OPEN-ACCESS UPLC/MASS SPECTROMETRY SOLUTIONS FOR ANALYTICAL SUPPORT LABORATORIES

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Marian Twohig, Paul Lefebvre, Darcy Shave, and Rob Plumb
Waters Corporation, Milford, MA 01757

INTRODUCTION

Open-access mass spectrometry has been successfully implemented by analytical chemistry support laboratories as an effective way of rapidly characterizing synthetic compounds and answering complex structural questions. These questions include issues such as correct formula (nominal mass), reaction completion (OA LC/MS), purity (UV and ELSD), and structural elucidation (HR-MS in conjunction with NMR).

Single quadrupole mass spectrometers are used routinely to answer less complex questions relating to nominal mass confirmation. Occasionally nominal mass is not sufficient to provide answers relating to less well characterized reactions. High resolution mass spectrometers can identify unexpected byproducts and complete unknowns. An elemental formula can be calculated using the measured mass with sufficient accuracy and precision. The normal definition of consistency is that the mass accuracy must be within 5 ppm[1-3].

Reaction monitoring studies take place once a chemical hit is found through a library screening process. Once the hit is verified optimization of the compounds' desired properties takes place. This step involves an iterative process of synthesis and reactivity measurement of the new compounds to further develop drug candidates into the lead phase. High-throughput approaches can provide important time savings in the optimization of process parameters. Open-access LC/MS is replacing TLC as a reaction monitoring tool[4].

Self service Ultra Performance LC™ (UPLC™)/MS systems were investigated for high throughput analysis of Nominal and Accurate Mass samples.

Figure 1. The Waters ACQUITY UPLC System with the SQD Mass Spectrometer (top) and LCT Premier.

METHODS

Chromatographic separations were carried out using a UPLC system coupled to an ACQUITY SQ, single quadrupole mass spectrometer or LCT Premier, Time of Flight mass spectrometer. PDA and ELSD signals were collected simultaneously. Samples were analyzed using gradients less than 1 minute.
Note: A low volume micro-tee was used to split the flow to the ELSD and SQD.

LC conditions

LC System:	Waters® ACQUITY UPLC® System
Column/s:	ACQUITY UPLC BEH C_8 Column 2.1 x 30 mm, 1.7 µm ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm, 1.7 µm
Column Temp:	45˚C
Flow Rate:	650-800µL/min.
Mobile Phase A:	0.1% Formic Acid in Water
Mobile Phase B:	0.1% Formic Acid in Acetonitrile
Gradient 1:	5-95% B/0.7 min @ 800µL/min.
Gradient 2:	5-95% B/1.0 min @ 650µL/min

Accurate Mass MS conditions

MS System:	Waters Micromass LCT Premier™ Mass Spectrometer
Ionization Mode:	ESI Positive/ESI Negative
Capillary Voltage:	3.0 KV
Cone Voltage:	25 V
Source Temp:	130 ˚C
Desolvation Temp:	450 ˚C
Desolvation Gas:	800 L/Hr
Cone Gas:	50 L/Hr
Acquisition Range:	100—1000 m/z
Acquisition Rate:	8 spectra sec^{-1}

Nominal Mass MS conditions

MS System:	Waters SQD™ Mass Spectrometer
Ionization Mode:	ESI Positive/ESI Negative
Capillary Voltage:	3.0 kV
Cone Voltage:	20 V
Source Temp:	150 ˚C
Desolvation Temp:	450 ˚C
Desolvation Gas:	800 L/Hr
Cone Gas:	50 L/Hr
Acquisition Range:	100—1000 m/z
Scan Speed:	10,000 amu sec^{-1}

PDA Conditions

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

ELSD Conditions

Gain: 500

N_2 Gas Pressure:	50 psi
Drift Tube Temp:	50 ^{0}C
Sampling Rate:	20 points sec^{-1}

RESULTS AND DISCUSSION

Open Access UPLC/PDA/ELSD/MS for Reaction Monitoring/Characterization

- By using a walkup UPLC/MS system, chemists were able to quickly and easily monitor their reactions, noting the relative amounts of starting materials, intermediates and products.
- 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. It runs as a complete system from sample introduction to end results. OpenLynx single page login is shown in Figure 2

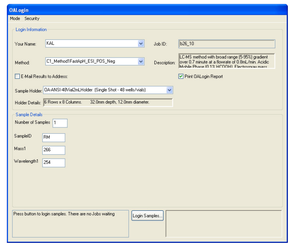

Figure 2. OpenLynx Single Page Login

Model Reaction: The Synthesis of Atenolol

To illustrate the functionality of such a system, the synthesis of Atenolol[5] was used as a reaction model.

Reaction Characterization

Low-resolution quadrupole LC/MS offers powerful reaction characterization capabilities, however problems can be encountered when isobaric substances need to be determined from complex reaction mixtures.
Tandem quadrupole LC/MS/MS offers the power of collision induced dissociation (CID) enabling the acquisition of information rich MS/MS spectra for the purpose of identification. Single or tandem quadrupole LC/MS offer useful molecular weight confirmation but do not provide any information about molecular formula.
The use of high resolution LC/MS allow the unequivocal determination of an elemental formula from an accurate mass measurement. The measurement can be made with a sufficient accuracy and precision. This results in the ability to discriminate between isobaric substances and increase confidence in the identification of unknown compounds. In addition to elemental composition using a quadrupule time of flight (Q-TOF) MS system allows accurate mass measurement of MS/MS spectra. This can greatly help with the identification of unknown reaction byproducts.

