MAKING A PURIFICATION SYSTEM MORE RUGGED AND RELIABLE

Paul M. Lefebvre, Warren B Potts III, Ronan Cleary, Robert S. Plumb
Waters Corporation, Milford, MA

INTRODUCTION

The demand for the number of samples requiring purification continues to grow. This increase forces purifications systems to run more efficiently 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 incorporating additional hardware to determine if liquid is present in a certain location and software monitoring tools such as counting 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.

Finally, we will show how a post- fraction collector detector can be used as a QC monitoring tool. This is used as an immediate confirmation tool to ensure fraction collection is consistent and efficient, with minimal sample loss. Previously, the alternative approach was to perform a recovery experiment which could require several hours. Now the system can be tested and the results determined immediately before the start of an unattended run.

SYSTEM CONFIGURATIONS

The configuration and the amount of solvent generated by a purification system can be variable. The number of preparative flow streams can range from a single stream, as shown in Figure 1, to 4 streams as in Figure 2. 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. Monitoring the status of the various the volume pumped, the solvent remaining, and the amount of waste generated is critical for unattended operation.

Figure 1. Waters AutoPurification™ Mass-Directed ZQ™- based system consisting of a 2525 Solvent Manager, 2767 Sample Manager, a Column Fluidics Organizer, and a PDA detector.

Figure 2. Waters Purification Factory™ consisting of 4 independent preparative flow stream with MS-based fraction collection from a single ZQ with MUX–technology™

SOLVENT MONTORING

Overview

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

Graphical solvent level indicators allow for easy viewing of the system status. Numerical tables list the status of the containers giving a more detailed status report.

Setup

The first step in setting up solvent monitoring is to define the configuration of the system. Figure 3 shows an example system with 3 solvents bottles, 2 for the gradient and 1 for makeup flow, and a single waste container.

Figure 3. Solvent monitoring interface with both graphical and numerical reporting of system status.

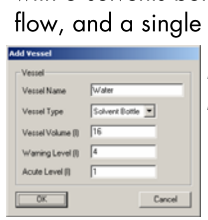

Figure 4. Solvent reservoir setup screen.

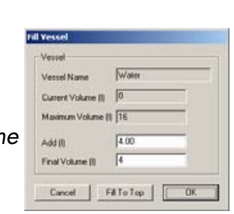

Figure 5. Vessel volume and "top up" option.

The options to be defined are shown in Figure 4 and include the vessels name, volume and the actions levels. For the warning level, an e mail will be sent by the system to the user, notifying them of the situation. For the acute level, a system level error will be generated and shutdown will occur as defined by the user. The quantity of solvent added to each needs to be entered, as shown in Figure 4. It is also possible to "top-off" the vessel, in which case, the "Fill to Top" button can be selected and the vessel's volume will be changed to the maximum. The user must define which of the configured pumps controlled in the Inlet Method Editor is associated with the appropriate solvent vessel.

Waste Drum

The pumps that are contributing solvent to the waste drum are also configured. The total volume and action levels are defined. In this example there is only 1 drum, so all pumps are considered waste contributors.

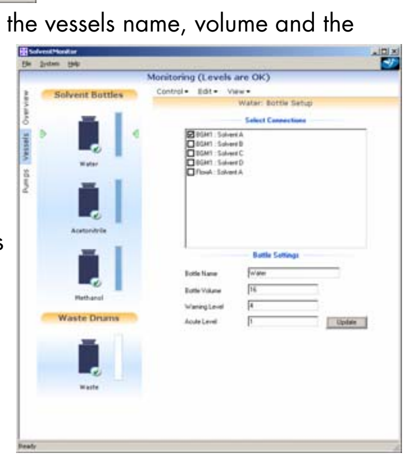

Figure 6. Defining waste drum contributors.

Running

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 account used to prime the pump will be considered. Figure 7 shows an example of vessels at various action levels. The acetonitrile vessel has passed the warning level. The waste drum has passed both the warning and acute level and a system level error has been generated.

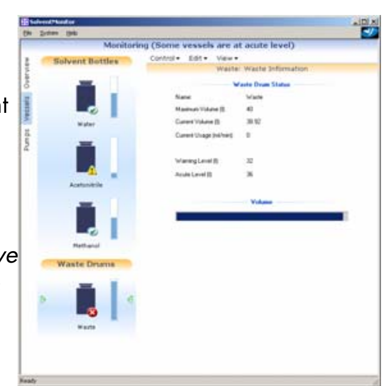

Figure 7. Warning levels have been reached and messages generated, which are then managed by shutdown.

