A COMBINED LC-MS/MS METHOD FOR THE ANALYSIS OF ALDOSTERONE AND PLASMA RENIN ACTIVITY FOR CLINICAL RESEARCH USING THE XEVO TQ ABSOLUTE MASS SPECTROMETER

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INTRODUCTION

The Renin-Angiotensin-Aldosterone System (RAAS) is critical in maintaining blood pressure homeostasis, either through increases in blood volume via the action of the mineralocorticoid steroid hormone, aldosterone, or increased vasoconstriction through activity of the renin - angiotensin pathway. Analysis of aldosterone and plasma renin (or plasma renin activity (PRA)) 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 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 at lower cost. Here we evaluate a single LC-MS/MS method for the combined measurement of aldosterone and PRA for clinical research purposes.

METHODS

Materials

- Aldosterone and its internal standard were purchased from Merck Life Science (Dorset, UK). Angiotensin I and its internal standard were purchased from Cambridge BioScience (Cambridge, UK)
- Calibrators were prepared in 2% (w/v) Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS) over the range of 28 – 6940 pmol/L and 0.23 – 58 nmol/L (0.08 – 19 nmol/L/hr) for aldosterone and angiotensin I (plasma renin activity), respectively.
- Different sets of Quality Controls (QCs) were prepared in-house using 2% (w/v) BSA in PBS for aldosterone and angiotensin I (plasma renin activity) and pooled human plasma (BioIVT, UK) for aldosterone. Lyphocheck™ Hypertension Markers Controls (Bio-Rad, Watford, UK) were used for precision assessments of PRA.

Angiotensin I Generation and Solid Phase Extraction

- Samples were rapidly thawed to minimize cryoactivation of plasma prorenin to renin. 125µL plasma was diluted with freshly prepared generation buffer and incubated at 37°C for 3 hours. Internal standard was added, followed by of 1% ammonia$_{(aq)}$.
- An Oasis™ MAX 96-well µElution Plate was conditioned and equilibrated and supernatant was loaded on to the SPE plate. Samples were washed with 1% ammonia in 20% methanol$_{(aq)}$.
- Aldosterone was eluted with 30% acetonitrile$_{(aq)}$ into the 1mL 96-well collection plate, followed by elution of angiotensin I into the same plate using 0.5% formic acid in 5% acetonitrile$_{(aq)}$.
- The samples were sealed, mixed and centrifuged prior to injection.

LC-MS/MS Parameters

- Injection was performed using an ACQUITY™ UPLC™ I-Class FL System. Separation was performed using a XBridge™ Premier BEH™ C_{18} Column with mobile phases of 0.2mM ammonium fluoride(aq) and acetonitrile, over a run time of 3.2 minutes.
- Polarity switching in MRM mode on a Xevo™ TQ Absolute Mass Spectrometer was used to detect aldosterone (ESI-), angiotensin I (ESI+), and their respective internal standards.

