**LC-MS/MS Analysis of Angiotensin I for Assessment of Plasma Renin Activity in Clinical Research**

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**Background:** We have evaluated an LC-MS/MS method for the measurement of angiotensin I (Ang1) enabling investigation of Plasma Renin Activity (PRA) for clinical research activities testing biomarkers of hypertension. Renin converts angiotensinogen to Ang1, which is subsequently converted to Angiotensin II (Ang2). Ang2 is a potent vasopressor and plays a central role in regulation of blood pressure. Measurement of Ang2 is challenging due to its low circulating levels and short half life. Ang1 is more stable, and therefore, a more suitable candidate to evaluate PRA. An analytical method was developed using µElution Solid Phase Extraction (SPE) in 96-well plate format, reducing sample preparation time and optimizing analytical sensitivity.

**Methods:** Ang1 purchased from Cambridge Biosciences (Cambridge, UK) was used to create calibrators and QC materials using Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS). Ang1 precision was performed using BSA/PBS QCs. Precision of the method for analyzing PRA was performed using high and low value assigned K₂EDTA plasma pools and PRA controls (PN:600, Bio-Rad, UK). Samples were treated with buffer containing: 0.5M sodium acetate pH 5.5, 18mM EDTA, 4mM PMSF and 0.02mM SBTI. Samples were incubated for 3 hours at 37°C to generate Ang1. Samples were diluted with Ammonium Hydroxide and Ang1-¹³C₁⁵N internal standard (Cambridge Biosciences, UK). SPE was carried out with a Waters® Oasis® MAX µElution 96-well plate, which negated the need for evaporation and allowed direct injection of the SPE eluate. Offline automated extraction was performed using a Tecan Freedom Evo 100. Samples were analysed using an ACQUITY UPLC I-Class system with Waters Xevo TQ-S mass spectrometer. Analytical separation was carried out using a 2.1 x 50 mm Waters ACQUITY UPLC HSS T3 column with pre-column VanGuard T3.

**Results:** The method was shown to be linear from 0.15 – 116 nmol/L for Ang1 (0.05 – 38.5 nmol/L/hr). Coefficients of variation (CV) for total precision and repeatability on five separate days for 1.5, 7.7 and 77.1 nmol/L Ang1 BSA/PBS QC samples were all ≤ 10.0% (n = 30). PRA low and high plasma pools and Bio-Rad controls were ≤ 15.0% over the five occasions. Analytical sensitivity investigations demonstrate a CV < 20% at 0.15 nmol/L (0.05 nmol/L/hr) for Ang1 with S/N >10:1.

**Conclusions:** We have developed an analytical method for clinical research to quantify Ang1 and evaluate the PRA in plasma utilizing SPE with LC-MS/MS. This offline automated method demonstrates good linearity, precision and accuracy, while providing high sample throughput capabilities.