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Choosing the best Open Access LC -MS Method Based on User-Selected Conditions

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Abstract

In this application, to simplify the process of selecting an appropriate method for each chemist's

compounds, new software features for the OpenLynx Open Access Application Manager for Waters MassLynx Software has been developed.

Benefits

- Allow users to filter and select specific methods for analysis of samples in an open access laboratory environment

- Obtain high quality and comprehensive data

Introduction

In today's medicinal chemistry laboratories, chemists synthesize many different classes of compounds in support of specific discovery projects. Once synthesized, the identity and purity of these compounds needs to be determined. The diversity of these compounds requires access to many different methods for analysis.

An open access liquid chromatography (LC) system may have many methods available for walk-up use by chemists. Methods can vary in mode (analytical or preparative), column size, run time, gradient conditions, solvents, modifiers, MS ionization mode, and other variables. Indeed, the variety of options can make it difficult for a chemist to choose the best method for their compound. This can mean delays in the development of the compound.

To simplify the process of selecting an appropriate method for each chemist's compounds, new software features for the OpenLynx Open Access Application Manager for Waters MassLynx Software has been developed.

In the Waters ACQUITY UPLC System with the SQ Detector used in this application, there are 108 available methods. The methods were sorted using five categories: lipophilicity, pKa, polarity, ionization mode desired, and speed or resolution. When the user logs in samples, he or she is given a series of choices within these categories. The system administrator has defined the series of categories and the choices under each category. With five choices, the user started with 108 possible methods but was easily able to select the one method that is most appropriate for the sample.

Figure 1. The ACQUITY UPLC System with the SQ Detector.

Experimental

The system was set up with a large number of methods, due to the flexibility available with the ACQUITY UPLC System configuration that includes a Column Manager and PDA, ELS, and SQ detectors.

The Column Manager allows for the use of four columns of varying chemistries and sizes.

The SQ single quadrupole mass spectrometer provides several ionization mode options, including electrospray, APCI, and ESi, with automated switching between modes using ESCi technology.

Two solvent systems, one acidic and one basic, were made available, as were three gradients and two run times.

The combination of these parameters led to the creation of 108 possible methods.

LC conditions

LC system:	Waters ACQUITY UPLC System
Column:	(1) ACQUITY UPLC BEH C$_8$ Column 2.1 x 30 mm, 1.7 µm
	(2) ACQUITY UPLC BEH C$_{18}$ Column 2.1 x 50 mm, 1.7 µm
	(3) ACQUITY UPLC BEH C$_{18}$ Column 2.1 x 100 mm, 1.7 µm
	(4) ACQUITY UPLC BEH C$_8$ Column 2.1 x 100 mm, 1.7 µm
Column temp.:	50 °C
Sample temp.:	8 °C
Injection volume:	2 µL
Flow rate:	650 µL/min
Mobile phase A1:	0.1 % formic acid in water
Mobile phase A2:	10mM ammonium formate (pH 9)
Mobile phase B:	0.1 % formic acid in acetonitrile
Gradient (1):	5 to 95% B/1.0 min
Gradient (2):	20 to 80% B/1.0 min
Gradient (3):	0 to 50% B/1.0 min
Gradient (4):	5 to 95% B/10.0 min
Gradient (5):	20 to 80% B/10.0 min
Gradient (6):	0 to 50% B/10.0 min

Weak wash:	10:90 MeOH/H_2O
Strong wash:	20:20:30:30 ACN/H_2O/MeOH/IPA

MS conditions

MS system:	Waters ACQUITY SQD
Ionization mode (1):	ESI Positive/ESI Negative
Ionization mode (2):	APcI Positive/APcI Negative
Ionization mode (3):	ESCi Positive/ESCi Negative
Capillary voltage:	3.0 KV
Cone voltage:	20 V
Desolvation temp.:	450 °C
Desolvation gas:	800 L/Hr
Source temp.:	150 °C
Acquisition range:	100 to 1000 *m/z*
Scan speed:	10,000 amu sec^{-1}

ELS conditions

Gain:	500
N_2 gas pressure:	50 psi
Drift tube temp.:	50 psi
Sampling rate:	20 points sec^{-1}

Range:	210 to 400 nm
Sampling rate:	20 points sec^{-1}
Software:	Waters MassLynx Software 4.1 SCN 627 with OpenLynx Open Access Application Manager

Results and Discussion

108 methods?

