OVERVIEW
The aim of the study was to show a fully automated open access software package from the initial set up of the optimisation parameters to the final calibration lines and QC data.

INTRODUCTION
There is a constant need for the rapid optimisation and acquisition of many samples in the pharmaceutical discovery environment. LC-MS and LC-MS/MS has become the method of choice due to increased selectivity and sensitivity. The high number of samples and the speed at which they need to be analysed has become ever more important. There is a need to keep the mass spectrometer working at all times and to allow users privileges to be defined. Here we show an OpenAccess system on a triple quadrupole mass spectrometer.

Analyses in the ADME environment with triple quadrupoles require multiple reaction monitoring (MRM) mode, which requires method development. In this study, we describe the use of MS and MS/MS within an OpenAccess environment were the method development and acquisition of calibration lines and QC’s are automated.

METHODS
Sample preparation
A mixture of four sulphonamides was prepared at a concentration of 1ng/ul (sulfaguanidine, sulfadimethoxine, sulfamethizole and sulfamethoxazole). OpenAccess used these for automated acquisition method optimisation.

A calibration line was prepared over the range 1pg/ul to 1000pg/ul

MS Conditions
Instrument: Quattro micro
Mode: Electrospray positive
Capillary Voltage: 3.0kV
Source Temp: 150 °C
Desolvation Temp: 350 °C
Collision Gas: Argon at 4.6e⁻³ mbar

LC Conditions
Column: Waters Symmetry C18 (2.1mmx100mm, 3.5um)
Flow Rate: 200ul/min
Mobile Phase: Acetonitrile/Water, 60/40,v/v

OPEN ACCESS SET-UP PROCEDURE
(1) QuanOptimise Parameters
The starting point for the OpenAccess-QuanOptimise and method development is to establish set methodology for the functions that are to be incorporated in the analysis. The first of these is the QuanOptimise method editor (Figure 1). Here the parameters are set for automated MS followed by MS/MS to establish a transition required for MRM analysis. The software then automatically defines the acquisition parameters and runs the pre-defined calibration series using the information from QuanOptimise.

Figure 1. QuanOptimise method editor
In the first injection of the tuning solution, an MS spectrum was acquired by using the molecular weight of the compound, over the range 10 to 40V for the cone voltage, with an increment of 5 volt for each acquisition. The automatically determined optimum cone voltage information is used to set the first quadrupole to the [M+H]+ ion.

The tune solution is injected again with the collision gas switched on and the collision energy ramped over the range 10 to 30eV. The product ion spectra that are then extracted out of the chromatogram are compared and the most intense product ion is chosen for the acquisition.

For this to occur, generic tune files are selected for MS and MS/MS as well as defined inlet methods for the optimisation stage and the final analytical stage where the calibration curves are acquired.

Adducts are defined from the method editor which can either follow the pseudo molecular ion or any other adducts such as the sodiated or ammoniated species.

Losses such as water and carbon dioxide can be excluded from the product ion spectra as they are non-specific.

(2) Configuring OpenLynx for OpenAccess Quantify

OpenLynx is now configured to allow the incorporation of QuanOptimise and Quantify for full method development and analysis. To convert OpenLynx into a suitable format for quantification the following dialogue box is defined.
The OpenAccess manager is displayed to give an update of all the relevant Jobs that are queued and ready to go (Figure 5). Importantly, here the bed layout is highlighted and confirmed as being the same as defined in the inlet method.

Figure 5. OpenLynx Manager

(3) Starting OpenAccess
When the parameters have been configured for OpenAccess the first stage is the logging in of the samples that are ready to run. The user is taken through a series of well defined intuitive dialogue screens. The first of which is the Login itself (Figure 6).

Figure 6. OpenLynx Login

By selecting login, the user is given several options as to how the samples are run and where that data will be stored or even if the data should be e-mailed to the relevant recipient (Figure 7).

Figure 7. User definitions and id

Only users that have permissions to enter samples can continue. This is taken from the drop down menu and a Job ID is required to run the samples. The e-mail address and storage directories are also stipulated in this section. If a single sample plate login is selected within OpenAccess QuanOptimise the user is prevented from continuation with this type of method. The type of method that is to be run is now selected;

Figure 8. Method Selection

Once a method has been selected then the compound list has to be defined for the optimisation. This is necessary as the optimisation will be carried out first and a MRM transition will be automatically generated. This transition will then be written directly into an acquisition method and the assay will be run. Figure 9 shows the four sulfonamides that were used in this experiment. This must be entered to continue the sample analysis.
The next logical stage is to enter the analysis sample list (Figure 10).

The OpenAccess dialogue screens then instruct the user where to place the samples (Figure 11).

RESULTS

The following shows the optimisation parameters that the software generated for MS and MS/MS:

- Sulfaguanadine 215.4>156, cv=30, ce=13
- Sulfadimethoxine 311.5>156, cv=35, ce=22
- Sulfamethizole 271.4>156, cv=30, ce=13
- Sulfamethoxazole 254.5>156, cv=35, ce=13

The chromatograms gave eight separate plots for the automatic generation of the molecular ion at different cone voltages (Figure 12). The software then generates the spectrum for each chromatogram and based on the most intense peak, will allocate the corresponding cone voltage.

A similar procedure is followed for the MS/MS optimisation and the largest peak in the spectra has collision energy allocated (Figure 14).
A calibration curve is plotted using the pre-defined Quantitation method within OpenAccess QuanOptimise. The data shows a good correlation coefficient over the range 1 pg/ul to 1000 pg/ul. This is repeated for all four compounds.

CONCLUSION

OpenAccess QuanOptimise allows rapid and easy optimisation and method development. From the initial stages of tuning solutions to the final calibration curves the data maintains a high degree of sensitivity and data integrity.

OpenAccess gives the chemist a walk-up system that is flexible for analytical assay development. It runs as a complete system from sample introduction to end results.
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