

SYSTEM MANAGEMENT TOOLS FOR A HIGH THROUGHPUT OPEN ACCESS UPLC/MS SYSTEM USED DURING THE ANALYSIS OF THOUSANDS OF SAMPLES

Ronan Cleary, Darcy Shave, Warren Potts, Michael D. Jones, Paul Lefebvre
Waters Corporation

OVERVIEW

Many compound libraries contain compounds which were synthesized several years prior or obtained from outside resources. It is important that the expected composition of each compound be confirmed. LC/MS has become the standard technique for confirming the purity and identification of a compound which has demonstrated activity in a biological screen. If the library store is not routinely checked, false positives in an activity screen are highly possible. This will lead to wasted time, effort and money on compounds which should not be advanced in the discovery process. Because these libraries may contain thousands, if not millions, of compounds, an open access Ultra Performance LC™ (UPLC™)/MS system was investigated for this high throughput library QC.

Enhancements to HPLC and LC/MS technologies have provided useful tools to improve the throughput and accuracy of these assays. Throughput can be increased with the use of UPLC/MS, which makes use of very small column particles ($<2\text{ }\mu\text{m}$) and high operating pressure ($>10,000\text{ psi}$). This can result in an up to 10 fold increase in throughput along with a 3 fold increase in sensitivity.

Due to the large amount of samples analyzed and data generated during this testing, a new software package was created which facilitated the administration of this open access system. It created new project directories for the open access users and moved the resulting project data (such as raw data files) across the network as it was created. Data processing could then be done on a separate dedicated computer. The software also monitored the instrument PC, providing on-the-fly information about its status and the status of its sample queue from a centralized location.

LC/MS CONDITIONS

All experiments were conducted using the Waters® Micromass® ZQ™ 2000 mass spectrometer, equipped with an ACQUITY UPLC™ system with Sample Organizer, Photo Diode Array detector, cooled autosampler and column heater. The ZQ-2000 was equipped with an ESCi® source, running in the ES+ ion mode. The instrumentation was controlled by Masslynx 4.1 with Openlynx and Openlynx Open Access application managers.

Eight micro-titre plates, each containing 96 pharmaceutical samples, were logged onto the system using Openlynx Open Access. The first and last samples in each plate were used for QC.

LC Conditions

Instrument: Waters ACQUITY UPLC System
Column: Waters ACQUITY C₁₈ BEH Column
1.7 um, 2.1 x 50 mm column, 30°C
Sample Temp : 15°C
Injection Volume: 5 uL
Mobile Phase: A. 0.1% Formic acid in AcN/Water 10/90
B. 0.1% Formic acid in AcN/Water 90/10
Gradient: Time A% B% Curve Flow
0.00 95% 5% 6 0.80 mL/min
1.00 5% 95% 6 0.80 mL/min
1.10 95% 5% 6 0.80 mL/min
Detector settings:
Wavelength Range: 210 to 400 nm
Resolution (nm): 1.2
Sampling Rate (spectra/s): 20

MS Conditions

Instrument: Waters Micromass® ZQ Mass Spectrometer

Tune Page Parameters:

ESI Capillary Voltage:	3.2 kV	Polarity:	ES Positive
Source Temp.:	120°C	Inter-scan Delay:	50 ms
Desolvation Temp.:	400°C	Dwell:	100ms
Desolvation Gas Flow:	800 L/Hr	Mass Range:	100 to 500 amu
Cone Gas Flow:	50 L/Hr		

RESULTS

By using an ACQUITY UPLC system with the optional Sample Organizer, we were able to analyze 3840 samples in under 7 working days on a single column. On a traditional HPLC system, this would take approximately 27 working days, assuming a 10 minute run time.

The open access interface allowed the users to log in the samples while providing a minimal amount of information. A series of methods, each including gradient conditions, MS conditions and processing parameters, was designed by the system administrator. The users simply chose a method from this list, imported their sample lists and placed their microtitre plates in the indicated positions.

The samples were then analyzed and the data was processed. Once processing was finished, the data was copied to a file storage PC. From here the users could do further processing, if desired. As well, a report file was generated from the processed file and converted to the XML format. This facilitated storage of the results in a database.

Part of the processing method included a quality control check. The first and last samples in the plates were QC samples. All QC samples were within the specified limits for retention time and UV peak area. Retention time varied by less than ± 0.2 minutes and peak area varied by less than 5%. If a QC sample had failed, the sample queue would have been paused.