The OpenLynx Open Access Application Manager for MassLynx Software offers the power of chromatography and mass spectrometry to chemists who are not analytical instrumentation specialists. The versatility of this software and of the ACQUITY UPLC System allows for the creation of a diverse set of methods.

The ACQUITY UPLC Binary Solvent Manager is capable of running two solvent systems. For our purposes, one of those systems was acidic (0.1% formic acid) and one was basic (10mM ammonium formate). The acidic methods were used for compounds with a low pKa, while the basic methods were used for compounds with a high pKa.

LC methods were designed for either speed (1 min) or resolution (10 min) and for optimal separation based on the polarity of the compound (generic, polar, and non-polar gradients). The generic gradient went from 5% B to 95% B and was used for separating compounds when the polarity was not known. The polar (0% B to 50% B) and non-polar (20% B to 80% B) gradients were more focused and allowed for better separation of the compound of interest.

The system's optional Column Manager allows for the use of up to four columns, running at an elevated temperature. The columns used were of two chemistries (C_8 and C_{18}) and various lengths (30 mm, 50 mm and 100 mm). The C_8 columns were used for very lipophilic compounds while the C18 columns were used for all other compounds.

The shorter columns were used for fast separations. The longer columns were used for both increased resolution (with 10 min run times) and a combination of resolution and speed (with a 1 min run time).

The ESCi source on the SQ Detector provides flexibility in choosing the ionization mode for mass detection.

The ability of the ESCi source to run in either electrospray or APCI modes, or a combination of these, permits the analysis of a wide range of compounds. The SQ Detector also allows for polarity switching in any of these modes. The high scan speed of the MS (10,000 amu/sec) facilitates the acquisition of a large number of individual acquisition modes in one run while maintaining adequate peak characterization.

By combining the choices supported by this system (two for lipophilicity, two for pKa, three for polarity, three for ionization modes, and three for gradient characteristics) and removing illogical methods (i.e., we would not run a 10-minute method on a 30 mm column), we end up with 108 possible methods.

