

Supplementary Information – Animals, Material and Methods

Plasma concentration (HPLC-MS)

Protein precipitation with 390 μL acetonitrile per 10 μl plasma was carried out as sample preparation. Afterwards plasma concentrations of propofol, ketamine, and rocuronium were measured by high-performance liquid chromatography coupled with a single mass spectrometer (HPLC-MS) [1]. HPLC-MS measurements were performed on an Agilent (Santa Clara, CA, USA) 1260 Infinity Liquid Chromatography system with an XSelect CSH C18 column (3.5 μm , 2.1 \times 100 mm, Waters, USA) at 40°C for propofol and with an XBridge BEH Amide XP HILIC column at 35°C (2.5 μm , 3.0 \times 150 mm, Waters, USA) for ketamine and rocuronium. The mobile phase for ketamine and rocuronium contained 4mM ammonium formate buffer pH 4.9 in water (A) and 4mM ammonium formate buffer pH 4.9 in 90% acetonitrile (B). A linear gradient with the following A:B volume ratio was used for the separation of ketamine and rocuronium: 0 min (0:100)→4 min (40:60)→8 min (40:60)→9 min (0:100)→14 min (0:100). Propofol was separated under isocratic conditions for 4 minutes with 0.025% ammonium hydroxide in 70% acetonitrile. The flow rate was set to 0.4 mL/min for both separation types. An Agilent 6130B quadrupole mass spectrometer with electrospray ionization was used for the detection of all analytes. Propofol was acquired in negative single ion mode, ketamine and rocuronium in positive single ion mode, using a 3 μL injection volume.

Propofol (Sigma Aldrich, Munich, Germany) standard samples with 0.25, 0.5, 1, 2, 3, 4 and 5 $\mu\text{g}/\text{mL}$, ketamine (Rotexmedica, Trittau, Germany) standard samples with 1, 2, 3, 4, 5, 6, 7, and 8 $\mu\text{g}/\text{mL}$ and rocuronium (Essex Pharma, Munich, Germany) standard samples with 1, 2, 3, 4, 5, 6, 7 and 8 $\mu\text{g}/\text{mL}$ were used for calibration. Calibration curves were calculated by plotting the concentration against the measured peak area. The peak areas were evaluated using OpenLab CDS C.01.05 Agilent. All curves yielded a correlation coefficient $R^2 > 0.985$. All back-calculated concentrations of the standard samples were at least $\pm 15\%$ of the nominal value or even better. Plasma concentrations represent the means of three replicate measurements.

[1] Shopova T, Kiefer D, Wolf B, Maurer F, Sessler DI, Volk T, et al. Simultaneous quantification of propofol, ketamine and rocuronium in just 10 μL plasma using liquid chromatography coupled with quadrupole mass spectrometry and its pilot application to a pharmacokinetic study in rats. *Biomed Chromatogr* 2019; 33: e4540.