Quantification of compound A in plasma by mass spectrometry

Mouse plasma sample preparation was carried out using solid-phase extraction kit (Impact, Phenomenex Inc.). Briefly, 800 µl of methanol was dispensed to the upper 96-well plate, and 200 µl of plasma was added directly into the methanol in each well. The sample was vortexed for 2 min and stood for 25 min. The plate was placed on a collection plate and applied 5 psi nitrogen gas using a positive pressure manifold to filtrate precipitated plasma proteins. The filtrate was dried with nitrogen gas before compound A was extracted using 0.1% formic acid in methanol (100 µl) into the lower 96-well plate for analysis. Quantification was carried out using external standards with control plasma and a calibration curve. The LC-MS/MS system was comprised of a HPLC system (ExionLC AD, AB SCIEX) coupled to a QTRAP6500+ mass spectrometer (AB Sciex) in electrospray ionization (ESI) mode. Compound A was analyzed via LC-MS/MS in positive mode. Ten microliters of the sample extract were injected onto a HPLC C18 column (Zorbax Eclipse XDB-C18 column, 3×100 mm, 3.5 µm, Agilent) with a guard column (ZORBAX SB-C18, 3×100 mm, 1.85 µm, Agilent) at 40°C using a 10 min solvent gradient employing 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Additional LC settings for LC-MS/MS are as follows: 0% B in 0.5 min; 0 to 95% B in 5 min; 95% B in 1 min; 95 to 0% B in 0.5 min; 0% B in 2.5 min at a flow rate of 0.5 ml/min. MS settings for LC-MS/MS mode are as follows: curtain gas, 30; ion spray voltage, 5500 V; temperature, 300°C; ion source gas 1, 50 psi; ion source gas 2, 80 psi; collision gas, 9 psi; declustering potential, 140 V; entrance potential, 10 V; collision energy, 31 V; collision cell exit potential, 18 V. Compound A was identified and quantified using multiple reaction monitoring (MRM) with Q1 and Q3 transition of xxx *m/z* and yyy *m/z*, respectively.