SIMPLIFYING THE PURIFICATION PROCESS WITH OPEN ACCESS

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INTRODUCTION

Mass spectrometry has proven useful in quickly assessing peak identity and homogeneity in complex chromatograms. Using the mass spectrometer to recognize the target peak and deposit it in a fraction collector tube, reduces processing steps.

Purification of compounds has increasingly become the responsibility of the chemist that has performed the synthesis. Frequently the knowledge and expertise required to perform a successful separation and collection, resides outside of the synthesis group.

In this poster we will describe some of the functionality of the FractionLynx™ purification software, and its use in an Open Access environment. This gives novice users access to the tools available to the experts, with a minimal amount of training and supervision.

OVERVIEW

The most critical areas where difficulties arise during purification are;

- Having software functionality to enable non specialists to operate systems
- Determining whether or not purification is required
- Creating methods and determining the threshold values to optimize the collection of the separated compound

The most important requirements for Open Access systems are;

- Ease of use
- Management of users

A superior approach is to use the Open Access capabilities within the Waters® FractionLynx™ Application Manager for MassLynx™ Software. This comprehensive informatics solution enables automation of the entire process from the initial evaluation and the automatic setting of the collection threshold values, through to the purification and analysis of the collected fraction. All of the functionality can be easily accessed from a single login by even the most novice user through the Open Access software.

SYSTEM

Figure 1. Waters® Mass–Directed Autopurification System:

Components

Waters 2545 Binary Gradient Module, 2767 Sample Manager, System Fluidics Organizer, 2996 Photodiode Array Detector, 3100 Mass Spectrometer, 515 Makeup Pump, and a Waters splitter.

AUTOPURIFY

AutoPurify is a component of the FractionLynx application manager that offers the system administrator levels of automation from the analytical analysis, to subsequent purification and fraction analysis.

Analytical Analysis

In this example, figure 2, the analytical screening of the sample has determined that the sample is not pure. The FractionLynx application manager can decide if purification is required, and what shallow gradient should be used, based on the sample purity (22%), and its analytical retention time.

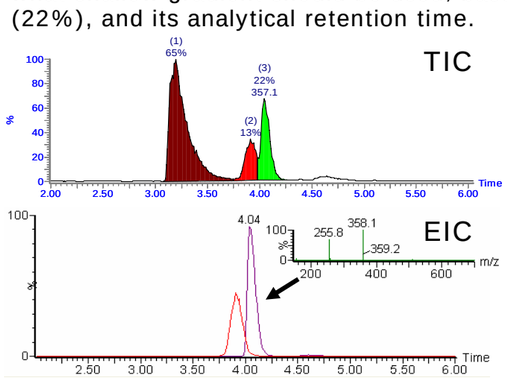


Figure 2. The compound of interest, mass 357.1 is co-eluting with another compound of mass 255.1, as shown in the TIC. An overlay of the two extracted ion chromatograms and the spectrum of the scans across the peaks, shows the co-elution more clearly.

Purification

Narrow or focused gradients, allow for optimal target separation from closely eluting impurities, thus improving the purity of the resulting fraction. The shallow gradient used is automatically chosen from an existing list of gradients, based on the analytical retention time of the target(4.04 min), figure 3.

The purification strategy, determined by the software can be automatically performed, or adjusted through the interactive browser, and then performed.

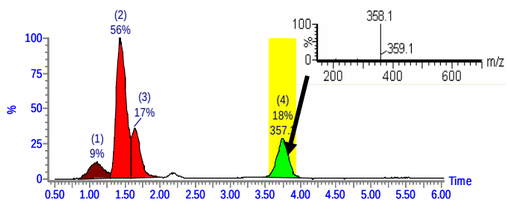


Figure 3. The fraction collected is displayed by the yellow box around the peak of interest. The spectrum reports none of the earlier co-eluting mass 255.1 present in the fraction.

Fraction Analysis

Mixing and analysis of the collected fraction can be performed to verify the success of the purification, figure 4.

