Angiotensin Peptide Quantification

Serum was incubated for 1 hour at 37°C. to establish equilibrium levels prior to stabilization with an inhibitor cocktail. The immediately stabilized plasma samples (see above) were thawed on ice. Both the serum and plasma samples were spiked with stable isotope labeled internal standards for each angiotensin metabolite and aldosterone at a concentration of 200pg/ml. Angiotensin peptides and aldosterone were then quantified using high performance liquid chromatography-mass spectrometry. Details of the method are presented in Supporting Information. The samples then underwent C18 based solid-phase extraction following standard protocol (Waters, Milford, MA, USA). The eluted samples were evaporated to dryness under a filtered, pre-warmed steady stream of nitrogen gas, reconstituted in 10% acetonitrile/0.1% formic acid and subjected to liquid chromatography tandem mass spectrometry analysis using a reversed phase analytical column (Acquity UPLC BEH C18 Column, 1.7µm, 2.1 mm Å~ 50 mm, Waters, Milford, MA, USA) operating in line with a Xevo TQ-S triple quadruple mass spectrometer (Waters, Milford, MA, USA). Component A consisted of water with 0.1% formic acid, while Component B was acetonitrile with 0.1% formic acid. A gradient program was used, where the concentration of component B was kept at 5% for 0.5 minutes initially and increased to 50% over 4 min. Component B was further increased to 95% for 1 min and then returned to 5% for 1 min. At least two different mass transitions were measured per analyte and internal standard signals were used to correct for matrix effects and peptide recovery of the sample preparation procedure for each angiotensin metabolite in each individual sample. Analyte concentrations were calculated by relating endogenous peptide signals to the calibration curve, provided that the integrated signal exceeded a signal-to-noise ratio of 10.