Determination of Methylphenidate in Human Plasma Using LC-MS/MS Techniques
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INTRODUCTION
Methylphenidate (MPH) is the most recommended medication for the treatment of attention-deficit hyperactivity disorder (ADHD), a commonly diagnosed neurobehavioral disorder among children. The drug is unstable in both plasma matrix and numerous extracted solvents. The purpose of this work was to develop and validate a sensitive, rapid, and reliable method with sufficient stability data for determination of methylphenidate in human plasma to support proposed clinical study.

METHOD
In this method, the plasma samples were thawed in ice water bath and treated with methanol for sample cleanup. The extract was chromatographed on a column (Pursuit C18, 50x2.0mm, 5µ) with gradient elution using methanol based mobile phase solutions. Methylphenidate and its internal standard methylphenidate-d9 were detected by monitoring ion transitions under positive MRM mode.

Liquid Chromatography
HPLC: Shimadzu 10A<br />
Autosampler: Perkin Elmer 200<br />
Column: Pursuit C18 (50 x 2.0 mm) 5 µ
MP A : 0.1% Formic Acid in DIH2O
MP B : 90/10 MeOH/10 mM ammonium formate, pH 3.0

Mass Spectrometry
AB Sciex API 3000 or API 4000<br />
Polarity: Positive
Scan type: Multiple Reaction Monitoring
Methylphenidate: m/z 234 → 84
Methylphenidate-d9: m/z 243 → 93 (I.S.)

RESULTS
Figures 1 through 3 show typical chromatograms for selected samples. The S/N ratio for Methylphenidate peak at LLOQ level was at least 5.

Figure 1. Chromatogram of Blank Plasma (No I.S.)

Figure 2. Chromatogram of a Processed LLOQ sample (0.10 ng/mL)

Figure 3. Chromatogram of a Processed ULOQ Sample (19.2 ng/mL)

The response was linear over a range of 0.100 to 19.2 ng/mL with weighted linear regression (1/x²). The correlation coefficients for three precision and accuracy batches were 0.998 or better (Figure 4).

Table 1. Precision and Accuracy Results for Methylphenidate Quality Control Samples (n = 6 replicates per batch)

<table>
<thead>
<tr>
<th>Plasma Lot</th>
<th>Avg drug area</th>
<th>Avg I.S. area</th>
<th>Avg area ratio</th>
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<tbody>
<tr>
<td>Lot 1</td>
<td>5368</td>
<td>129759</td>
<td>0.0414</td>
</tr>
<tr>
<td>Lot 2</td>
<td>5434</td>
<td>125798</td>
<td>0.0432</td>
</tr>
<tr>
<td>Lot 3</td>
<td>5767</td>
<td>129588</td>
<td>0.0444</td>
</tr>
<tr>
<td>Lot 4</td>
<td>5433</td>
<td>127400</td>
<td>0.0427</td>
</tr>
<tr>
<td>Lot 5</td>
<td>5277</td>
<td>124978</td>
<td>0.0422</td>
</tr>
<tr>
<td>Lot 6</td>
<td>5526</td>
<td>125438</td>
<td>0.0441</td>
</tr>
<tr>
<td>Mean</td>
<td>5468</td>
<td>127198</td>
<td>0.0430</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>SD</td>
<td>168</td>
<td>2197</td>
<td>0.0911</td>
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<tr>
<td>% CV</td>
<td>3.1</td>
<td>1.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Table 2. Matrix Effect in Human Plasma at Low QC (0.300 ng/mL)

Matrix stored in polypropylene | Stability
Freeze/Thaw (-70°C/Ice-water bath) | 3 cycles
Bench-top (RT) | 2 hr
Bench-top (Ice-water bath) | 6 hr
Long-term (-20°C) | 64 days
Long-term (-70°C) | 64 days

Table 3. Summary of Stability Studies

<table>
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<th>Study ID</th>
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<th>2</th>
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<tbody>
<tr>
<td>Numbers within ± 20.0 %</td>
<td>30</td>
<td>30</td>
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<tr>
<td>% of Samples within ± 20.0 % *</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* % Difference (% Diff) = [(Re-analysis value-Original value) / (average of re-analysis value, original value)] x 100 %

Table 4. Summary of Incurred Repeats in Two Clinical Trials

CONCLUSION
During sample cleanup, methanol played an important role in stabilizing methylphenidate by deactivating plasma enzymes and minimizing the hydrolysis of drug. As a result, a reliable and robust assay was achieved with simple operation. The method was fully validated over a range of 0.100 to 19.2 ng/mL with weighted (1/x²) linear regression. This method showed acceptable accuracy, precision, selectivity, stability, and reproducibility. The validated method can be demonstrated to be simple and reliable in supporting drug development and two clinical trials.

ACKNOWLEDGEMENTS
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