DISCUSSION

Instrumentation

Throughput was increased with the use of Ultra Performance Liquid Chromatography™ (UPLC™). This technique made use of 1.7 μm column particles and high operating pressure (12,000 psi). These properties resulted in a 5 fold increase in throughput. Sensitivity was not investigated.

Due to the large number of samples being run, an ACQUITY UPLC Sample Organizer was also used. This thermally conditioned sample storage compartment extended the capacity of the system by adding space for 7 deep well microtitre plates (or 21 shallow well plates). Total sample capacity was increased from 192 samples (two plates) to 768 samples (eight plates) when using 96 well plates. If using 384 well plates, maximum capacity would be 8064 samples.

An added advantage of the Sample Organizer in an open access environment is the ability to add samples to the system without pausing the sample queue. When the door to the Sample Manager is opened, any movement, whether of the sample plate or of the needle, are paused for safety considerations. This pause does not occur when loading the Sample Organizer.

Software

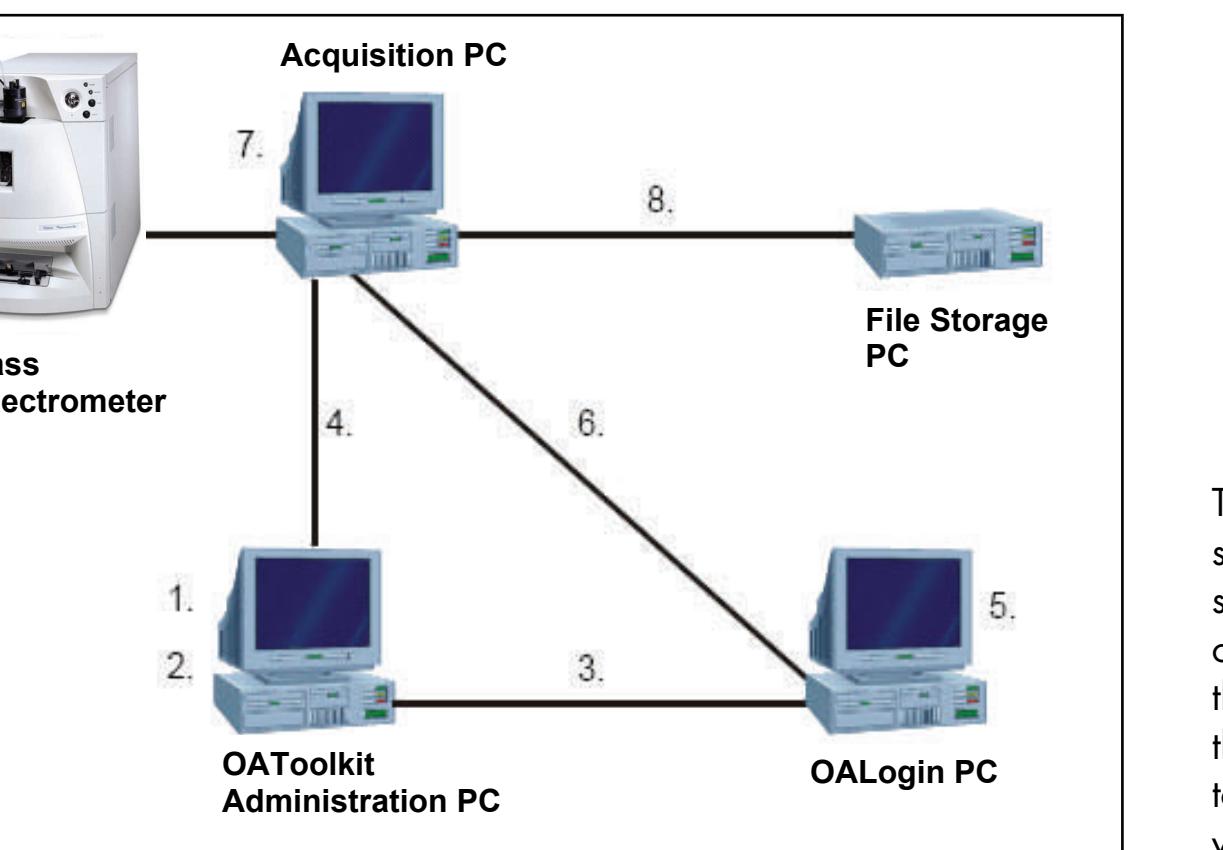
Administrator Tools

The open access software allowed chemists to 'walk-up' to a terminal and log in samples onto an instrument, whilst inputting the minimum of information needed for the sample run. It also allowed the system administrator to maintain control over the open access systems and to track the performance of each system. It facilitated the batch processing and reporting of results.

