SUPPLEMENTARY DATA 1. PLASMA DEXMEDETOMIDINE ANALYSIS

Chemicals and supplies

Dexmedetomidine reference stock solution was a pharmaceutical product Dexdor 200 µg / 2 ml (Orion Pharma). Internal standard (Medetomidine-d3 Hydrochloride, 100 µg·ml^{-1}, Lot number2-GJR-98-2) was a commercial solute on manufactured by Toronto Research Chemicals, Inc., Canada. HPLC-grade methanol was purchased from Honeywell, Riedel-de-Haën, Germany, Water was purified using a Merck Millipore Milli-Q® Advantage A10® Synthesis water purification system.

Preparation of calibration standards and quality control samples

Stock solutions of dexmedetomidine and the internal standard were first diluted with a mixture of water and methanol (1:1, v/v) and then with water to achieve desirable concentrations to be used for spiking of calibration standards and quality control samples. For preparation of calibration standards at nine concentration levels and quality control samples at four concentration levels appropriate volumes of dexmedetomidine dilutions were added into drug-free human K2EDTA plasma. The lower limit of quantitation (LLOQ) was 0.02 ng·ml^{-1}. The concentrations of the calibration standards were 0.02, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 4.5 and 5.0 ng·ml^{-1}, and the concentrations of the quality control samples were 0.06, 0.15, 1.0 and 4.0 ng·ml^{-1}. Calibration standards, quality control samples and actual study samples were stored at -70 °C and processed in an identical manner.

Sample Preparation

Sample preparation was performed using SPE (Solid Phase Extraction). The frozen plasma samples were thawed and tempered at room temperature. 0.250 ml of plasma sample was transferred into a 12 ml PP-tube. 0.675 ml of 0.1 % formic acid in water and 0.075 ml of internal standard solution (medetomidine-d3, 1 ng/ml) were added. In the case of blank samples, 0.075 ml of water was added instead of the internal standard solution. The tube was gently shaken for about 5 s and the sample was extracted with Sep-Pak® tC18 100 mg 96-Well Plates using an Oasis 96-well plate extraction manifold.

First, the SPE cartridges were activated with 1.0 ml of methanol and equilibrated with 1.0 ml of 0.1 % formic acid in water. 1.0 ml of the plasma mixtures were added and then cartridges were washed with 1.0 ml of water. The cartridges were dried with air for ten seconds. The samples were extracted with 1 ml of methanol. The extracts were evaporated to dryness in a sample collection well plate at 50 ºC under a gentle stream of nitrogen. The residue was dissolved in 100 µl of a solution containing 30 % of methanol and 70% of water. After having been dissolved on a microtiter plate shaker for ten minutes, the samples were transferred into autosampler vials. Finally, the samples were centrifuged with a microcentrifuge at the speed of 2000 rpm for 2 minutes and then the samples were injected (20 µl) into the LC-MS/MS system.

Mass spectrometric detection was carried out using an AB Sciex 4000 QTrap® triple-quadrupole instrument using positive Turbo Ion Spray (TIS) ionization and multiple reaction monitoring (MRM) mode. The TIS temperature was 500 °C. The nebulizer gas (Gas 1) setting was 50 and the turbo gas (Gas 2) setting was 50, too. The TIS voltage setting was 5000 V. The declustering potential was set at 55 V. Entrance potential was 10 V and the collision energy was 22 V. Curtain gas (nitrogen) was set to 16 and the collision gas (nitrogen) was set to 7. The precursor ion –

fragment ion pairs detected were m/z 201.2 – 95.05 for dexmedetomidine and m/z 204.2 – 98.05 for the internal standard. The dwell time for both molecules was 300 ms.

Data analysis

The quantitation of the analyte was accomplished with HPLC-MS/MS using an internal standard method. The calculations for the quantitations were based on the peak area ratios of the analyte and the internal standard. The data from the HPLC-MS/MS analyses were collected using AB Sciex Analyst 1.6.1 software. The peak integrations, calibration curves and quantitations were generated with the same software. The statistics were calculated with Microsoft Excel 2010. The standard curves were generated using weighted (1/x2) linear regression. The inter-assay accuracies of the quality control samples (at four different concentration levels: 0.06, 0.15, 1.0 and 4.0 $ng \cdot ml^{-1}$) were 100.4 %, 101.4 %, 102.9 % and 98.7 %. The corresponding inter-assay coefficients of variation were 2.8 %, 2.6 %, 2.9 % and 3.4 %.