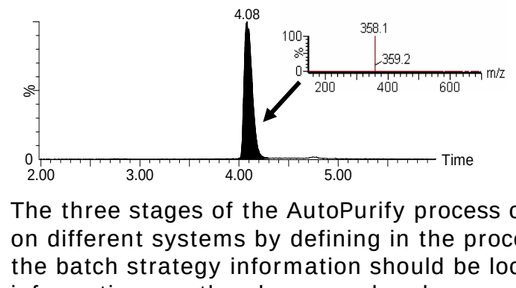


Figure 4. TIC of the collected fraction indicating successful purification. The fraction purity is now 100%.

The three stages of the AutoPurify process can also be performed, on different systems by defining in the processing method where the batch strategy information should be located. The batch information can then be opened and run, once the samples have been loaded onto the particular system.

AUTOMATIC THRESHOLD DETERMINATION

In order for the collection of a fraction to be successful, it is important that the correct collector threshold value be set. The threshold must be high enough so it is not effected by changing baselines, yet it must not be too high, as it could prevent the sample from being collected. Frequently threshold values may have to change during the run to compensate for chromatographic changes.

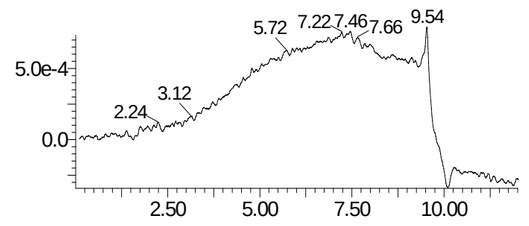


Figure 5. Over the course of the gradient, the background intensity of the detectors can change, especially in UV mode, requiring the need of multiple threshold setting.

Determining what those changing threshold values should be, requires prior knowledge of the chromatography is, but this information is not always available to the person performing the purification.

The AutoMIT capability in FractionLynx, determines the threshold values from information generated by the preparative injection of a blank. The software calculates the threshold values for any of the masses, wavelengths or other detectors signal acquired during the blank run. The threshold values are saved in a "read only" data file, that is then referenced during the purification run.

When a purification sample list is created, it must include this "blank" reference file. FractionLynx then uses the threshold values in the reference file to automatically assign a threshold setting for the trigger in the sample list.
The software is then able to perform the purification, figure 6, using the thresholds from the reference file for any masses, wavelengths or analog signals.

Example:

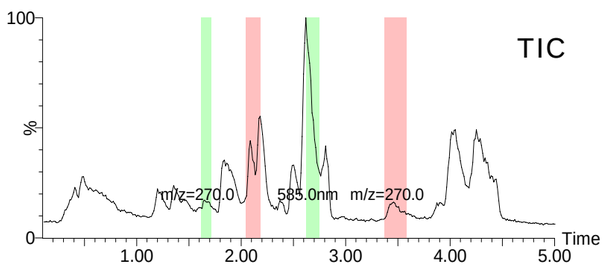
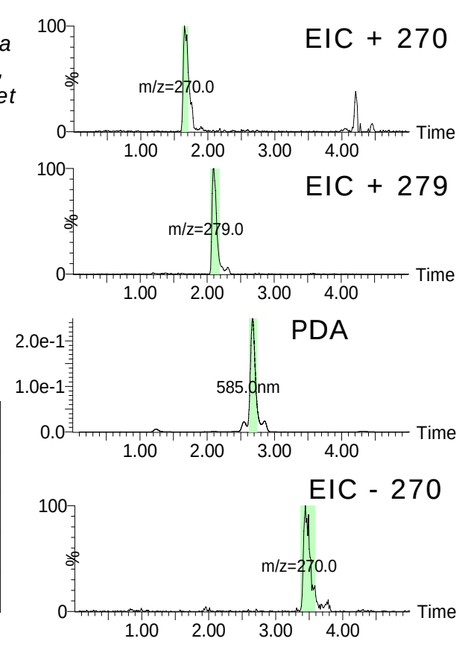


Figure 6. TIC of a complex sample, with the four fractions collected marked by the colored lines.

Figure 7. Different masses and ion modes, as well as a wavelength were collected, with no threshold values set by the analyst.
Each of the extracted chromatograms shows collection with a different trigger. Each trigger has a different threshold setting, table 1, which was automatically determined by the software.



