Flexibility of Multichannel HPLC with a Single Mass Spectrometer to Simplify Workflow Complexity and to Improve Throughput of LC-MS
INTRODUCTION

The recent growing interest of LC-based MS in clinical laboratories is mainly because this analytical technique can provide definitive identification and accurate quantitation of targeted compounds. Studies show that MS is the preferred method to detect and quantify drugs thanks to its high specificity and sensitivity. However, the use of this technology is often hindered by the laborious and time-consuming sample preparation steps required for MS analysis. These include sample dilution, solid phase extraction, and waste cleanup procedures that can take up to several hours. A highly efficient and robust sample preparation method can thus significantly increase sample throughput for MS analysis.

MATERIALS AND METHODS

Sample Preparation

(1) Sample Preparation for Testing by Two Channel HPLC

Calibration Standards were prepared by spiking stock solution in crashed synthetic serum and urine. The calibration standards were prepared at eight concentration levels: 2, 5, 10, 20, 50, 100, 200, and 500 ng/mL.

(2) Sample Preparation for Testing by Four Channel HPLC

Calibration, QC samples, and test samples were prepared with analytic column and stable isotope labeled internal standards (IS) in synthetic urine and synthetic serum as shown in Table 3. In this study, all samples were prepared in crashed synthetic serum (CPS) and crashed synthetic urine (CSU). QC samples were prepared at 2, 5, 10, 50, 100, 200, and 500 ng/mL.

RESULTS

The precision studies of both instruments (n = 120, 10 replicates, 4 channels) used four different methods using this four channel HPLC TurboFlow™ (2 channel HPLC) and Thermo Scientific™ Prelude MD™. Results of Prelude MD™ and Thermo Scientific™ Prelude LX™ are shown in Table 5.

Table 5. Preliminary MD Parameters for Robustness Study – HESI in TLX Mode

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<tr>
<th></th>
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<tbody>
<tr>
<td>Warfarin</td>
<td>0.5 ml/min</td>
<td>0.5 ml/min</td>
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<tr>
<td>Estradiol</td>
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<tr>
<td>Alprazolam</td>
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</table>

(2) Four-Channel HPLC

The second design is a four-channel HPLC, which includes a laminar flow only, and is designated as LX mode.

Table 6. Preliminary LX Parameters for Robustness Study – HESI in LX Mode

<table>
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Table 7. Preliminary LX Parameters for Robustness Study – APCLI in LX Mode

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DISCUSSION

The results suggest that the LX mode provides a significant improvement in sample throughput and method flexibility. Multichannel optimization ensures the quantitation of each compound is less than 5% in both concentration and retention time; Between 5 channels, Cross correlation of 1976 samples were run unattended and continuously for about 60 hours. QC samples were binary mobile phases from its own solvent bottles, and the same type of analytical column. A total of 4 MD samples were run unattended and continuously for about 30 channels. QC samples were run less than 5% in both concentration and retention time; Between 5 channels, Cross correlation of 1976 samples were run unattended and continuously for about 60 hours. QC samples were binary mobile phases from its own solvent bottles, and the same type of analytical column. A total of 4 MD samples were run unattended and continuously for about 30 channels. QC samples were run 10% in retention time, and less than 1% in concentration measurements, as shown in Table 13.

Table 13. Identical Method – Prelude LX-MD (1800 injections; 60 hours)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/mL)</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Test 5</th>
<th>Test 6</th>
<th>Test 7</th>
<th>Test 8</th>
<th>Test 9</th>
<th>Test 10</th>
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<td></td>
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<td></td>
<td></td>
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<tr>
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</tr>
<tr>
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</table>

CONCLUSIONS

Both designs (Prelude MID: 2 channels HPLC; Prelude LX-MD: 4 channels HPLC) can continuously operate unattended for about 36 hours. Prelude MD can run four different methods or clinical methods with LX mode only. Multichannel optimization can remove this limitation, with less than 5% in concentration measurements, as shown in Table 13.