Setting up the Method Filter

Two wizard pages, accessible from the main OALogin window (Figure 2), cover the setup of the Method Filters. The first page allows the specification of multiple categories and the creation of lists of options associated with them. The second page lists all the available method files and allows the administrator to assign one option from each category to each method file.
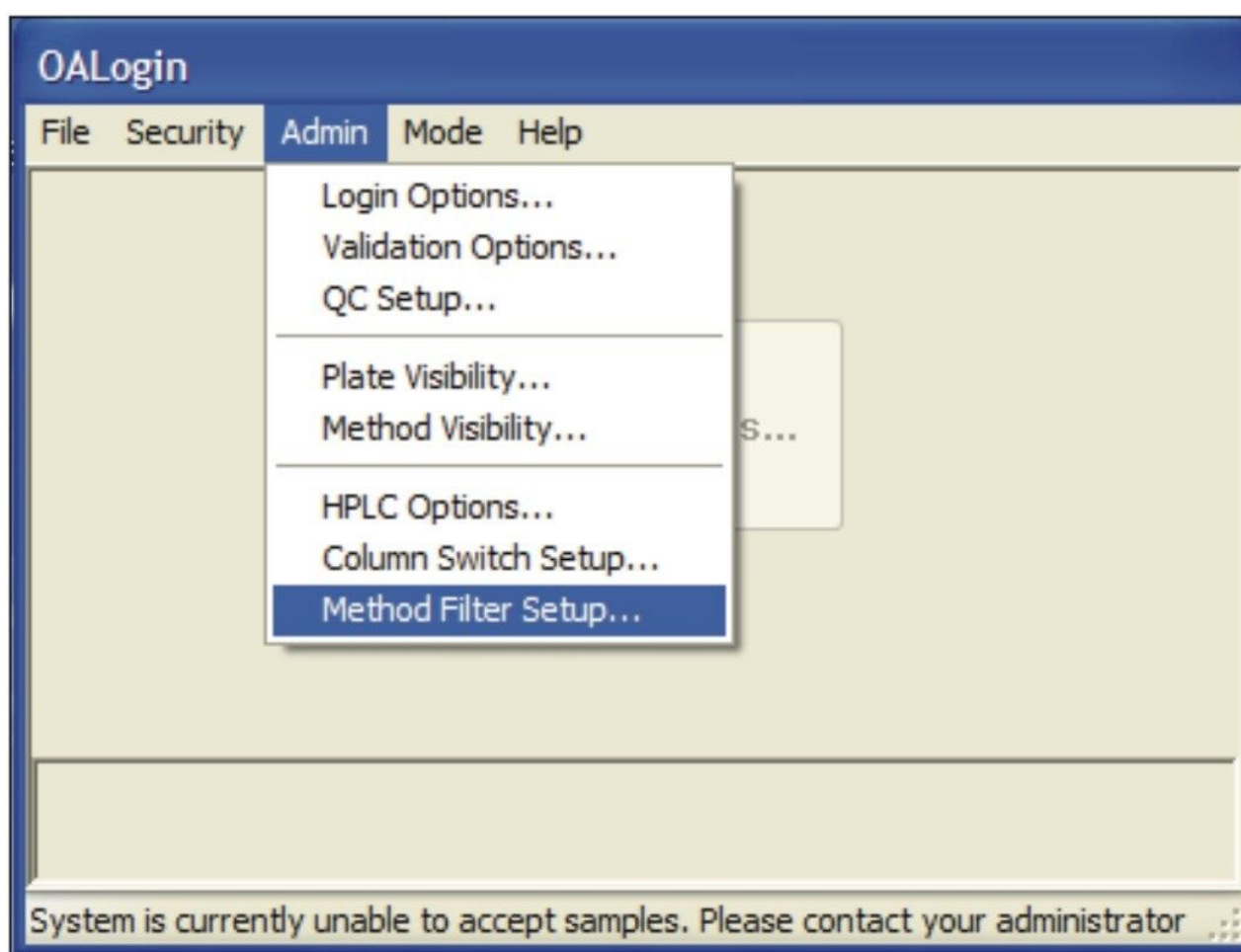
Figure 2. Accessing the Method Filter setup feature.

On the first page, the administrator specifies each category as they wish the users to see them. These could be worded as questions, as seen in Figure 3, or could simply be a list of options (such as column, run time, solvent system, etc.).

Figure 3 OpenLynx Method Filter setup wizard, first page.

Once the desired categories have been entered into the left-hand column of the method filtering setup, options can be specified in the right-hand column. The options are the choices that the user will make while logging in their samples. In the example seen in Figure 3, the options are the acceptable answers to the questions posed in the Categories column.

Once all the categories and options are specified, the methods need to be classified. This page is also where the administrator enables/ disables the use of method filtering during Open Access login.

On page 2 of the Method Filter setup wizard (Figure 4), all the available method files for the current mode of operation (OpenLynx or AutoPurify) are listed in the left-hand column and all the categories are listed as the column headers. Initially, all the category columns display "Not set." The system administrator then double-clicks each instance to change "Not set" to one of the options specified on the previous page. When all methods are classified, the administrator clicks the Finish button.