The administrator selected the fields that appeared when remote users logged in samples. The administrator designated fields as mandatory so that login would not proceed unless the remote users entered values for these fields. They also defined upper and lower limits for the values of numeric fields. In addition, the administrator defined the format for text that remote users entered in the text fields.

A toolkit service ran on the Acquisition PC and copied open access users' batch files and raw data to remote locations once their samples were run. The information about these users, and the locations to which their data was to be sent, is contained within the administration tool. This information is uploaded to the service on the Acquisition PC.

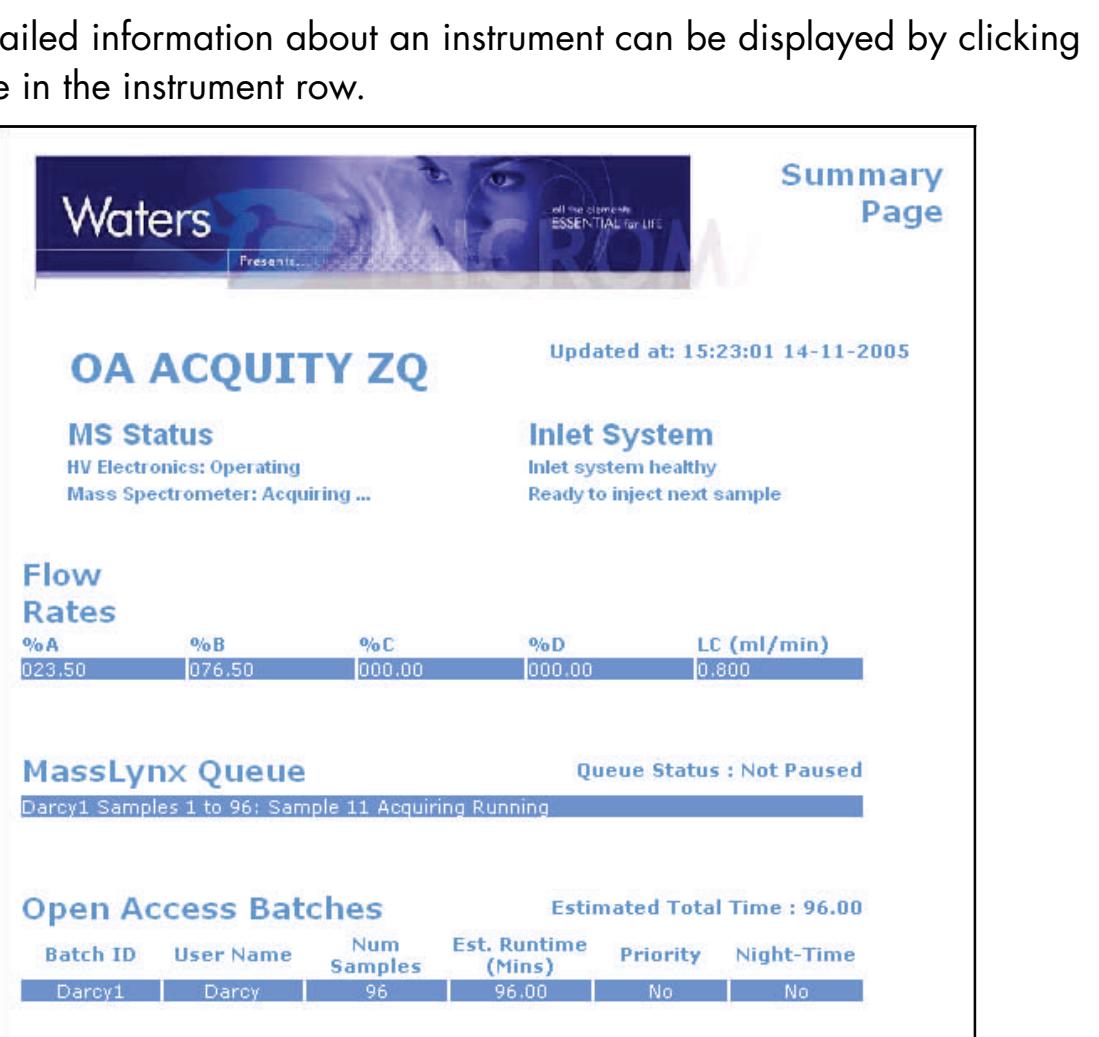
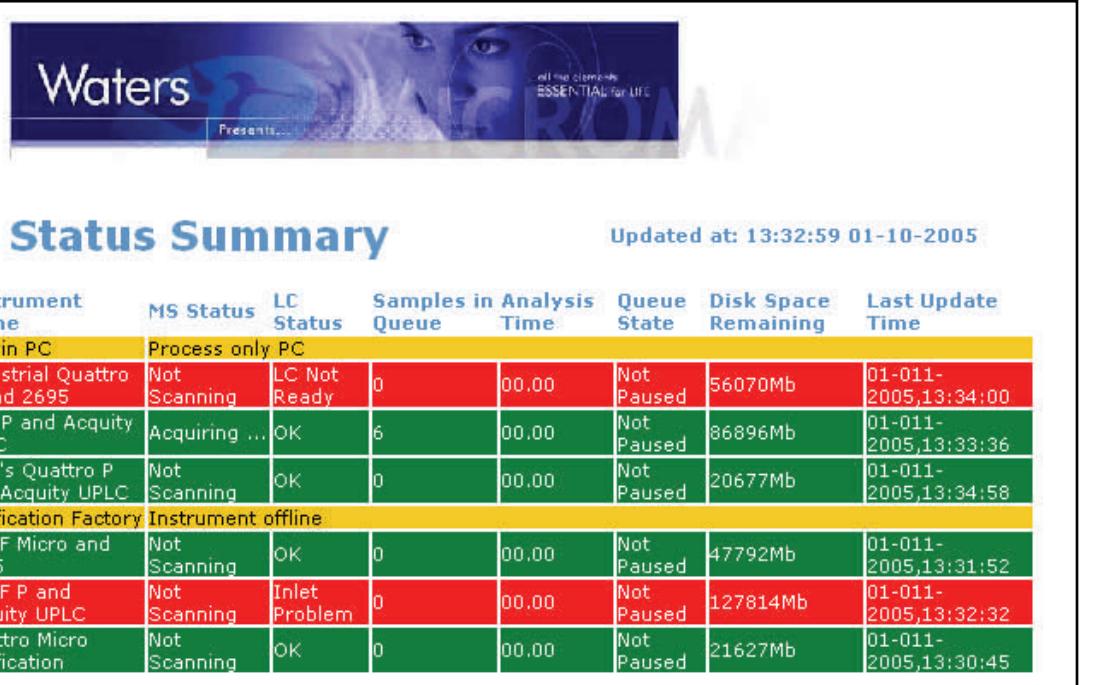
The next illustration and procedure describes the order of events during typical operation.



