References


Quantification of the Aminosteroidal Non-Depolarizing Neuromuscular Blocking Agents Rocuronium and Vecuronium in Plasma with Liquid Chromatography-Tandem Mass Spectroscopy, Ursula Gutteck-Amsler and Katharina M. Rentsch* (Institute of Clinical Chemistry, University Hospital Zürich, Rämistrasse 100, CH-8091 Zürich, Switzerland; * author for correspondence: fax 41-1-255-4590, e-mail: rentsch@ikc.unizh.ch)

Rocuronium (RO) and vecuronium (VE) are widely used aminosteroidal non-depolarizing neuromuscular blocking agents. There are few methods published for the determination of VE, its metabolite 3-desacetyl-vecuronium (OHV) (1–4), and RO (5, 6) that use HPLC with ultraviolet, fluorescence, or electrochemical detection or gas chromatography with nitrogen-sensitive detection. To date, the methods published for the determination of VE and OHV use an exotic detection system (1), a time-consuming derivatization step (3), or a very laborious analytical technique (2, 4). The published methods for RO need a very sophisticated instrument that requires post-separation extraction of the drugs (6) or include a very time-consuming derivatization step (5). Despite the great analytical effort, the reproducibility (CV > 10%) and detection limits (> 10 μg/L) of these methods are not satisfying. To perform pharmacokinetic studies of RO, VE, and its metabolite OHV, we have established a robust, sensitive, and specific liquid chromatography electrospray ionization-tandem mass spectrometry method.

Immediately after blood collection into heparin-containing tubes and centrifugation at 4 °C, 1 mL of plasma was added to a tube containing 0.2 mL of a 1 mol/L sodium hydrogen phosphate solution to inhibit the degradation of the drugs. The samples were kept frozen at −70 °C until analysis. Before extraction of the plasma samples, 0.25 mL of a 1 mol/L sodium hydrogen phosphate solution and 50 μL of the internal standard pancuronium (PA; 10 mg/L) were added to 0.5 mL of plasma. After the addition of 1 mL of 6 mol/L potassium iodide, the drugs were extracted with 5 mL of toluene on a horizontal shaker (Infors HAT; Infors) for 20 min. After centrifugation for 5 min at 1000g, the organic layer was separated and dried by evaporation (Rotavapor; Büchi), and the residue was dissolved in 100 μL of mobile phase.

The HPLC system consisted of a RHEOS 2000 pump (Flux Instruments AG), an A200S autosampler (CTC) and a LCQ ion trap mass spectrometer (Thermoquest). The ionization mode was positive electrospray with a spray voltage of 3.8 kV and a capillary temperature of 230 °C. PA was detected by the most intense product ion of its monovalent cation (m/z 279.2), RO by the most intense product ion of its divalent cation (m/z 286.4), and OHV by the most intense product ion of its monovalent cation (m/z 529.4). VE and OHV were detected by the most intensive product ions of their protonated molecules (divalent cations; VE, m/z 279.2; OHV, m/z 258.5).

The different neuromuscular blocking agents were separated using a Nucleosil C18 HD column (12.5 cm × 4 mm; 5 μm particle size; Macherey-Nagel) protected with a...
Nucleosil C18 HD guard column (8 × 4 mm; 5 μm particle size; Macherey-Nagel). The mobile phase consisted of 50 mmol/L ammonium formate buffer (pH 3):methanol (73:27, by volume). The flow rate of the mobile phase was set at 0.8 mL/min, and a postcolumn flow-splitting device was used to send sample through the mass spectrometric detector at a flow rate of 100 μL/min.

The retention times were 6.4 min for RO, 8.2 min for PA, 9.7 min for OHV, and 15.4 min for VE. Examples of chromatograms of patients’ plasma samples containing RO and VE are shown in Fig. 1, A and B, respectively.

Seven calibrators were prepared by adding to human plasma corresponding amounts of RO or VE and OHV at concentrations of 0, 20, 100, 200, 500, 1000, and 2000 μg/L, respectively. The calibration curves were obtained by plotting the peak-area ratio for the m/z 487.4 (RO), 249.4 (VE), 100.2 (OHV), 236.7 (PA) ion pairs vs the concentration. Calibration curves were analyzed by unweighted least-squares linear regression analysis and were linear over the range studied. The precision of the calibration curves is shown in Table 1.

Within-run imprecision was determined for a series of six plasma samples supplemented with each compound; the CVs (mean concentration) were 2.9% (100 μg/L) and 5.3% (2000 μg/L) for RO, 9.0% (75 μg/L) and 1.0% (750 μg/L) for VE, and 10% (75 μg/L) and 1.0% (750 μg/L) for OHV. The between-run CVs were 7.4% (50 μg/L) and 5.3% (750 μg/L) for RO, 7.2% (75 μg/L) and 2.7% (750 μg/L) for VE, and 15% (75 μg/L) and 4.0% (750 μg/L) for OHV. The mean accuracy of the presented method was 106% for RO, 90% for VE, and 89% for OHV.

To determine the extraction recovery of the method, different plasma samples to which RO, VE, and OHV had been added were analyzed and compared with calibrators in mobile phase containing the same amount of drug. The recovery of added RO was 66%. The recoveries for VE and OHV were 78% and 70%, respectively.

The limit of quantification for the method was calculated using a signal-to-noise ratio of 3. For this purpose, the noise signal was obtained as the amplitude of the peaks from a segment of the chromatogram that preceded each peak. The quantification limits were 5 μg/L for RO and 15 μg/L for VE and OHV.

In conclusion, the method described met all of the analytical requirements necessary to analyze a large series of patient samples, e.g., pharmacokinetic studies. The reproducibility and sensitivity for the determination of RO was markedly improved compared with previously published analytical techniques. This method was applied to three kinetic studies in humans and to one study in rats.

We gratefully acknowledge René Bühler for excellent technical assistance.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Slope, (μg/L)^{-1}</th>
<th>Intercept</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO</td>
<td>0.0038 ± 0.0002</td>
<td>-0.0094 ± 0.0100</td>
<td>0.9999 ± 0.0001</td>
</tr>
<tr>
<td>VE</td>
<td>0.0028 ± 0.0004</td>
<td>0.0507 ± 0.0438</td>
<td>0.9994 ± 0.0003</td>
</tr>
<tr>
<td>OHV</td>
<td>0.00054 ± 0.00013</td>
<td>0.0046 ± 0.0108</td>
<td>0.9990 ± 0.0004</td>
</tr>
</tbody>
</table>

Table 1. Least-squares regression data for RO, VE, and OHV (n = 5).

All values are the mean ± SD.

References