Figure 4. OpenLynx Method Filter Setup wizard, page 2

Using the Method Filter

The method filtering page allows the user to select the options specified by the administrator during the Method Filter setup, which was used to reduce the number of method files displayed. Upon logging into OALogin and selecting their user name, the user was presented with the Method Selection window (Figure 5).
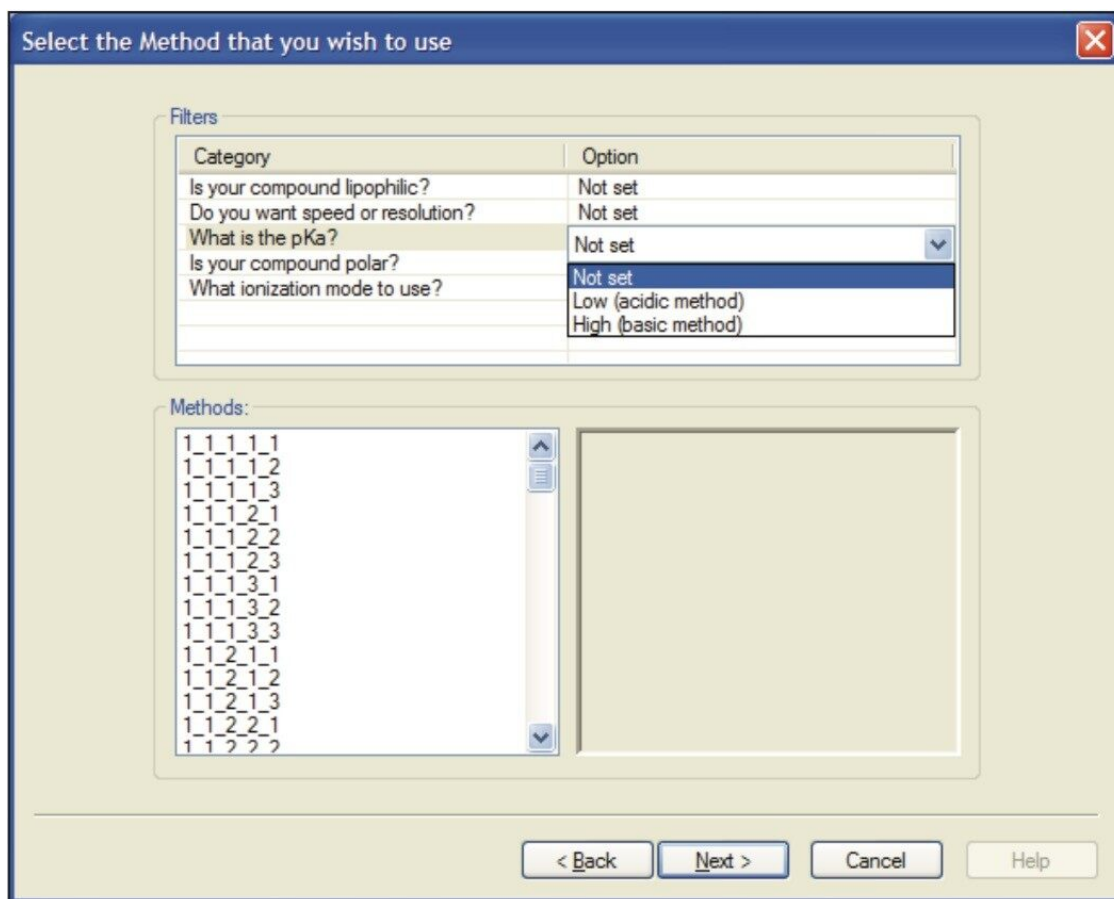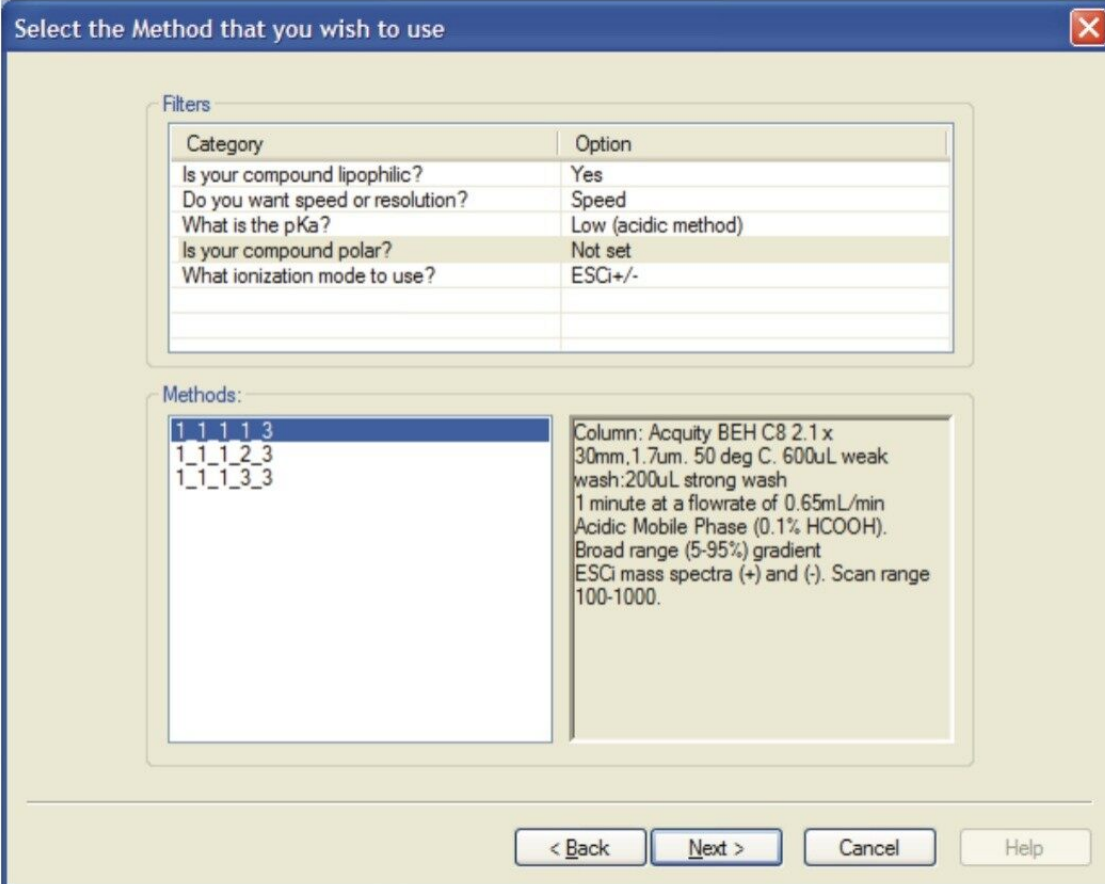
Figure 5. Method Selection window.

