OVERVIEW
A simple and rapid LC-MS/MS method has been developed which allows the simultaneous quantification of a panel of commonly prescribed psychotherapeutic drugs in human plasma and whole blood.

INTRODUCTION

- Recent figures suggest that approximately a quarter of the world's population will suffer from a diagnosable mental disorder at some point in their lives \(^{(1)}\).
- Depression, schizophrenia, anxiety and substance abuse are amongst the most common conditions in the developed countries.
- 78% of affected people suffer from more than one mental disorder at the same time \(^{(2)}\).
- First-line treatment generally comprises psychotherapy and psychotherapeutic medication.
- Since the relationship between dose and clinical response is often poorly delineated, as a result of wide inter-individual variations in ADME, therapeutic drug monitoring (TDM) provides a valuable means by which to establish individual target therapeutic concentrations, to determine potential toxicity and to verify compliance.
- Current methods for TDM involve extraction of the drugs from plasma followed by analysis using LC-ECD (or LC-UV). However, these procedures are frequently problematic or insensitive due to the co-elution of contaminants, which frequently persist, even following lengthy sample preparation techniques.

In order to address this problem we have developed an alternative method. Drugs were isolated from plasma using simple protein precipitation step and subsequently analysed using LC-MS/MS. The procedure requires only 50µL of biological sample and has a total analysis time (including sample preparation) of less than 20 minutes. The method allows the simultaneous quantification of several of the most commonly prescribed psychotherapeutic drugs, in plasma or whole blood. Limits of detection of 1µg/L or better were achieved.

METHODS AND INSTRUMENTATION

**LC conditions**

HPLC System: Waters Alliance 2795  
Column: Waters Symmetry 300 C\(_{18}\)  
(2.1mm x 150mm, 5µm) maintained at 30 °C  
Mobile phase: (A) = 2 mM ammonium acetate containing 0.1% formic acid  
(B) = Acetonitrile containing 0.1% formic acid  
Isocratic elution (60:40)  
Flow rate: 0.35 mL/min  
Injection volume: 10 µL

**MS conditions**

Mass spectrometer: Micromass Quattro micro tandem mass spectrometer  
Ionisation mode: ES positive ion  
Capillary voltage: 1kV  
MS/MS: Collision gas: Argon at 4.5 x 10\(^{-3}\) mbar

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Table 1: MRM transitions and conditions for the measurement of psychotherapeutic agents. The deuterated analogues of imipramine and nortriptyline were included as an internal standards.

RESULTS AND DISCUSSION

MRM transitions were determined for the psychotherapeutic drugs (Table 1). Figure 2 shows some examples of precursor ion and product ion spectra.
A series of calibrators (0.1-500µg/L) were prepared by addition of the psychotherapeutic drugs to blank plasma. Following isolation from the matrix using a simple protein precipitation step which also incorporated the addition of internal standards (Figure 3), samples were analysed using LC-MS/MS. Figure 4 shows the MRM chromatograms acquired simultaneously during a single injection of a 50µg/L plasma calibrator. Quantification was achieved by integration of the area under the specific MRM chromatogram. In all cases the responses for the psychotherapeutic agents were calculated in reference to the integrated area of a deuterated internal standard.

Responses were linear, for all compounds in plasma, over the range investigated ($r^2 > 0.99$). A typical standard curve is shown in Figure 5a.

The precision of the assay was assessed by performing replicate ($n = 6$) extractions of plasma samples containing low, medium and high concentrations of the psychotherapeutic compounds (i.e. 2, 20 and 200µg/L respectively). Coefficients of variation (%CV’s) were found to be highly satisfactory (see Table 2).
The utility of the developed method was assessed by the analysis of actual plasma samples collected from patients currently receiving the various psychotherapeutic drugs. The procedure was demonstrated to be sufficiently sensitive for routine TDM studies. Our initial studies were extended to investigate the quantification of these drugs in whole blood. The described method was found to be suitable for this matrix also. **Figure 5b** shows a typical standard curve for risperidone in whole blood.

![Figure 5b](image)

**Figure 5.** Typical linearity of response for plasma (a) and whole blood (b) containing risperidone. In all cases, drugs were quantified by reference to the internal standard.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CV (%)</th>
<th>Low</th>
<th>Med</th>
<th>High</th>
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</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>13.1</td>
<td>3.5</td>
<td>1.9</td>
<td></td>
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<tr>
<td>Citalopram</td>
<td>3.4</td>
<td>2.9</td>
<td>4.1</td>
<td></td>
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<tr>
<td>Clomipramine</td>
<td>11.9</td>
<td>7.1</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Dibenzoepine</td>
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<td>3.1</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
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<td>1.9</td>
<td>3.4</td>
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<tr>
<td>Imipramine</td>
<td>14.0</td>
<td>9.8</td>
<td>5.6</td>
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<tr>
<td>Nortriptyline</td>
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<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
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<td>3.9</td>
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<tr>
<td>Risperidone</td>
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<td>5.7</td>
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<tr>
<td>Sertraline</td>
<td>18.6</td>
<td>3.1</td>
<td>3.3</td>
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</table>

**Table 2.** Precision of LC-MS/MS method for the analysis of psychotherapeutic agents in plasma.

**CONCLUSION**

Drug monitoring is advocated for individuals who are receiving psychotherapeutic medication in order to establish target therapeutic concentrations and to evaluate compliance. Thus, we have developed a simple and rapid HPLC-MS/MS method that allows the simultaneous quantification of a several commonly prescribed psychotherapeutic drugs during a single injection. The procedure has been successfully applied to whole blood and plasma samples collected from patients currently receiving treatment with various psychotherapeutic agents and offers several advantages over the existing methods i.e. it is more sensitive, faster and less labour-intensive.

**FUTURE AIMS**

- To continue to assess the utility of this method in routine TDM studies carried out in the clinic.
- To assess the feasibility of using alternative specimens such as saliva.
- To extend the profile of psychotherapeutic agents.

**REFERENCES**

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