Trigger	Threshold
ES + 270	8.5 e6
ES + 279	11 e6
PDA 585	5.4 e4
ES - 270	9.1 e4

Table 1.

OPEN ACCESS PURIFICATION

OA Login

The functionality of AutoPurify and AutoMIT can easily be utilized through the OpenAccess functionality of MassLynx, to make walk-up purification simple.

The single screen login, figure 8, is used for single samples, for fast easy access to the system. The user can simply choose the analysis method to be used from the list, define the type of sample container being used, the reaction compounds to monitor and the trigger required to perform the collection, Mass, UV, ELSD, or analog. All three stages of the process can be performed from a single login.

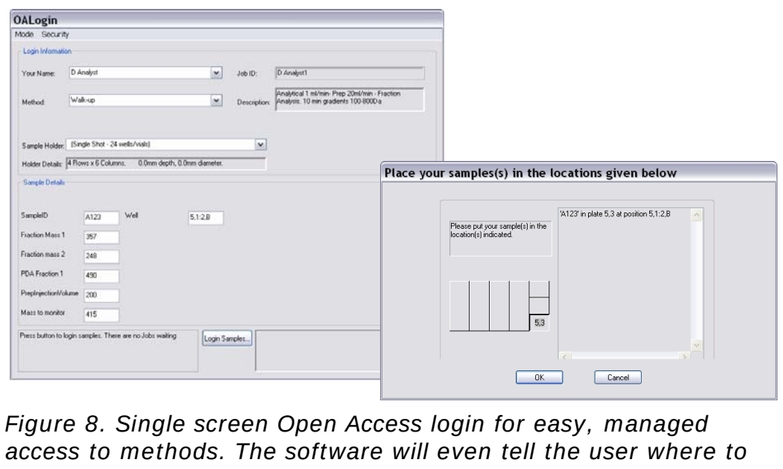


Figure 8. Single screen Open Access login for easy, managed access to methods. The software will even tell the user where to put the sample to be analyzed.

FractionLynx Browser

Lists of samples that are already in txt or excel formats, can be imported into the wizard login for easy loading of large batches.

FractionLynx Browser reports, figure 9, are generated automatically after analysis is completed, and show the locations of the fractions, as well as chromatograms and spectra. Any of the information in the reports can be exported in different formats such as xml, csv and tab, to easily interface with sample handling software packages, such as liquid handlers or weighing devices.

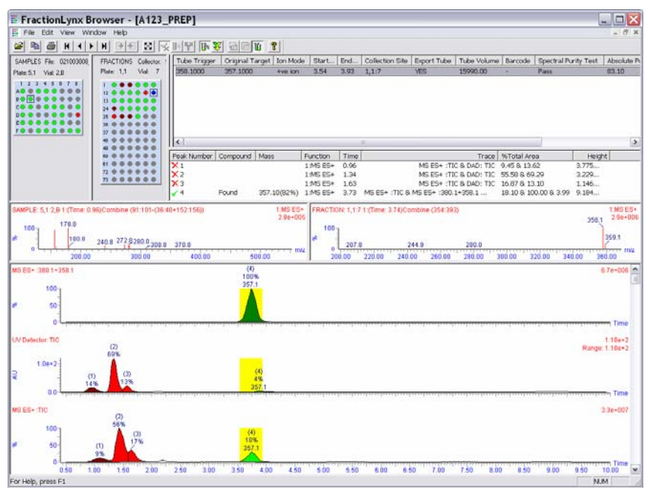


Figure 9. FractionLynx Browser showing relevant sample and fraction information. It is interactive to allow for editing of the software determined decisions, if necessary.

Barcodes

This export file can also include barcode information. For example, there can be a unique barcode for each tube or a common barcode associated with a collection plate, figure 10. Before the system can track the barcodes all the tubes and plates in the collector must be assigned a barcode. Once assigned, the collection reports will indicate fraction positions by barcode and tube number.

