Strategic Approaches for Compound Purification and Isolation

Waters

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Overview

Over the past years the role of purification has expended rapidly in both the number and types of compounds to be isolated. The requirements placed on the purification chemist are getting stricter, requiring them to generate purified product more efficiently and with greater quality. Due to these requirements, it is necessary to select the appropriate technology to accomplish the specified goals and plan the purification experiments correctly.

This poster will examine the key decisions required to successfully meet the given purification requirements. The initial decision is selecting the correct instrumentation to form the chromatographic system. These includes the sample and solvent delivery systems, detectors, fraction collectors and columns.

The sample amount is the major driver in determining the capabilities of the appropriate components to use. It is the basis for which the column size is selected, which defines the flow rate and pressure requirements of the system. The remaining components of the system are based on the throughput and level of automation required.

The other major component of the purification process is sample and data handling. In this poster we will explore how the combination of software and hardware can improvement the functionality and efficiency of the overall process. We will also show the key aspects which can be automated and how summary data review can expedite the data management process.

Sample Analysis

Sample Amount

The quantity of crude material to be injected will be the primary consideration in identifying the appropriate column. Table 1 shows the relationship between sample load, column size and flow rate:

Mass Capacity (mg)

	Diameter (mm)											
Length (mm)	3.9	4.6	7.8	8	10	19	20	25	30	40	47	50
50	2	3	8		15	45	50		110			310
100	4	5	15	15	25	90	100	155	225	400		620
150	6	8	25		40	135	150		335			930
200				30				310		795		
250	10	13	40		60	225	250		560			1550
300	12	16	45	50	75	270	300	470	670	1195	1650	1860
Reasonable Flow Rate(ml/min)	1.0	1.4	4.0	4.2	6.6	24	27	42	60	105	145	164
Reasonable Injection Volume(µl)	15	20	60	65	100	350	390	610	880	1565	2160	2450

Many factors affect the mass capacity of preparative columns.
The listed capacities represent an "average" estimate

Table 1: Reasonable mass capacity of small molecule pharmaceutical-like compounds, dissolved in approx 30% MeOH based on column size.

Analytical Information

Initial analytical information can be used to define the purification strategy. The most question to be answered are;

Does the sample require purification?
What are the separation conditions?

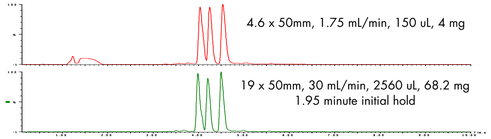
The analytical retention time indicates the % organic composition in which the target elutes from a gradient separation. The preparative gradient can then be automatically focused around this composition, increasing the chromatographic resolution and throughout. Refer to poster "Purification Workflow Management with Automated Gradient Strategies and Enhanced Fraction Analysis."

Separation Scaling

The separation achieved analytically can be maintained in the preparative run without increasing the gradient time. This is done by keeping the particle size constant and then scaling up the mass load and flow rate proportional y.

$$M_2 = M_1 * L_2/L_1 * D_2^2/D_1^2 \qquad \text{and} \qquad F_2 = F_1 x\, D_2^2/D_1^2$$

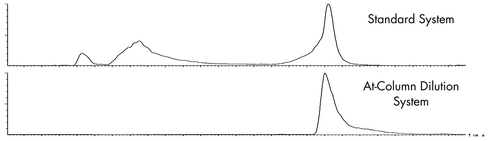
M = mass, L = length, D = column diameter, F = flow rate, and the subscripts 1 and 2 indicate the analytical and preparative scales respectively.



Chromatograms of a scaled separation from a 4.6 to a 19mm column.

At-Column Dilution *(US Patent #6,790,361)*

At-Column Dilution (ACD) is a techniques that was developed specifically for injecting relatively large volumes of relatively strong sample diluents. Such injections may distort the chromatography in a conventional system. If injection artifacts are limiting mass capacity or chromatographic resolution, the effects can be reduced by applying At-Column Dilution. In addition, ACD often increases system ruggedness and column life by preventing bulk precipitation in the sample loop or in the column itself. For more information on ACD, refer to application note 71500078010 at Waters.com.



800 mg loaded on a 19mm column for a standard system and one with At-column dilution

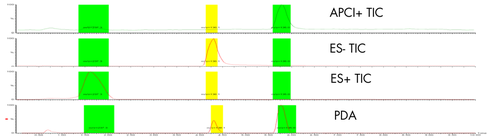
Fraction Trigger

Some detectors are less specific than others, UV and ELSD for example, are less specific than Mass Spectrometry. The need for increased specificity will help to determine the type of detectors included in a system. For example, with a non selective trigger, like UV, the collected fractions have to be analyzed to locate the target. However, with mass-directed purification only the target mass is collected, so no additional work is required.
The selectivity of the trigger will determine how many fractions for each sample will be collected and if additional analysis is required. This allows for improved throughput and saves the collector capacity for only the necessary fractions.

Detector options, with varying selectivity include UV, ELSD, CLND, MS and MS/MS directed purification. Furthermore, enhanced software functionality allows for the use of mixed trigger algorithms which can combined signals from multiple detectors.

