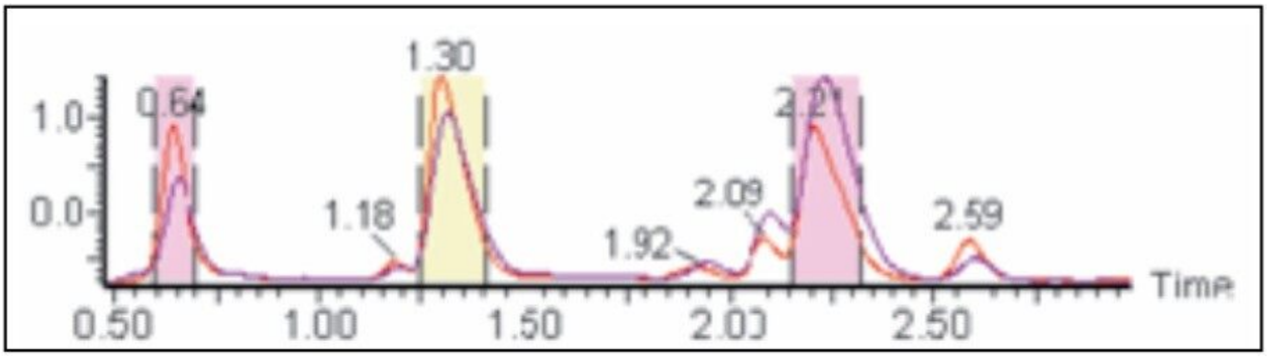


Figure 11. Overlay of the trigger and collected fraction trace using a Waters splitter. The collected fraction is the purple trace, and shows little or no peak dispersion.

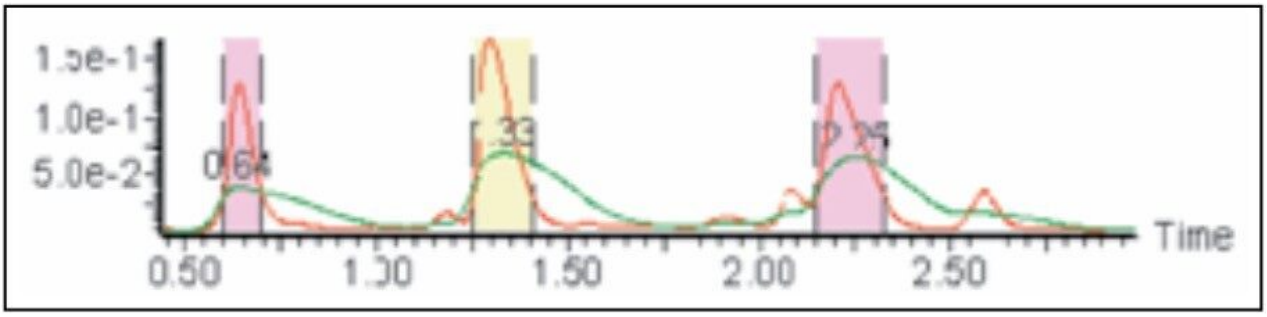


Figure 12. Overlay of the collections with the vertical axis linked. The green trace shows what would have been missed if a non-Waters splitter had been used.

