A novel uPLC-MS/MS method to quantify oestrogens and their sulphates optimised using MUSCLE software

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Introduction
Oestrogens are implicated in many diseases and drive cell proliferation in breast, ovarian and endometrial cancer. Sulphated oestrogens are inactive and represent a circulating reservoir for active oestrogens. LC-MS/MS methods are the gold-standard for steroid measurements1.2). Thus, MS methods that can accurately quantify oestrogens and their sulphates are vital for understanding oestrogen-related disease.

Methods
The method was developed from Owen et al 20132. Samples with representative internal standards were extracted using isolute solid phase extraction columns and analysed by Waters Xevo LC-MS/MS in negative ion mode with 0.3mM ammonium fluoride (aqueous phase). Optimal separation of oestrone (E1), oestradiol (E2), oestrone-sulphate (E1S) and oestradiol-sulphate (E2S) was performed using MUSCLE software (Multi-objective Unbiased optimisation of Spectrometry via Closed Loop Experimentation), (Figure 1 and Table 1). MUSCLE software is a recent development by Bradbury et al. (3) (www.muscleproject.org) and involves automated optimisation of targeted LC-MS/MS analyses.

To test the method, colorectal cancer (CRC) cell lines HCT116, HT-29 and Colo205 were treated for 1 hour with E1, E2 and E1S (20, 100, 200, 400 and 800 nmol/l). Oestrogen metabolism was then compared across cell lines.

Figure 1.

Table 1. Table showing the changes in methanol (%) over time and gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow</th>
<th>Water (%)</th>
<th>Methanol(%)</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.45</td>
<td>70</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>2.20</td>
<td>0.45</td>
<td>50</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>5.00</td>
<td>0.45</td>
<td>0</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. Low concentration (CV%) at 18nmol/l for E1 and E2 and 14nmol/l for E1S and E2S and high concentration (CV%) at 554nmol/l for E1 and E2 and 427nmol/l for E1S and E2S.

Oestrogen metabolism was detected in the three CRC cell lines

Table 2. Table showing the changes in methanol (%) over time and gradient

<table>
<thead>
<tr>
<th></th>
<th>E1</th>
<th>E2</th>
<th>E1S</th>
<th>E2S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Concentration (CV%)</td>
<td>5</td>
<td>15</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>High Concentration (CV%)</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2. Low concentration (CV%) at 18nmol/l for E1, E2 and 14nmol/l for E1S, E2S and high concentration (CV%) at 554nmol/l for E1 and E2 and 427nmol/l for E1S and E2S.

Results
The MUSCLE optimised method analysed E1, E2, E1S and E2S over the linear range 0.5-500 ng/ml in less than 5 minutes (Figure 2). Average recovery for all oestrogens was approximately 100%. Variability of repeated extractions at high and low concentrations (CV%) are shown in Table 2.

Figure 2.

Figure 3.

Figure 3. Oestrogen metabolism in CRC cell lines after treatment with E1, E2 and E1S.

Conclusions
• MUSCLE software enabled the rapid development of this novel highly specific, high throughput method that accurately quantifies E1, E2 and their sulphates together in less than 5 minutes.
• Validated and tested on cell cultures
• Could also be applied to tissue, serum and urine based research.
• In the future other oestrogen metabolites, such as 16β-hydroxyestrone and 2-hydroxyestrone, could be added to this method.
• This method will significantly benefit future oestrogen-related research.

References
2. Owen LJ, Frederick W, Keevil BG. ANN Clin Biochem. 2013
4. www.muscleproject.org

Acknowledgements