A Single Extraction and Analysis for Both Aldosterone and Plasma Renin Activity

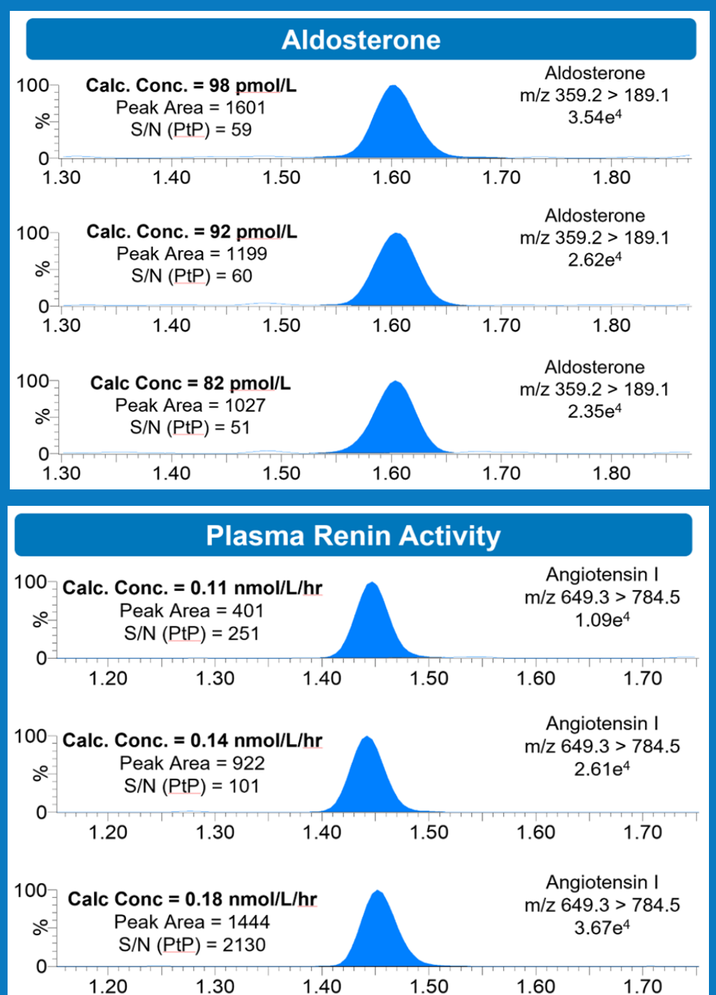

Figure 1. *Low-level concentrations of aldosterone and PRA demonstrating analytical sensitivity of the method*

RESULTS

Chromatography and Analytical Sensitivity

- Functional sensitivity tests (three runs, n=30 at each level) demonstrate precision of <20% with S/N > 10:1 for aldosterone and PRA at 28 pmol/L and 0.08 nmol/L/hr, respectively.
- Low-level aldosterone (82 – 113 pmol/L) and PRA samples (0.10 – 0.18 nmol/L/hr) from the method comparison demonstrate excellent analytical sensitivity for this method (Figure 1).

QC Precision

- Reproducibility and repeatability were determined by extracting and quantifying five replicates of three level QC material per day over five analytical runs (n=25).
- Reproducibility and repeatability for 2% (w/v) BSA in PBS QCs at 83, 833 and 5553 pmol/L for aldosterone and 0.23, 2.3 and 15.4 nmol/L/hr for PRA were ≤6.8% CV.
- Reproducibility and repeatability for human plasma QCs at 133, 963 and 5450 pmol/L for aldosterone were ≤5.6% CV.
- Reproducibility and repeatability for Bio-Rad QCs at 2.0, 5.7 and 12.7 nmol/L/hr for PRA were ≤7.5% CV.

EQA and Method Comparison

- Analysis of 39 aldosterone EQA samples from UK NEQAS was performed to assess agreement to the scheme LCMS mean.
- Deming fit analysis provided agreement of y=0.93x +2.14 (Figure 2) and Altman-Bland agreement provided a mean method bias of -6.1%, compared to the EQA LCMS mean values for aldosterone.

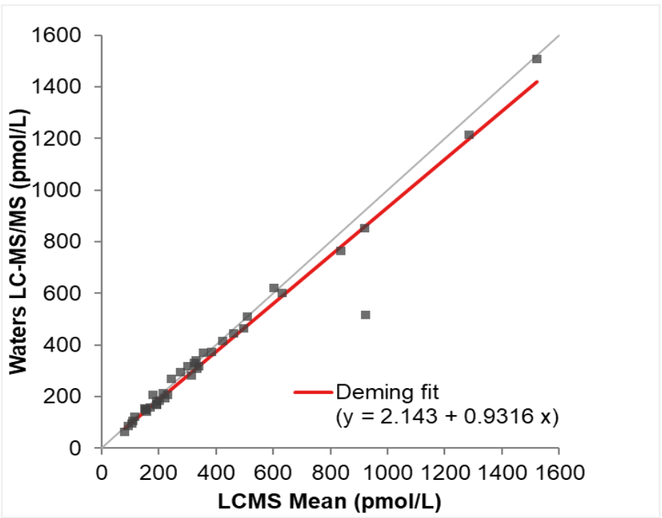

Figure 2. *Deming fit of the aldosterone samples analyzed using the LC-MS/MS method by Waters compared to the EQA scheme LCMS mean*

- A method comparison was performed using samples previously analyzed by two separate independent LC-MS/MS methods for aldosterone and PRA.
- Comparison of 58 aldosterone samples provided a Passing-Bablok fit of y = 0.96x – 2.49 with an Altman-Bland agreement mean method bias of -6.0%.

Note: Waters, Oasis, ACQUITY, UPLC, XBridge, BEH, and Xevo are trademarks of Waters Technologies Corporation. Lyphocheck is a trademark of Bio-Rad Laboratories, Inc.

CONCLUSION

A method for the simultaneous analysis of aldosterone and plasma renin activity has been developed for clinical research using the Xevo TQ Absolute Mass Spectrometer

- **Using 125µL plasma, analytical sensitivity of 28 pmol/L and 0.08 nmol/L/hr for aldosterone and PRA can be achieved.**
- **Excellent reproducibility and repeatability of ≤7.5% across QC samples for aldosterone and PRA.**
- **Assessment of aldosterone EQA samples has shown the method provides excellent agreement to the scheme LC-MS mean, and comparison to independent methods for aldosterone and plasma renin activity demonstrate good agreement.**

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