For each category, the user selects an option. Each one of these choices reduces the number of available methods. By selecting an option for each category, the number of methods available was reduced to one.

For the system configured for this experiment, upon logging in the sample, the first choice the chemist was given was whether their compound was lipophilic. When "Yes" was chosen, all methods within that category were shown (54 in total). The chemist then chose whether to prioritize speed or resolution (or a combination), reducing the number of methods to 18. The third and fourth choices dealt with pKa (now down to 9 methods) and the polarity of the compound (3 methods left). Finally, the user chooses what ionization mode to use, leaving one method.

If one or more of the options is not chosen (i.e., left at "Not set"), multiple methods are available. A detailed method description, written by the system administrator, then assists in the final choice of method.

Figure 6 illustrates what happens when an option is not chosen. Here, the user has not specified the polarity of the compound, so three method choices remain. By reading the method description, the user

finds that the methods they vary in their gradient characteristics and thus an appropriate method can be chosen based on that information.

Figure 6. Method Selection window showing method description.

Conclusion

New software features allow users to filter and select specific methods for analysis of samples in an open access laboratory environment. OpenLynx Open Access' new Method Filter simplifies the steps that chemists take to apply the most appropriate LC-MS method, and allows the system administrator to create many tailored methods, instead of a few generic ones.

The Method Filter allows users to choose an LC-MS method based on the properties of their compound. In the experiment used to illustrate this capability, by answering a few simple questions, a list of 108 possible methods was reduced to just one.

In this example, the method filter choices made available were lipophilicity, pKa, polarity, ionization mode, and speed or resolution of the separation. These choices were designed by the system administrator. Other options can be used, especially if more methods are desired.

By using methods tailored to their compounds, chemists are able to obtain high quality and comprehensive data about their compounds by selecting the most suitable method, thus reducing the need for reanalysis. This flexibility in compound screening speeds up the discovery process.

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SQ Detector 2 <https://www.waters.com/134631584>

MassLynx MS Software <https://www.waters.com/513662>

OpenLynx Open Access <https://www.waters.com/10008851>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

ACQUITY UPLC ELS Detector <https://www.waters.com/514219>

720002799, September 2008