MAKING A PURIFICATION SYSTEM MORE RUGGED AND RELIABLE

Waters

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INTRODUCTION

Overview

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 multiple 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 poster will highlight the various ways in which the Waters® Purification hardware and software can be utilized to ease theses concerns. Examples to be demonstrated include software tools for monitoring solvent usage and software that can monitor the number of injections without fraction collection. We will also show how the system can be efficiently shutdown in case of error to minimize the risk of sample loss.

We will show how a new splitter can increase recovery rates and how a post- fraction collector detector can be used as a QC monitoring tool.

SYSTEM CONFIGURATION

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

The requirement for chemists to be able to make analytical injections to evaluate the sample before purification, led to the development of the 2767 autosampler which has two separate flow paths, one analytical and one for preparative. A separate additional flow path allows for fractions to be collected on to the instrument bed for further analysis. This injector/collector then 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 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.

Figure 1. Waters AutoPurification™ Mass-Directed system consisting of a 2545 solvent manager, 2767 Sample Manager, a System Fluidics Organizer, and a PDA detector.

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.

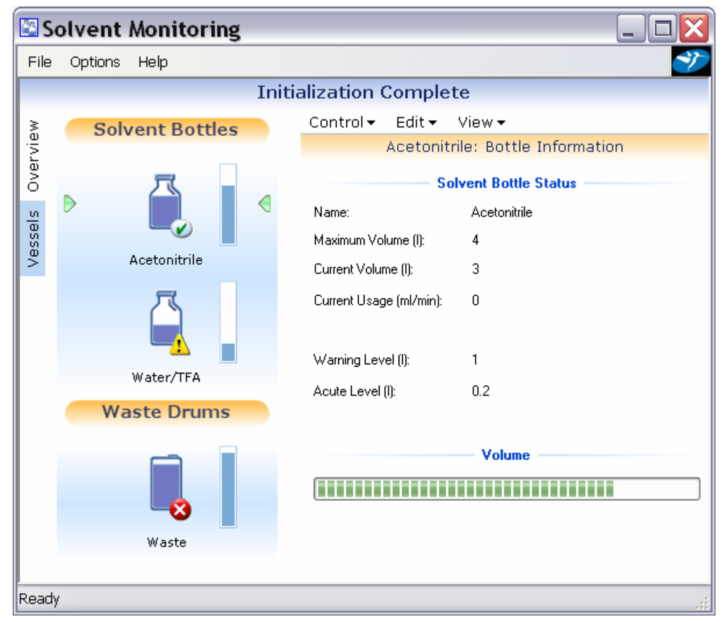


Figure 2. Solvent monitoring interface with both graphical and numerical reporting of system status.

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

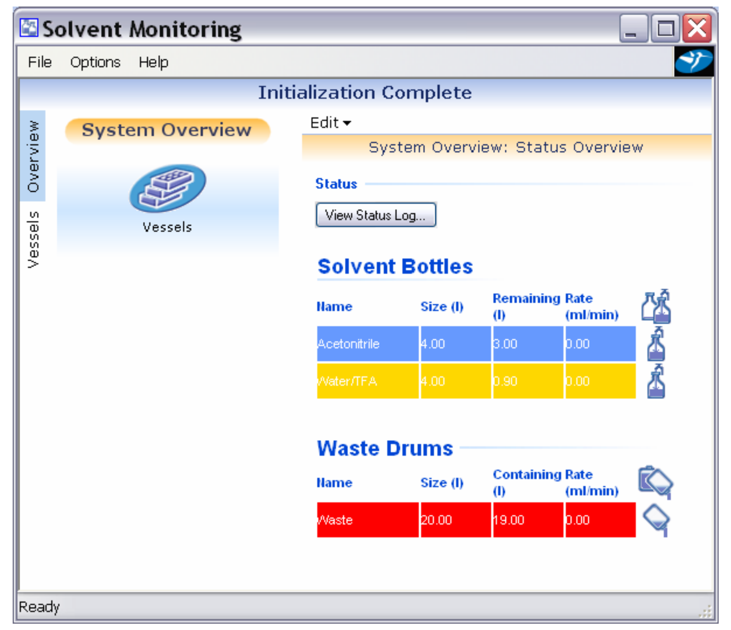


Figure 3. Color coded system status page, with icons to refill or empty the containers.