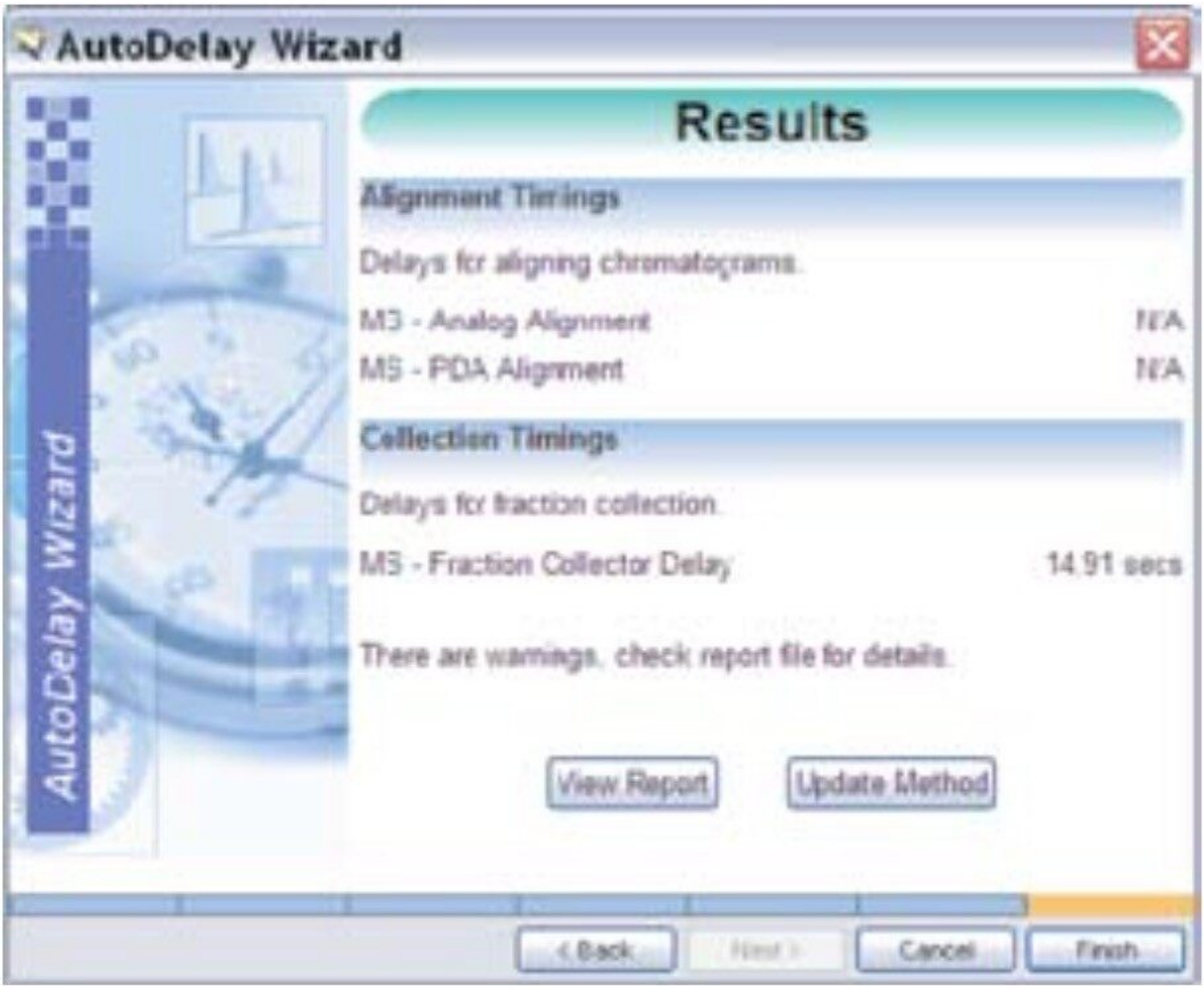


Figure 13. AutoDelay results page with delay time and results export.

Collector Delay Time

Delay time determination can be easily accomplished with the use of AutoDelay software, which will perform injections to determine the delay time and confirm injection for the determined delay time.

Figure 14 shows the effect of delay time on the amount of missed fraction detected in the waste detector. The larger the detected peak corresponds to a lower recovery or increased sample loss. When the delay time is set optimally there is only a small peak, just above the noise. But as the delay time drifts from 1 to 3 seconds away from the optimal, the increase signal becomes more and more substantial. The measured recovery is greater than 99% at the optimal delay time. With the 3 seconds too early, the recovery is only 60%.