LEAK DETECTION

Having the ability to monitor the solvent levels does not ensure that a leak will not occur. Leak sensors can be easily integrated into the system. The leak detection output signal can be wired into leak detector input on the pump. When the signal is received by the pump, a system level error is generated.

There are 2 options for configuring a system for leak detection, a single or multiple point. Figure 8, Waters P/N 205000152, is a single point liquid sensor. All possible leak sources are directed into a single vessel. When any liquid is detected, a signal is sent to the 2525 to shutdown. Though this approach requires only a single sensor, it does not give any information as to the source of the leak.

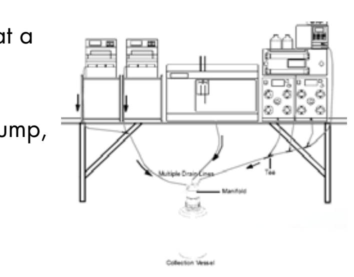

Figure 8. Diagram of leak sensor attached to the drip trays of the instrumentation.

Another approach is to use multiple leak sensors. In this case, leaks can be detected at or close to the point of origin. An example of this type of sensor, incorporated in the system, is shown in Figure 9. With this approach, multiple sensors are placed throughout the system. When any sensor sees a leak, a signal is sent to the control box. From there the signal is sent to the 2525 and the leak error is generated.

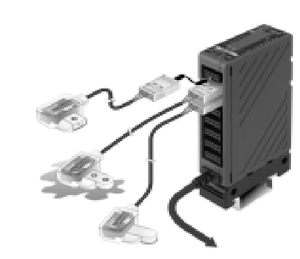

Figure 9. The leak sensor connector unit. LED's on the unit indicate which of the sensors has tripped.

INTELLIGENT SHUTDOWN

Shutting down the system in the event of an error is a critical component of a rugged system. Figure 10 shows the options available in the MassLynx startup and shutdown control window. In this window, the startup and shutdown methods set to run at the beginning and end of a batch are defined. Figure 11 shows the "Shutdown On Error Configuration" window. There are 5 different sources of system level errors, shown at the left. With each error, the system can shutdown at different user defined times. The options include shutdown immediately, after a delay, the batch, or the sample.

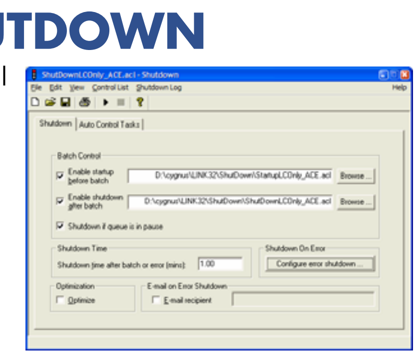

Figure 10. Shutdown page options.

Figure 11. Action on error settings including solvent monitoring options.

FRACTION COLLECTION QUALITY CONTROL

The time that the fraction collector valve is opened and closed is dependent upon the detector of choice. However, there is an additional factor critical for optimal fraction collection. It is the time it takes for the peak to travel from the triggering detector to the fraction collector valve, commonly referred to as the delay time. Having even the smallest of error can result in significant loss in recovery. Missing 1 second from a 10 second peak could cause a 10 % loss of fraction. The percentage lost increases as the peak width decreases.

An additional detector, located after the fraction collection valve, can be used to monitor the quality of fraction collection. By monitoring this data channel, a standard can be injected and collected with varying delay times. The injection with the minimum area detected in the waste channel corresponds to the optimal delay time. The area that is detected can then be used as a quality control (QC) reference. Therefore, if a standard is injected and collected days later and the response detected is greater than this value, the QC fails. Further investigation is required to determine what has changed with the system, i.e. a check valve in the pump is causing the flow rate to be off.

Figure 12 shows the chromatogram of the waste detector when the delay time is set optimally. The green box indicates the fraction collection time. The peak size, shape and quantity will change with changes in delay times and collection settings.

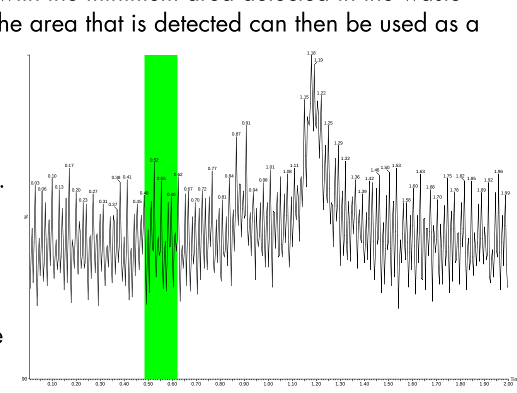

Figure 12. Optimal collection with almost no sample passing through to the waste detector.

