AN AUTOMATED HIGH THROUGHPUT APPROACH TO QUANTIFICATION OF MULTIPLE COMPOUNDS USING ULTRA PERFORMANCE LC COUPLED TO TANDEM MASS SPECTROMETRY

INTRODUCTION

In the Drug Discovery arena, there is an increasing need for high throughput screening and quantitation of thousands of compounds in order to rapidly determine the compounds most likely to progress through development and into the clinic. To allow the desired throughput there is a requirement to use a fast, generic analytical method with a high degree of automation. Although generic chromatographic conditions can be developed to cope with a high percentage of the compounds encountered through drug discovery, the specificity of MS/MS demands that detection methods are developed on a compound-by-compound basis. When large numbers of diverse compounds need quantitative analysis in small sample volumes, it is possible to consume individual optimising the MS response of each compound.

In this work, we show a fast, generic separation using Ultra Performance LC coupled to tandem quadrupole with automated MS/MS optimisation allowing a reduction in both the method development and analytical run times. Optimised MRM transitions are automatically created by the software and stored in a customised optimisation library. This library facilitates the re-use of MS methods for future experiments on the same compounds, thus reducing method development requirements further. Due to the diverse nature of the compound libraries in drug discovery the software also investigates both peak and noise ion response and judges the best technique based on signal to noise of the raw data, therefore providing the best possible detection levels.

Using this approach we have been able to reduce method development time significantly and reduced the sample to sample analysis time by greater than a factor of 2, compared to a traditional HPLC/MS/MS approaches, without compromising data quality.

METHODS

Sample Preparation

Samples were prepared from stock solutions of each compound made up at 1 mg/mL in 1:1 Water/Methanol. Stock solutions were diluted to 10 µg/mL in 95:5 Water/Methanol for use as optimisation solutions. Sparing solutions were prepared at the concentrations shown in Table 1. Separate calibration curves for each compound were prepared in pooled human plasma: 10 µg/mL in 1:1 Water/Methanol. Stock solutions were diluted to 100 µg/mL added to 100 µL water and vortexed, this solution is used for injection.

EXPERIMENTAL DESIGN

QuanOptimise and OpenLynx application managers were configured to accept a list of compounds and their molecular weights as well as a list of samples to be analysed. The lists are input into the open-access interface in either a tab-delimited text file or imported and pasted into the application from a program such as Microsoft Excel. The QuanOptimise software then uses the optimisation samples in the compound list to generate optimisation conditions for the compounds as well as appropriate MRM methods and quantitation methods to run the analysis list.

Optimisation was carried out using a linear isocratic method (99% Eluent B) through the runs of 3 min, all temperature, offset and MS conditions are as recommended above. The software automatically optimises the cone voltage (EV), ion mode, positive or negative ion product ion selection and collision energy (ICE) from this run. MRM methods are then created for each compound (or group of compounds) and a quantitation method is automatically generated for data processing. The optimisation results for all compounds used are displayed in Table 2.

For each compound we prepared 2 replicate extractions of each of the calibration point in human plasma and 2 blank extractions, the analysis list comprised of four blank injections and two 7-point calibration curves for each compound. This list was run in an automated fashion after the optimisation run was complete.

Analysis of Atenolol and Metoprolol was repeated by HPLC/MS/MS for each compound. This list was run in an automated fashion after the optimisation run was complete.

RESULTS

All data produced were analysed in an automated fashion by the software after completion of the analysis list.

A total of 10 compounds produced good linearity with r2 values of greater than 0.96 with all but three giving better than 0.995 by UPLC/MS/MS. Linear range and r2 values for each compound were at least 0.25—50 ng/mL by UPLC/MS/MS. Full details of these figures are shown in Table 2. Calibration curves for each compound by UPLC/MS/MS are shown in Figure 2 below.

Table 2. Spiking solution concentrations and equivalents.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Concentration</th>
<th>Equivalent</th>
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</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>250 ng/mL</td>
<td>&gt; 135.8 ESP +ve</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>125 ng/mL</td>
<td>&gt; 135.8 ESP +ve</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>100 ng/mL</td>
<td>&gt; 137.8 ESP +ve</td>
</tr>
</tbody>
</table>

Table 1. Spiking solution concentrations and equivalents.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear Dynamic Range</th>
<th>r2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>0.25—50 ng/mL</td>
<td>0.999</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.25—50 ng/mL</td>
<td>0.999</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>0.25—50 ng/mL</td>
<td>0.993</td>
</tr>
</tbody>
</table>

DISCUSSION

The methodology used to generate the data shown in this paper is designed to allow new and inexperienced users of the triple quadrupole instrumentation to generate high quality experimental data with minimal user input. flavin experiments using a simple interface to speed up the submission of routine sample sets.

We have used a generic 1.5 minute UPLC gradient along with automatic software to allow the user to simply input a list of compound information and a list of samples, the software will then automatically optimise the response for each compound, generate the necessary method, run a sample list and automatically generate a quantitation results file.

We have shown good linearity and dynamic range (> 0.999) and dynamic range > 0.25—500 ng/mL for pre-processed human plasma samples heated by UPLC/MS/MS.

CONCLUSION

- We have demonstrated the feasibility of generating high quality quantitative data using a highly automated UPLC/MS/MS methodology from samples in dirty matrices
- From the data available UPLC/MS/MS gave between 2x and 3x improvement in signal/noise when compared to HPLC/MS/MS.
- The UPLC/MS/MS gave 3x the throughput of the HPLC/MS/MS method while giving highly comparable results.

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