Risperidone (Risperdal, Janssen-Cilag, Oyonnax, France), is an atypical antipsychotic introduced in 1993 that is licensed in the UK for the treatment of schizophrenia. Risperidone is metabolized to 9-hydroxyrisperidone (9-OH-risperidone), which has a similar pharmacological profile to that of the parent compound and accumulates in plasma on chronic dosing. Some studies have demonstrated significant potential for drug-drug interactions with risperidone [1], hence therapeutic monitoring may help not only in assessing compliance with oral dosage, but also in dose optimization.

Early methods for risperidone/9-OH-risperidone generally lacked sensitivity and specificity [2,3]. Other more recent methods either use HPLC-MS [4] or involve high specificity [2,3]. Other more recent methods either use HPLC-MS [4] or involve high performance liquid chromatography with electrochemical detection [5]. The aim of the current study was to develop and validate a simple, rapid, and sensitive method for risperidone/9-OH-risperidone assay in human plasma/serum. The limit of accurate measurement is 2 µg/L.

We have developed a relatively simple and rapid method for risperidone/9-OH-risperidone assay in human plasma/serum. The limit of accurate measurement is 2 µg/L for both risperidone/9-OH-risperidone, using a 1.0 mL sample. Hence the method has sufficient sensitivity to measure the concentrations of these compounds attained during low-dose risperidone treatment.

Materials and methods

Risperidone and 9-OH-risperidone were gifts from Janssen (Beerse, Belgium). The internal standard (benzimidazole), Tris (tri(hydroxymethyl)aminomethane) and newborn calf serum were from Sigma-Aldrich and ammonium perchlorate from Fluka. Coarsely filtered human serum was from Scipac (Sittingbourne, Kent). Methanol and methyl tert.-butyl ether (MTBE) were HPLC grade.

HPLC: PU-1580 intelligent pump, AS-950 autosampler and AS-1550 UV detector (280 nm) (all Jasco, UK). Data collection was via an Atlas chromatography data system (Thermo, UK). The analytical column (125 x 4.6 mm i.d.) contained Phenomenex SCX silica (5 µm) (Phenomenex, Cheshire, UK). The eluent was 35 mmol/L ammonium perchlorate in methanol adjusted to apparent pH 5.0 (flow-rate of 1.0 mL/min). Silica (5 µm aps) (Phenomenex, Cheshire, UK). The eluent was 35 mmol/L ammonium perchlorate in methanol adjusted to apparent pH 5.0 (flow-rate of 1.0 mL/min).

Calibration solutions were prepared in newborn calf serum by appropriate dilution of stock risperidone and 9-OH-risperidone working solutions (10 and 1 mg/L free base in methanol) to give concentrations of 2, 5, 10, 20, 50, 100 and 200 µg/L both analytes. Calibration standards and IQCs were stored at -20°C.

Samples were measured in duplicate. Internal standard solution, aqueous benzimidazole (2 mg/L, 50 µL) and Tris buffer (2 mol/L, pH 10.6, 200 µL) were added to plasma serum (1.0 mL). After extraction into MTBE by vortex-mixing (30 s) and centrifugation (1000 g, 4 min), the extract was evaporated to dryness. The residue was reconstituted in methanol and a portion (100 µL) was analysed by HPLC.

Results and Discussion

The calibration graphs for risperidone and 9-OH-risperidone were linear over the calibration range (r > 0.99, Figure 1). The recovery of risperidone and 9-OH-risperidone from calf serum and human plasma/serum were compared by replicate analyses (n=6) of calibration solutions spiked in calf serum and human plasma/serum. There was no significant difference in the extraction efficiency of the analytes from calf serum and human plasma/serum (paired t-test, p>0.05).

Table 1. Intra-assay precision at three concentrations (n = 5)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Nominal (µg/L)</th>
<th>Measured (µg/L)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risperidone</td>
<td>6</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>9-OH-Risperidone</td>
<td>70</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>158</td>
<td>156</td>
<td>8</td>
</tr>
</tbody>
</table>

Intra- and inter-assay precision were evaluated at three different concentrations (n = 5) (Tables 1 & 2). Individual batch analyses were accepted if the IQC results were within ± 10% of the nominal values, or ± 15% for the lowest IQC.

Conclusions

The method described is simple, economical, and sensitive with no interferences from commonly co-prescribed psychoactive drugs, and has adequate sensitivity to measure risperidone and 9-OH-risperidone concentrations in plasma/serum from patients prescribed low dose risperidone.

References