Once all the solvents are defined, the 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.

- Warning notification on the instrument page,
- Color coded notification on the remote monitoring software
- E mail condition report to responsible party
- Terminate the analysis or batch
- E mails can be sent out to different individuals notifying them of the condition of the particular system.

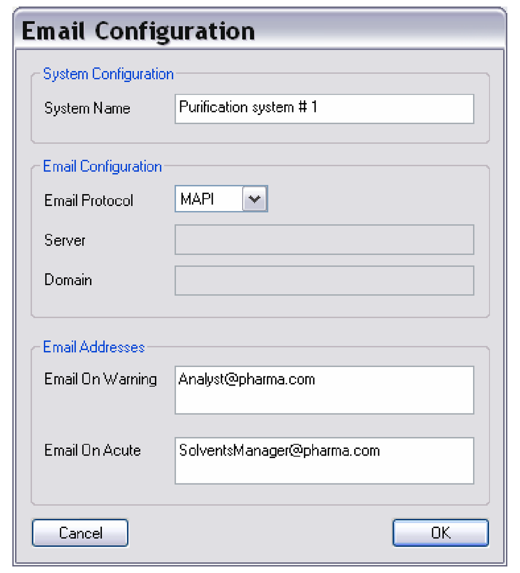
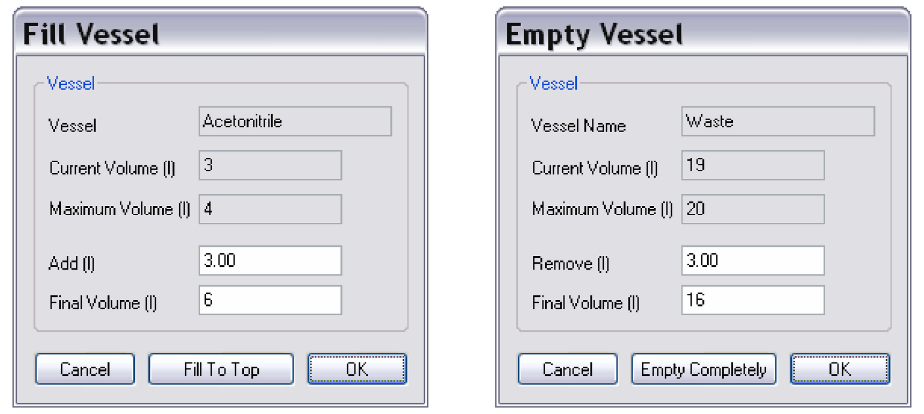


Figure 4. E mail configuration with primary and secondary e mail contacts

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 + 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.

- Shutdown immediately
- Shutdown after delay
- Shutdown after sample
- Shutdown after batch
- Ignore the warning

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

TRACKING FAILURES

A critical component to insure 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. Some examples include a blocked splitter or MS sample cone so nothing can be detected, or a blocked injection port, so no sample is getting loaded onto the column. User error can also be a contributing factor. Incorrect information such as mass or wavelength can contribute to fractions not being collected.

