SIMULTANEOUS QUANTITATIVE ANALYSIS OF MULTIPLE ATYPICAL ANTIPSYCHOTIC DRUGS IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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AIM

Development of a rapid, robust and sensitive method for the therapeutic monitoring of antipsychotic drugs widely used in the UK for the treatment of schizophrenia and schizo-affective disorders.

INTRODUCTION

- Schizophrenia is a serious illness that affects about 1 in every 100 people at some time in their life.
- Estimated cost of treatment is 2.5% of healthcare costs in developed countries (for the NHS in England 2004-5 = ca. £2 billion).
- Atypical (or second generation) antipsychotics have an effect on the negative and positive symptoms

Why is TDM of antipsychotics required?

- Compliance (adherence, concordance): poor or partial compliance with antipsychotic medication is common possibly due to adverse reactions
- Optimisation of therapeutic dosage
  - Is dosage adequate?
  - Is the patient at risk from dose-related toxicity (adverse drug reactions)?
- Investigation of drug interactions/metabolic influences (renal failure, liver disease)

METHODS

SAMPLE PREPARATION

Protein precipitation using methanol containing internal standard Imipramine-D3.

CHROMATOGRAPHY

ACQUITY UPLC system

Column: Waters X Terra MS C18 (2.1 x 50mm, 3.5µm), 30°C
Mobile phase: A=5mM Ammonium acetate, pH 10
B=Acetonitrile
Gradient: 20% B up to 80% B over 4 minutes (5.5 minute cycle time)

 MASS SPECTROMETRY

Waters Quattro Premier XE mass spectrometer used in electrospray positive ionisation mode with a collision cell pressure of 3.5 x 10⁻³ mbar (argon)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>CV (%)</th>
<th>CE (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amisulpride</td>
<td>370</td>
<td>242</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>448</td>
<td>176</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Dehydroaripiprazole</td>
<td>506</td>
<td>80</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Clozapine</td>
<td>327</td>
<td>270</td>
<td>45</td>
<td>20</td>
</tr>
<tr>
<td>Norclozapine</td>
<td>313</td>
<td>192</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>313</td>
<td>256</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>Risperidone</td>
<td>411</td>
<td>191</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>9-OH Risperidone</td>
<td>427</td>
<td>207</td>
<td>45</td>
<td>25</td>
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<tr>
<td>Sulpiride</td>
<td>342</td>
<td>112</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>384</td>
<td>253</td>
<td>40</td>
<td>25</td>
</tr>
</tbody>
</table>

RESULTS

For all compounds, responses were linear over the investigated range (1–200 µg/L). Intra-assay precision and accuracy were acceptable with CV’s for spiked QC samples < 11% and > 86%, respectively. The use of a simple protein precipitation step was demonstrated to be very efficient and gave reproducible extraction recoveries > 75% for all analytes. The matrix effects were assessed and found to be acceptable with the sulpiride response being most affected (~31%). All compounds gave satisfactory stability in prepared samples over 24 hours (except for olanzapine that showed a loss of 33% over the assessed time period). The method was applied to the analysis of spiked calibration standards and authentic clinical samples (n = 100) which were also analysed externally against an established HPLC/UV method. EQA samples (n = 6) were also assessed and all gave a deviation of < 22% from the method mean.

CONCLUSIONS

- Rapid and sensitive solution for the high-throughput monitoring of schizophrenia and schizo-affective disorder patients
- Simple protein precipitation sample preparation step
- Method validation demonstrates robustness with adequate linearity for all compounds over the investigated range
- Developed method shows good comparison to an established HPLC/UV method against the developed HPLC/MS/MS method
- Approximate LOD’s range from 0.5 – 0.05 µg/L

REFERENCES

2. NICE. Treating and managing schizophrenia— information for people with schizophrenia, their advocates and carers, and the public. 2007; http://www.nice.org.uk

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