1. The administrator uses the Administration Tool to create an user.
2. The administrator uses the Administration Tool to add extra information about the OALogin user. For example, that the RAW data of any of the user's samples should be moved to the File Storage PC whenever a user's sample is processed.
3. The administrator uploads the user information to the OALogin PC. This adds the user's name to the drop-down list in the login screen on the OALogin PC.
4. The administrator uploads the user information to the OAToolkit service on the Acquisition PC. The service now contains the instructions of how to proceed if the OALogin user logs in a batch.

System Monitoring

On the Administration PC, the Remote Status Monitor (RSM) monitored the status of the open access Acquisition PC, along with other Acquisition PCs on the network and wrote that monitoring information to an XML file. The information could then be read and interrogated remotely in a browser.



CONCLUSIONS

Waters Open Access Systems give chemists the ability to analyze their own samples close to the point of production by simply walking up to the LC/MS system, logging their samples, placing their samples in the system as instructed, and walking away. As soon as the analysis is completed, sample results are emailed or printed as desired. System configuration and setup is enabled through a System Administrator who determines login access, method selection, and report generation.

Openlynx Toolkit enables administrators to manage Open Access users from a central point, assign detailed configuration information and attributes for these users and then export these details to multiple OALogin PCs and Acquisition PCs. Openlynx Toolkit also enables administrators and users to remotely monitor the status of Acquisition PCs.