COLLECTOR DELAY TIME

Figure 13 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 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%.

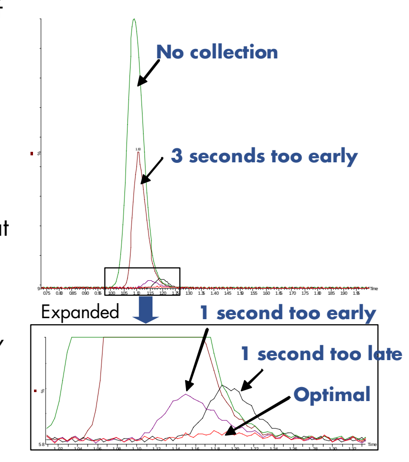

Figure 13. Different collection delay values have different responses in the waste detector.

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.

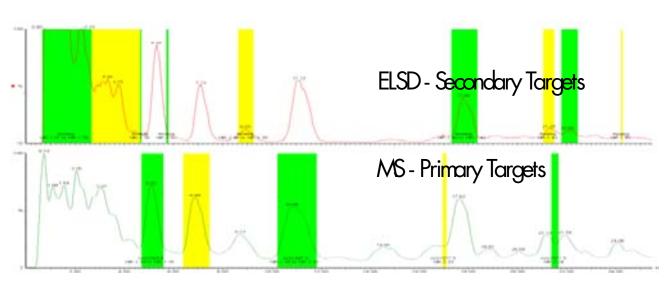

Figure 14. Secondary collection based on peak in the ELSD. Primary collection is mass-directed

Waste Collection

There is no such thing as a universal detector, so it is possible that some compounds may not be detected. For increased protection from sample loss, a waste collector can be added to the system enabling all column eluent not diverted for collection earlier, to be collected separately.

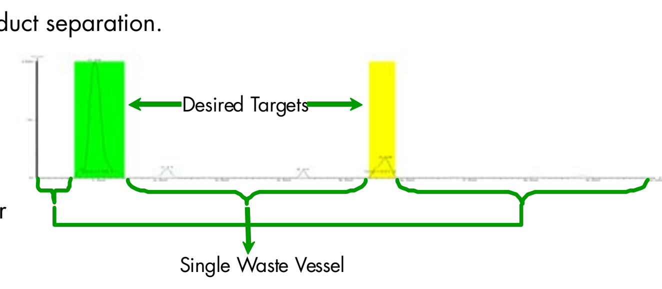

Figure 15. Waste collection with mass-directed purification.

N # OF FAILURES

Another 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, 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, as incorrect information such as mass or wavelength can contribute to the system not collecting fractions.

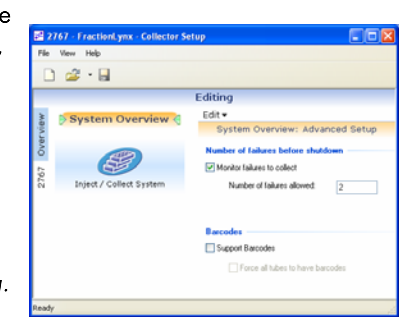

Figure 16. Collection monitoring enables the user to automatically end a sequence if a defined number of injections have been made without collection occurring.

CONCLUSIONS

A rugged and reliable preparative system is critical, especially during unattended operation.

Solvent Monitoring

The major areas of solvent management to consider include:

- Accommodating various preparative pump configuration
- Monitoring the level contained within each of the different vessels and waste generated
- Notifying the user responsible for the system that solvents are running low or the waste is getting full without interruption to the sample queue
- Shutting the system down if error conditions are met

Leak Sensing

System level leak sensing can be accomplished with a single point sensor, while multiple sensors can give more accurate information about the source of the leak.

Intelligent Shutdown

Based on the system level error generated, the system is capable of shutting down immediately or after a delay, the sample, or the batch, based on the user's definition.

Waste Detection

An additional detector placed after the fraction collector valve can provide useful information about the quality of fraction collection. It can be used for quality control or to help set the appropriate time delay.

Fraction Collector Control Options

Additional collectors can be added to the system for added security against losing samples. These collectors can be configured for secondary or waste collection. The system can also be programmed to generate an error after a user defined consecutive number of samples are injected without having any fractions collected.

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS