NEW TOOLS FOR IMPROVING DATA QUALITY AND ANALYSIS TIME FOR CHEMICAL LIBRARY INTEGRITY ASSESSMENT

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Marian Twohig, Paul Lefebvre, Darcy Shave, Warren B. Potts III, and Rob Plumb
Waters Corporation, Milford, MA 01757

INTRODUCTION

The identity and purity of a candidate pharmaceutical is critical to the effectiveness of the drug screening process. LC/MS is employed extensively in drug discovery in order to exclude false positives and maintain the high quality of the product. This process can be very time consuming and can potentially delay the progression of a drug through the discovery process. Thus sample throughput is a critical issue in moving compounds from the hit to lead status. Ultra Performance LC® leverages sub 2 µm LC particle technology to generate high efficiency faster separations.
When a PDA/ELSD/MS detection scheme is used in conjunction with multiple-mode ionization the potential for peak detection is greatly improved. Pharmaceutical chemical libraries often contain a great diversity of small molecules to cover a broad range of biological targets[1]. In this environment the ability to obtain information pertaining to multiple Mass Spectrometer acquisition modes in addition to PDA and ELSD in a single injection is invaluable.

- Open access software offers the power of chromatography and mass spectrometry to chemists who are not analytical instrumentation specialists.
- It allows them to quickly and easily know what they've made and allows the experts to work on the difficult analytical problems.
- An open access Ultra Performance LC® (UPLC®)/MS system was investigated for high throughput library QC.

Figure 1. The Waters ACQUITY UPLC System with the SQD Mass Spectrometer.

METHODS

Experimental

LC conditions

LC System:	Waters® ACQUITY UPLC® System
Column:	ACQUITY® UPLC BEH C_8 Column 2.1 x 30 mm, 1.7 µm
Column Temp:	50 °C
Sample Temp:	8 °C
Injection Volume:	2 µL
Flow Rate:	800 µL/min.
Mobile Phase A:	0.1 % Formic Acid in Water
Mobile Phase B:	0.1 % Formic Acid in Acetonitrile
Gradient:	5-95% B/0.70 min.

MS conditions

MS System:	Waters SQD™ Mass Spectrometer
Ionization Mode:	ESI Positive/ESI Negative, Multi-mode Ionization.
Capillary Voltage:	3.0 kV
Corona Current	8.0 µA
Cone Voltage:	20 V
Desolvation Temp:	450 °C
Desolvation Gas:	800 L /Hr
Source Temp:	150 °C
Acquisition Range:	100—1300 m/z
Scan Speed:	2,500, 5,000 and 10,000 amu sec^{-1}

Note: A low volume micro-tee was used to split the flow to the ELSD and SQD.

ELSD conditions

Gain:	500
N_2 Gas Pressure:	50 psi
Drift Tube Temp:	50 °C
Sampling Rate:	20 points sec^{-1}

PDA conditions

Range:	210-400 nm
Sampling Rate:	20 points sec^{-1}

RESULTS AND DISCUSSION

Maximum efficiency is essential for labs challenged by throughput requirements and the management of data from multiple systems and users. Waters Open Access suite of software streamlines the integration of analysis with data acquisition, processing and reporting.

Sample Login

- **Simple Login Procedure:** The MassLynx™ OpenLynx™ Open Access application Manager is designed to allow chemists to walk up to a terminal and log in samples while entering the minimum information required to run the samples (Figure 2).
- **Method Selection:** A series of methods each including gradient and MS conditions and processing parameters, are initially set up by the system administrator. The users choose an appropriate method from the list, and place their samples in the position designated by the software. Desired sample analysis follows.

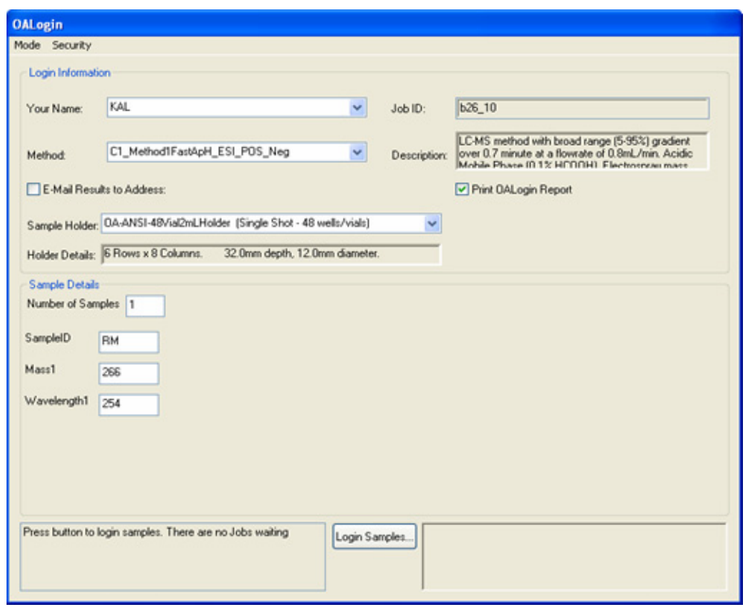

Figure 2. OpenLynx Single Page Login

Open Access System

Chromatographic separations were carried out using a UPLC system coupled to an ACQUITY SQD single quadrupole mass spectrometer, with PDA and ELSD signals collected simultaneously. For additional system flexibility the UPLC system was configured with a Sample Organizer and a Column Manager.

- **Sample Capacity:** The sample capacity of the system totals twenty two 384 well plates, 8448 library samples in total. This extends the overall walk-away time for the system.
- **Column Flexibility:** The column manager allows four UPLC columns to be installed, heated and switched into line based on the method requirements.

Sample Analysis

- **Increased Sample throughput:** Samples were analyzed using gradients less than 1 minute in length with a flow rate of 800 µL min^{-1}. The total cycle time of the method was 1 minute 20 seconds facilitating increased sample throughput.
- **Increased Chromatographic Peak Definition:** When analyzing the narrow peaks generated by the UPLC/MS system, the data collection rate can compromise the number of points across the LC peak, resulting in a poor definition of the eluting peak (Figure 3).

Data Quality

As illustrated by Figure 3, the result of operating at lower data collection rates can compromise the chromatographic resolution. Operating at 10,000 amu sec^{-1} allows greater chromatographic definition and facilitates the acquisition of a large number of individual acquisition modes in one run while maintaining adequate peak characterization.

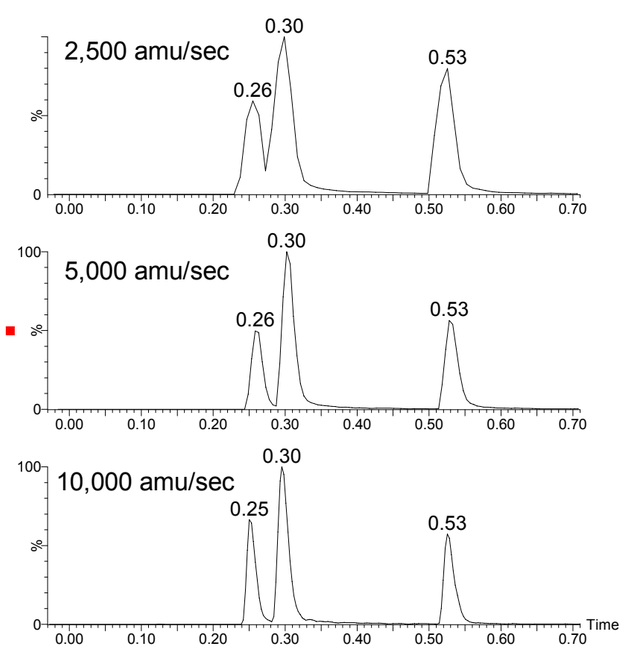

Figure 3. Chromatograms shown at 2,500, 5,000, 10,000 amu sec^{-1} .

- **Spectral Data Quality:** The spectral data quality of scanning experiments carried out from 2,500-10,000 amu sec^{-1} were found to be comparable, thus providing confidence that operating at these rapid data collection rates does not compromise the spectral data quality.
- **Isotope Ratios:** Comparison of an acquired spectrum with a software generated isotopic model showed isotope ratios of data collected at 10,000 amu sec^{-1} were within 1% of the isotopic model, again ensuring data fidelity is not compromised.

UPLC/PDA/ELSD/MS

In addition to obtaining mass confirmation by multiple MS modes, it is possible to add PDA and ELSD detectors to obtain auxiliary information.
A single run can then provide UV spectral information and an estimation of compound purity at low wavelengths. Chromatograms illustrating the use of "triple detection" (PDA/ELSD/MS) are shown in Figure 4.

- The signal from an ELSD can give a tentative estimation of the relative quantities of the components present. It has been known to give rise to similar responses for related compounds[2].
- The chromatographic peak widths of the MS and ELSD increased by 25-30% when compared with the PDA trace. This can be attributed to the use of a low volume micro-tee after the PDA.

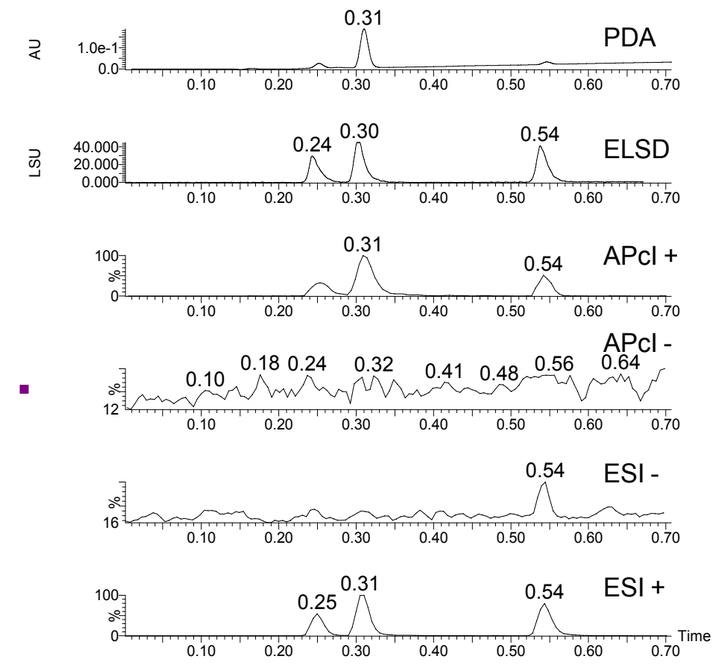

Figure 4. UPLC/PDA/ELSD/MS with Multi-Mode Ionization

Data Processing

As soon as the analysis is complete, data is automatically processed and a sample report is generated. OpenLynx can report results using printed reports or through the OpenLynx browser. The browser presents a summary of the results as a color coded (found/not found/tentative) map for clear interpretation of the results. Chromatograms, spectra, sample purity, peak height, peak area, retention time and other information can easily be viewed by the browser. The OpenLynx browser, shown in Figure 5.
displays the results for the entire 384 well plate. The report can automatically be emailed, converted to pdf or printed as desired[3].

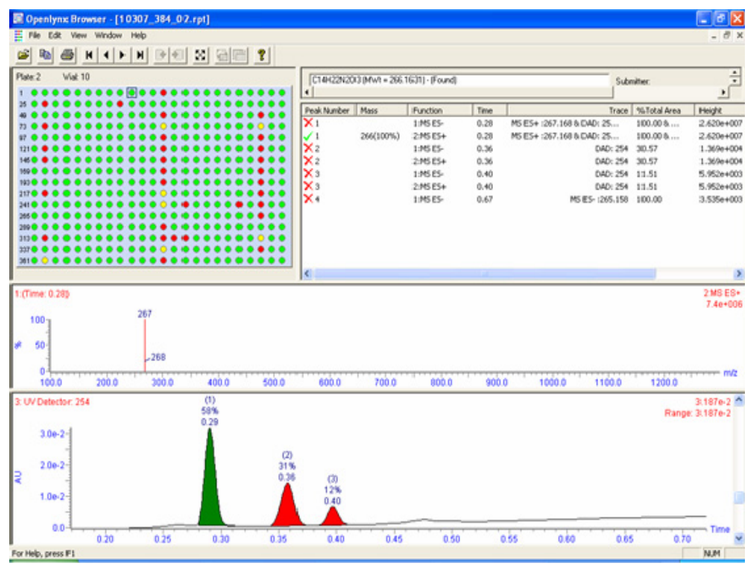

Figure 5. OpenLynx Browser

CONCLUSION

- **Faster Data Turnaround:** The described system and software combination can autonomously evaluate large numbers of samples with a cycle-time of 1 min 20 seconds. Data can then be automatically processed and a summary report can be generated.
- **Elevated Scan Speeds:** The scan speed capabilities of Waters ACQUITY SQD make it possible to better characterize narrow chromatographic peaks.
- **Automated Analysis and Data Handling:** The use of the fast scanning MS along with the throughput of UPLC technology allows the chemist to obtain high quality comprehensive data about their compounds in the shortest possible time. This combined with intelligent open access software allows informed decisions to be made faster, thus supporting the needs of the modern drug discovery process.

REFERENCES

1. LC/MS Applications In Drug Development, *Wiley-Interscience series on Mass Spectrometry.*, **2002** *Mike S. Lee.*, Chapter 6. p96-106.
2. *Kibbey, C.E.* Mol. Diversity, **1995**, I *247-258.*
3. Technical Note; Author: Darcy Shave; Source: **2006**; Library Number.: 720001594EN

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

720002266EN