A Combined LC-MS/MS Method for the Analysis of Aldosterone and Plasma Renin Activity in Plasma for Clinical Research using the Xevo TQ Absolute

Dominic Foley, Waters Corporation

Background:

Primary aldosteronism (PA) is a common cause of hypertension, whereby uncontrollable amounts of aldosterone are produced by a benign tumour or hyperplasia of the adrenal glands. Excess aldosterone results in significant sodium reabsorption in the kidneys, increasing water retention and blood volume, thereby causing hypertension. The renin-angiotensin-aldosterone system (RAAS) regulates the production of aldosterone and in this system, renin and aldosterone should move in synchronicity with each other throughout the day. Therefore, these two components are used to assess the status of the RAAS, particularly in the evaluation of new therapies in clinical research studies. Historically, the assessments of aldosterone and plasma renin activity (PRA) have been performed using separate methods using immunoassay or more recently liquid chromatography – tandem mass spectrometry (LC-MS/MS) platforms. One of the benefits of using LC-MS/MS for clinical research is the ability to measure multiple analytes across the proteome and metabolome using the same system and even in the same analysis to provide more information in less time and save costs. Here we evaluate a single LC-MS/MS method for the combined measurement of plasma aldosterone and renin activity for clinical research purposes.

Methods:

Aldosterone certified reference material (Merck, UK) and Angiotensin I (Cambridge BioScience, UK) were used to create calibrators in 2% Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS). In-house QC material prepared in both 2% BSA in PBS and K2EDTA plasma (BioIVT, UK), were used to evaluate method precision. Plasma samples were analyzed using the newly developed method and the quantified results were compared to separate independent LC-MS/MS methods for aldosterone and plasma

renin activity. Plasma samples were treated with generation buffer (Sodium acetate, EDTA, acetic acid, SBTI and PMSF) and mixed for three hours at 37°C. Samples were precipitated, diluted and centrifuged prior to SPE. Sample supernatant was transferred to a Waters Oasis™ MAX µElution 96 Well Plate, followed by a wash and elution. Using an ACQUITY™ UPLC™ I-Class System, samples were injected onto a Waters XBridge™ C8, 2.5µm, 2.1 x 50 mm Column using a water/methanol/ammonium fluoride gradient elution profile and quantified with a Waters Xevo™ TQ Absolute Mass Spectrometer.

Results:
The method demonstrated no significant carryover or matrix effects and was shown to be linear from 10 – 2500 pg/mL for aldosterone and 0.1 – 25 ng/mL/hr for PRA. Analytical sensitivity investigations indicate the analytical sensitivity of this method would allow precise quantification (<20%) at 10 pg/mL and 0.1 ng/mL/hr, for aldosterone and PRA, respectively. Coefficients of variation (CV) for total precision and repeatability on 5 analytical runs for low, mid and high QCs were all < 10% (n = 25) for aldosterone and PRA. Comparison with samples previously analyzed by an independent LC-MS/MS method demonstrated good agreement for aldosterone and PRA.

Conclusions:
We have successfully quantified aldosterone and renin activity in plasma in a single method using an SPE protocol with LC-MS/MS analysis, for clinical research purposes. The method demonstrates excellent linearity and precision, with minimal matrix effects.

For Research Use Only. Not for Use in Diagnostic Procedures.

Waters™