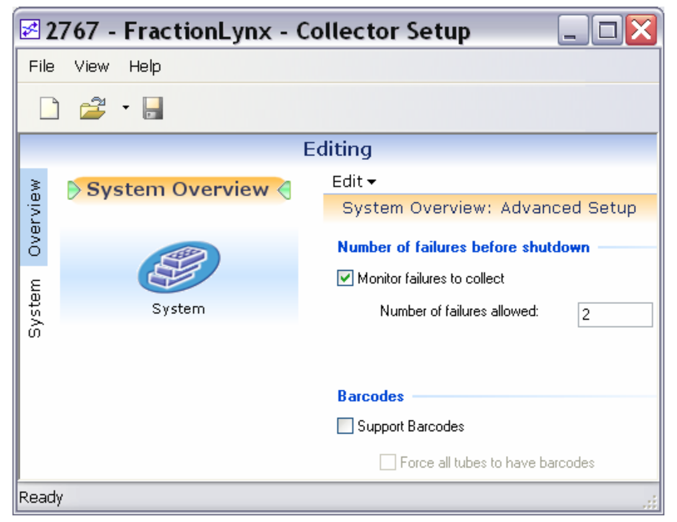


Figure 7. The user can define the number of injections that can occur without fraction collection, before the run is ended.

ADDITIONAL COLLECTORS

Secondary Collection

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

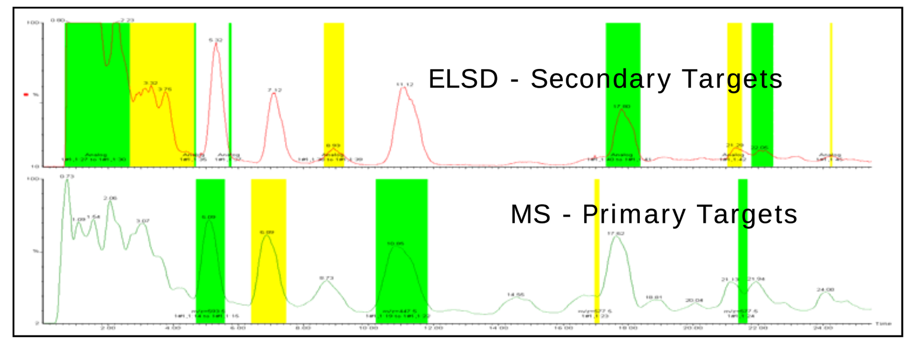


Figure 8. The top chromatogram shows collection of peaks detected by the ELSD. The lower chromatogram shows the peaks detected by the MS and collected by mass trigger.

Waste Collection

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 collected 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 10. The new Waters splitter is matched to column dimensions for optimized performance.

The most important requirement of the splitter is that the 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 fractions 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 Water splitter against another commercially available splitter to highlight the improvements that have been made with the splitter technology.

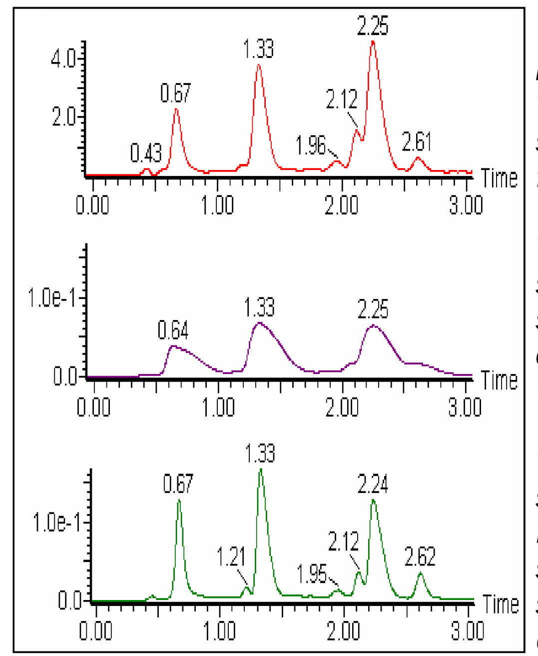


Figure 9.
The upper chromatogram shows the low flow split to the fraction trigger detector.

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 same sample after it has passed through the Waters splitter into the high flow stream and into the waste detector.