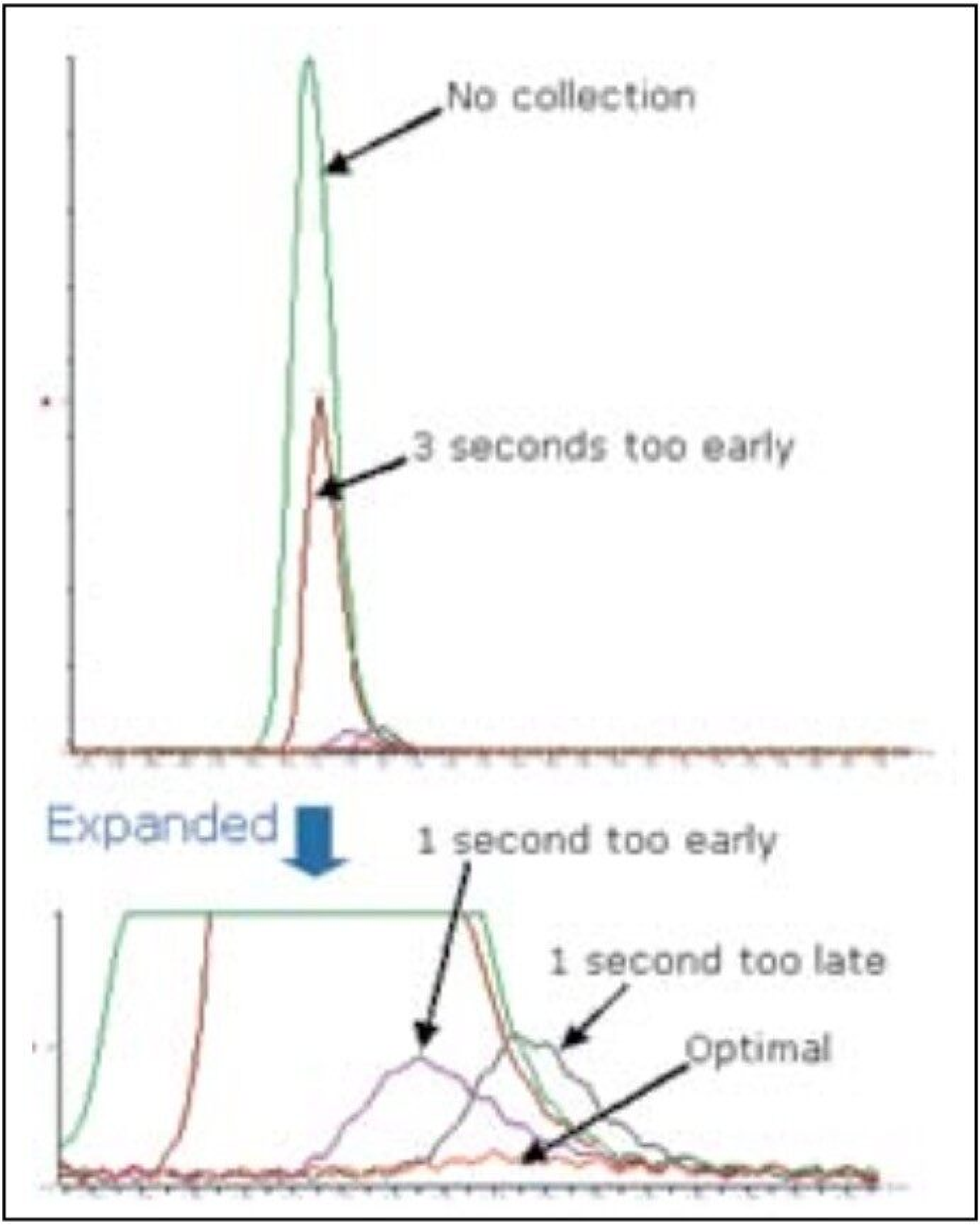


Figure 14. Different collection delay values have different responses in the waste detector.

Conclusion

Purification systems should include functionality that allows for unattended operation such as:

- Solvent monitoring with tiered responses such as email notification
- Solvent monitoring with intelligent shut down
- Remote system monitoring
- Secondary fraction collection for use with other detectors
- Waste collection to enhance user confidence

Flow splitters should not increase band broadening and decrease fraction recovery rates. The new Waters flow splitters maintain equal peak shape for both the high and low flow for optimal fraction recovery and purity.

The AutoPurification System, with technology that allows for rugged and reliable operation, is available from Waters.

Featured Products

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

720002285, June 2007