Multi-Mode Ionization

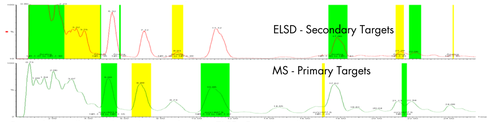
For increased MS detection capabilities, a multiple-mode ionization source can be used because not all compounds ionize under the same conditions. FractionLynx™ with the ESCi™ Multi-Mode Ionization Source allows mass-directed purification to be driven from ESI+, ESI-, APCI+ or APCI- data in a single run. The benefits of this include a reduced number of missed targets and eliminates the need to split samples and run them under separate conditions. As no source changes are required by the user, ESCi is ideal for Open Access.



Mass-directed purification with ESCi. Each peak requires a different ionization mode to be detected.

Secondary Collection

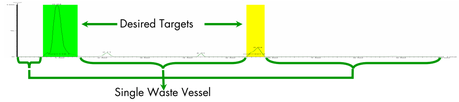
Frequently, analysts find that the compounds other than the primary compound of interest, are of importance, so it may be necessary to collect them into a separate collector. Some examples include, collection of a starting material or impurity along with the primary target. Another example is to collect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



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

Waste Collection

Due to the fact that there is no such thing as a universal detector, 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.



Waste collection with mass-directed purification.

AutoPurification System

Waters® Mass-Directed AutoPurification™ System

Level of Automation

The most important factor to consider when determining the automation required, is the number of samples that need to be purified. Systems can range from handling a single sample to hundreds of samples per day. The major components impacted are the injector and the collector. Injector styles can range from simple manual injectors to a large open bed autosamplers, with the capacity of performing hundreds of injections with no manual intervention.

The fraction collection system can also have a wide range of sample and volume capacity. The simplest of these is a single small-capacity collector. The larger collection systems can consist of a series of multiple large primary collectors or a series primary, secondary and waste collectors.

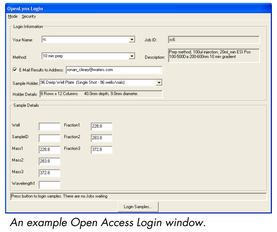
To maximize on efficiency and workspace, combinational injectors and collectors are available. These instruments can perform analytical analysis on the sample before purification, the purification itself, and the analysis of the collected fractions.

The system photograph shows a typical system with a sample manager that can perform both injection and collection.

Data Management

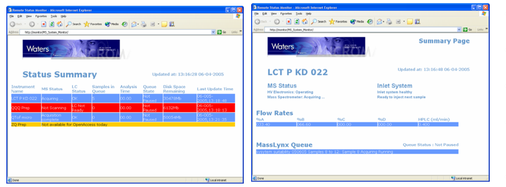
Open-Access Tools

An option to make it easier to log in samples is Open Access. This provides a simple user interface for data entry and allows the system to be used by multiple user with minimal experience. Thus, the OA systems can handling the routine analyses, freeing up experienced prep chromatographers and allowing them to handle the more challenging samples.



An example Open Access Login window.

In many instances, the Open Access systems are not located in the same lab as the users, so checking queue and instrument status requires a trip to the instrument. Now chemists can remotely determine what instruments are currently available for use, and can also monitor the status of their samples.

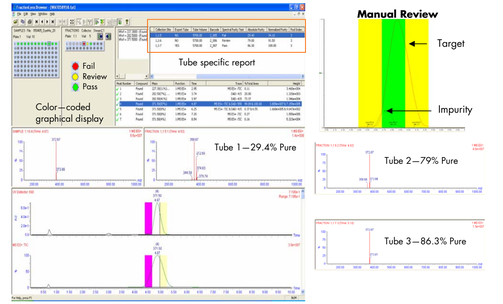


Example Remote Status Monitor windows. One shows the status of all the instruments. The other shows the system specific queue information.

Fraction Data Handling and Quality Interpretation

One of the primary efficiency burden is data processing and reviewing. The FractionLynx Browser reports displays all critical information about the samples and fraction in an interactive graphical format. This allows for rapid data review and handling. Futhermore, the AutoPurify capabilities can automatically carry the sample information from the analytical run, through the purification, to the fraction analysis and beyond, as necessary.

The FractionLynx Browser has the ability to determine the quality of each tube for each fraction. This determination is based on spectral purity. In the example shown below, the collected target, m/z = 372.5, has a closely eluting impurity coming out at the very front edge of the target. The browser displays the spectrum for each tube collected, and calculates the target's spectral purity. The user-defined purity threshold allows the Browser to display each tube as a Pass, Review, or Fail. Also, the easily reviewed summary data can can be manually modified and the new report updated, as necesary.



FractionLynx Browser window indicating the different spectral purity classifications for each tube, along with the appropriate chromatograms and spectra to support the summary results.

Conclusion

- The amount of sample is the primary consideration for the type of purification system.
- The number of samples will dictate the level of automation required.
- Analytical information can be intelligently applied to the preparative scale for increased performance for separation scaling and for using narrow preparative gradients.
- The selectively fraction trigger of the fraction trigger will determine how much if any additional work is required to locate the target.
- Various collector configurations are possible, including the secondary and waste collectors.
- Open Access provides a simple user interface for multiple non-experienced users. Additional tools allow for increased easy-of-use.
- The spectral purity assessment and thresholding, simplifying the task of isolating the quality tubes of a multiple tube collection, without extensive data mining.
- Summary data reporting with the FractionLynx Browser presents the data in simple graphical format for rapid data review and manipulation.

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