- A Waters Micromass LCT Premier TOF MS was combined with the enhanced speed of UPLC technology. MassLynx OpenLynx Open Access application Manager was used to automate sample login, data analysis, data processing and reporting.
- Figure 3 shows OL browser from the analysis of the Atenolol reaction mixture. PDA (top) and MS chromatograms are shown. Atenolol (Rt=0.41 min, 0.7 PPM, $C_{14}H_{22}N_2O_3$). The reaction intermediate 4-Hydroxyphenylacetamide (Rt= 0.50 min, -0.7 PPM, $C_8H_9NO_2$) and reaction byproduct, 4-Hydroxyphenylacetic acid (Rt=0.56 min, -2.0 PPM, $C_8H_8O_3$). The peak visible in the PDA chromatogram at Rt= 0.73 min did not ionize in positive or negative Ion ESI.
- OL Software uses the raw data to calculate the exact mass, the mass measurement error and the iFit (isotope fit) value. The software automatically corrects for detector saturation that can be encountered near the apex of high concentration peaks, thereby assuring that accurate mass measurement is possible even on very intense peaks.

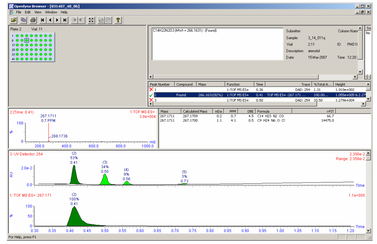

Figure 3. OpenLynx Browser Report

Reaction Monitoring

- A new single quadrupole mass spectrometer was used to perform reaction monitoring and optimization using the synthesis of atenolol as a reaction model.
- As shown in Figure 4. operating at lower data collection rates can compromise the chromatographic resolution.
- The ACQUITY SQD single quadrupole is capable of scan speeds up to 10,000 amu sec^{-1} allowing a large number of scan functions in a single run ensuring adequate peak characterization. In situations where nominal mass is sufficient, this allows confirmation of compound synthesis to be obtained on reaction components whether they ionize in Positive Ion Mode or Negative Ion Mode, ESI or APcI.
- By configuring auxiliary detectors like PDA and ELSD. A single run can also provide UV spectral information and an estimation of compound purity. ELS detection can give a tentative estimation on the relative quantities of the components present. It is also an alternative detector to UV which depends on the presence of a chromaphore. . Chromatograms illustrating the use of "triple detection" (PDA/ELSD/MS) are shown in Figure 5.

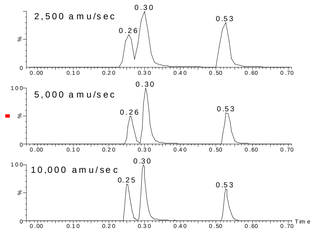

Figure 4. MS Chromatograms shown at Various Scan Speeds, 2,500, 5,000, 10,000 amu sec^{-1}

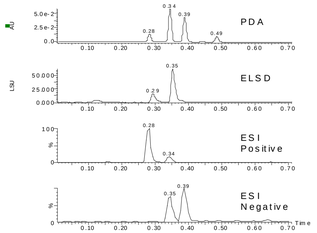

Figure 5. UPLC/PDA/ELSD/MS with Positive Negative Switching

The increase in the formation of Atenolol was monitored, as was the decrease in the intermediate 4-Hydroxyphenylacetamide (Figure 6).

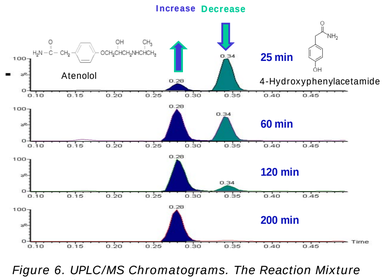

Figure 6. UPLC/MS Chromatograms. The Reaction Mixture was sampled at various time points.

CONCLUSION

- **With Low-resolution LC/MS ambiguity can arise when isobaric compounds are to be characterized. This can occur during the initial stages of reaction optimisation when the structural substitutions may lead to reaction end products with the same nominal mass. In this instance the use of high resolution MS technology is highly desirable. Allowing isobaric substances to be distinguished and to increase confidence in the identification of the analytes of interest using elemental composition and isotope characterization (iFit).**
- **By using a walkup UPLC/MS system, chemists were able to quickly and easily monitor their reactions, noting the relative amounts of starting materials and products. They were also able to note the formation of any side products and make necessary alterations to their reaction protocol to minimize these.**
- **Using the Acquity SQD configured in a walkup fashion The system can evaluate large numbers of samples with a cycle-time of 1min 20 seconds. Data can then be automatically processed and a summary report can be generated.**
- **The scan speed capabilities of Waters ACQUITY SQD make it possible to better characterize narrow chromatographic peaks. This has become a necessity since the advent of sub 2 µm particle technology where chromatographic peaks can be 1 second wide or less .**
- **Configuring multiple detectors (PDA, ELSD, MS) has the advantage of measurement of multiple parameters simultaneously.**

References

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