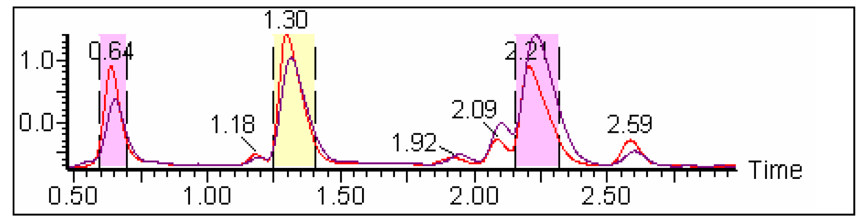


Figure 10. Overlay of the trigger and collected fraction trace using a Waters splitter. The collected fraction is the purple trace, and shows little or no peak dispersion.

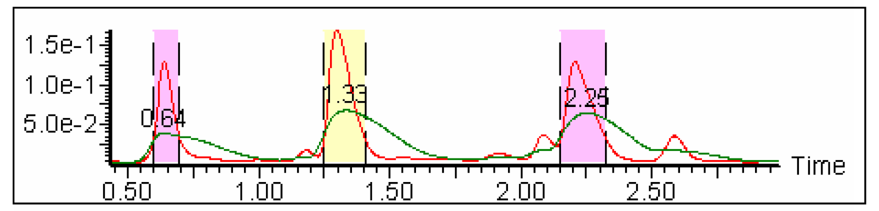


Figure 11. Overlay of the collections with the vertical axis linked. The green trace shows what would have been missed if a non Waters splitter had been used.

COLLECTOR DELAY TIME

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

Figure 12. AutoDelay results page with delay time and results export.

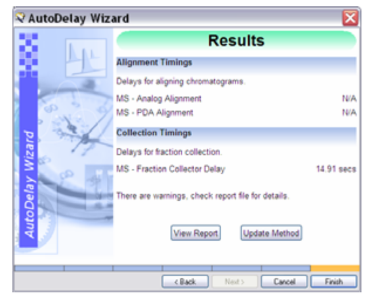


Figure 14 shows the effect of delay time on the amount of missed fraction detected in the waste detector. The larger the detected peak corresponds to lower recovery or increased sample loss. When the delay time is set optimally there is only a small peak, just above the noise. But as the delay time drifts 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%.

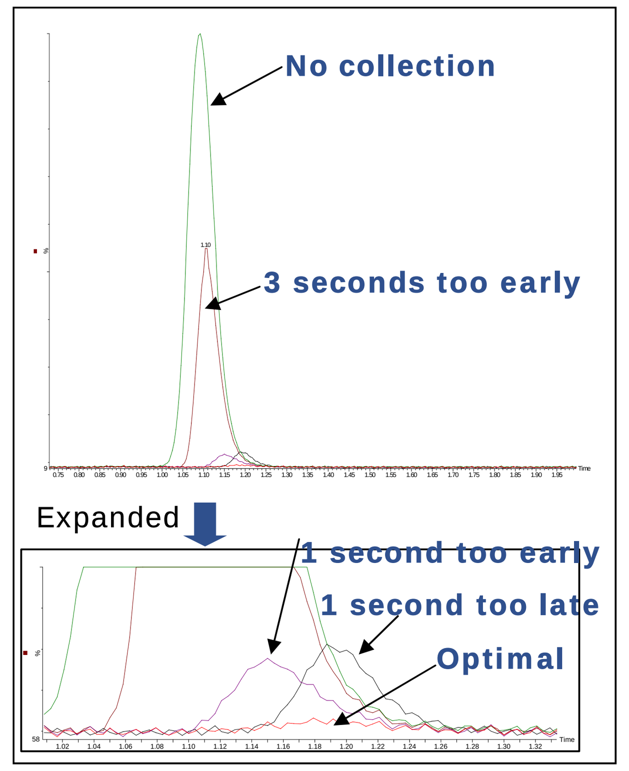


Figure 13. Different collection delay values have different responses in the

CONCLUSIONS

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

- Solvent Monitoring with tiered responses such as e mail notification
- Solvent Monitoring with intelligent shutdown
- Remote system monitoring
- Secondary fraction collection for use with other detectors
- Waste collection to enhance user confidence

Flow splitters should not add 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.

A Purification system with technology to allow for rugged and reliable operation is available from Waters.

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