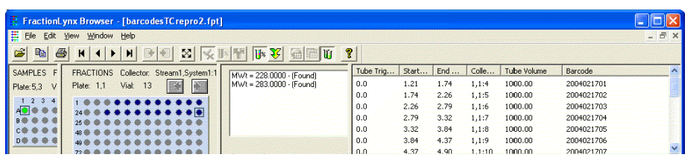


Figure 10. The barcode is simply the name of the tube, with all the other associated information.

SYSTEM ADMINISTRATION

Management of users and their data can be accomplished through Open Access Toolkit. The administrator can define different users and privileges for different systems from a central location, figure 11, and then "push" those settings across the network, to the different login computers.

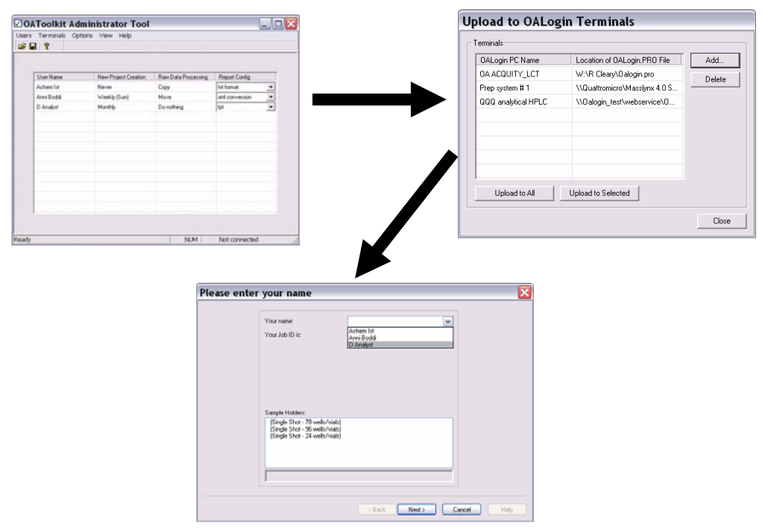


Figure 11. Different data and report settings are enabled for different users. The settings are sent to the specific system that the user needs access to. Different systems can have different sets of users, with passwords required for access.

SYSTEM MONITORING

Frequently systems are located in areas not close to users, or the administrator, so checking on instrument and queue status can be inconvenient. With the Remote Status Monitor component of OA Toolkit, systems monitoring can be enabled, and the information accessed through a web browser, figure 12. The functionality is available for any instruments operating under MassLynx.

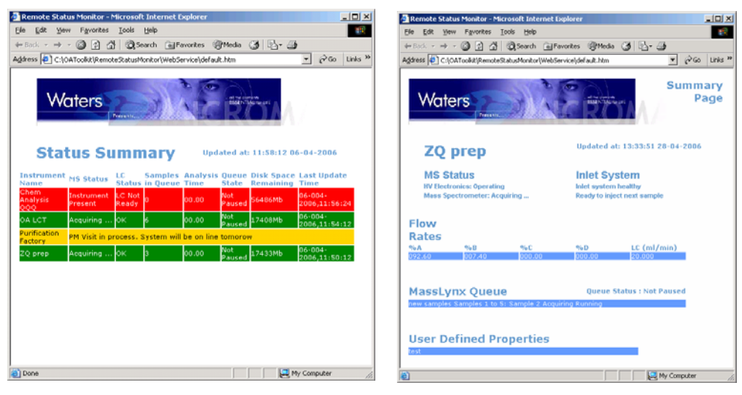


Figure 12. Remote Status monitoring screens allow users access to status information on any of the systems currently operating MassLynx. Two levels of detail are available, allowing access to both MassLynx queue and OA Login information. Color codes indicate instrument status, per system messaging is available also.

CONCLUSIONS

- **The AutoPurify capabilities of FractionLynx allow for automation of the entire purification process, from the initial screening, through purification, to the analysis of the collected fractions.**
- **Automatic determination of the collection threshold on a per sample basis, removes the interference from drifting baselines or high background signals, and increases ease of use.**
- **Open Access software allows even the most novice user to be able to utilize the full potential of the system, with the minimal amount of training.**
- **OA toolkit software allows for remote management of users and their privileges, and allows users to remotely monitor systems across the network.**

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