THE NEXT GENERATION IN AUTOMATED PURIFICATION

Waters

Ronan Cleary, Paul Lefebvre, Warren B. Potts III
Waters Corporation, 34 Maple Street, Milford MA 01757

OVERVIEW

Traditionally preparative sample separations are monitored using UV detection with the collection of many fractions over the course of the entire chromatographic program, necessitating labor intensive post-run analysis and processing. Mass spectrometry has proven useful in quickly assessing peak identity and homogeneity in complex chromatograms. Mass-directed isolation of compounds uses the mass spectrometer to recognize the target peak and deposit it in a fraction collector tube, reducing processing steps.
Purification of compounds has more and more 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 discuss automation of the purification process without sacrifice to compound resolution, optimizing the collection parameters for maximum performance, and enabling the process with minimal amount of training and supervision.

INTRODUCTION

The most critical areas where difficulties arise during purification are;

- Determining whether or not purification is required
- Creating a gradient to target separation from closely eluting impurities
- Determining the threshold values to optimize the collection of that now separated compound
- Having software functionality to enable no specialists to operate systems

A superior approach is use the 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 by even the most novice user through Open Access software.

SYSTEM

Waters® Mass–Directed Autopurification System:

Components

Waters 2525 Binary Gradient Module, 2767 Sample Manager, Column Fluidics Organizer, 2996 Photodiode Array Detector, ZQ™ Mass Spectrometer, 515 Makeup Pump, and a LC Packings 1:1000 Splitter

Sample

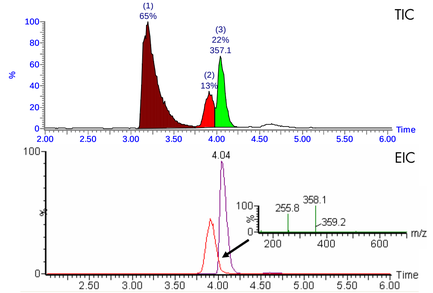
100 mg/mL mixture drug like test compounds in DMSO

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.

In this example, the analytical screening of the sample has determined that the sample is not pure, and indeed only represents 22% of the total sample.

Normally the chemist would have to decide if purification of the sample was required, and then what shallow gradient is necessary to improve the separation. The FractionLynx application manager can make both of decisions easily, based on the sample purity (22%), and by its analytical retention time.



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

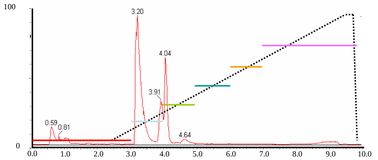
Shallow gradients, also known as narrow gradients, allow for optimal target separation from closely eluting impurities, thus improving the purity of the resulting fraction.

With the relationship between the analytical retention time and the organic composition already established, the shallow gradient to be used is automatically chosen from an existing list of gradients, based on the analytical retention time of the target.

In this example the sample eluted at 4.04 minutes under analytical conditions, which corresponds to about 25% organic. The shallow gradient automatically chosen to be used, changes from 24% to 37% organic, and is the one denoted by the green line in the next figure.

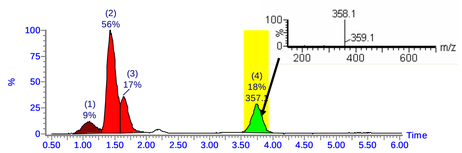
The purification strategy, determined by the software can be automatically performed, or adjusted and then performed, through the interactive browser.

With 5 other shallow gradients available, samples that elute at other times may be purified using gradients that focus on other parts of the analytical composition change.



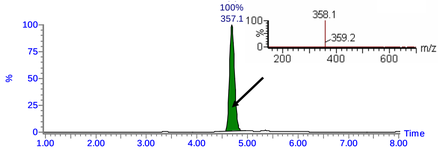
Multiple preparative gradients are created to focus on the different analytical gradient elution compositions.

Successful collection of the fraction is indicated in the chromatogram, with the yellow box indicating the collected fraction. From the extracted spectrum we can see that the collected fraction does not contain any other masses.



The fraction collected after the automatically chosen shallow gradient is displayed by the yellow box around the peak of interest. The spectrum reports none of the earlier co-eluting mass 255amu present in the fraction.

Analysis of the collected fraction can be performed if necessary to verify the success of the purification and to provide a baseline analysis of the fraction before it is moved to the next stage of the process. Mixing of the fraction in the collection tube by the autosampler prior to injection, will remove the effect of any layering that may have occurred during the collection.



TIC of the collected fraction indicating successful purification.

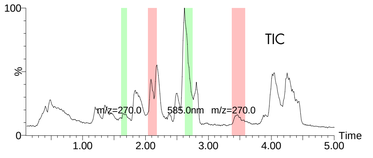
AUTOMATIC THRESHOLD DETERMINATION

In Order for the actual collection of the fraction to be successful, it is important that the correct collection threshold be set. The threshold must be high enough so it is not to be effected by changing baselines, yet it must not be too high, as that could cause some sample not to be collected. 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.
Prior knowledge of the chromatography is required to set multiple thresholds throughout the run, but this information is not always available to the person performing the purification.

The threshold value for the prep could be estimated from the results of the analytical analysis, however, requiring the analysis of the analytical sample in order to calculate the preparative threshold can be restrictive.

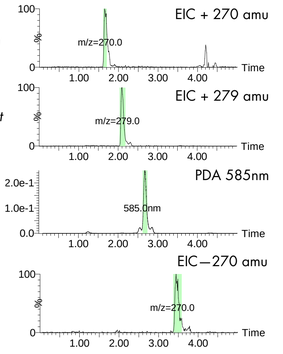
Using the AutoMIT capabilities available in FractionLynx, the software can determine the required threshold values from information generated by a preparative blank injection. The values for any of the masses, wavelengths or other detectors that might be used to trigger collection are determined. FractionLynx uses this information to set the threshold values required to trigger collection. This means that chemists can bring a sample that was screened on any analytical instrument, and still be able to utilize the automatic threshold determination functionality.

The software is able to perform the automatic determination and use of the thresholds for multiple masses, wavelengths and analog signals.



The TIC shows a complex sample, with the fractions collected marked by the colored lines. Each of the extracted chromatograms shows collection with a different trigger, with each trigger having a different threshold setting, automatically determined by the new software.

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

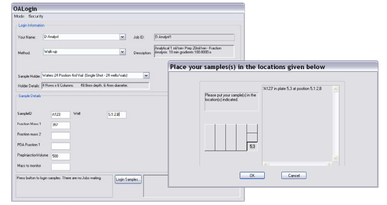


OPEN ACCESS PURIFICATION

The capabilities of the other application managers can all be utilized with the easy to use single page Open Access format.

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.

Administrator management of users, privileges, and system monitoring is available remotely through Open Access Toolkit.



Single screen Open Access login for easy, managed access to methods.

RESULTS

The automatically generated report shows the locations of the fractions, as well as chromatograms and spectra. The information in the reports can then be easily 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.

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

CONCLUSIONS

- The AutoPurify capabilities of FractionLynx allow for automation of the entire purification process from the initial QC, through purification, to the analysis of the collected fractions.
- By culling the samples that do not need to be purified, un-necessary purifications are not performed, increasing throughput.
- The software control of the preparative method determination makes it unnecessary for individual methods to be created, saving development time.
- Automatic determination of the collection threshold on a per sample basis removes the interference from drifting 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.

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