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Application Note

Simultaneous LC-MS/MS Analysis of Aldosterone and Plasma Renin Activity Using the Xevo™ TQ Absolute Mass Spectrometer for Clinical Research

Dominic Foley, Lisa J. Calton

Waters Corporation

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Abstract

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), which measures the rate of angiotensin I generation) 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

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measurement of plasma aldosterone and renin activity for clinical research purposes.

Benefits

- A single method for the measurement of aldosterone and plasma renin activity that reduces time and cost associated with these analyses

- Analytically sensitive method for the analysis of aldosterone and plasma renin activity

- An extraction method that is amenable to automation using liquid handling robots

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, which measures the rate of angiotensin I generation) are used to assess the status of the RAAS, particularly in the evaluation of new therapies in clinical research studies.

Analysis of aldosterone and plasma renin (or plasma renin activity) have traditionally been performed using separate methods, typically using ligand-binding techniques. More recently, LC-MS/MS has become a sought-after technique in steroid analysis, as it has been established that it can overcome the limitations observed in ligand-binding methods, while providing similar levels of analytical sensitivity. 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 at lower costs. We have developed a method using the Oasis™ MAX 96-well µElution plate and the ACQUITY UPLC I-Class FL with Xevo™ TQ Absolute Mass Spectrometer that allows extraction and analysis of the steroid hormone, aldosterone, and the peptide, angiotensin I for the evaluation of RAAS status in clinical research.

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Figure 1. The Waters Xevo TQ Absolute Mass Spectrometer.

Experimental

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

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(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 purchased for precision assessments of plasma renin activity.

To convert SI units to conventional mass units divide by 2.774 for aldosterone (pmol/L to pg/mL) and 0.771 for angiotensin I (nmol/L to ng/mL).

Samples were rapidly thawed to minimize cryoactivation of plasma prorenin to renin. Samples were mixed and then centrifuged prior to use.
Angiotensin I Generation: 125 µL of plasma was diluted 1:1 with freshly prepared generation buffer. Samples were sealed, mixed, and incubated at 37 °C for 3 hours. Internal standard (SIL) was added, followed by addition of 1% (w/v) ammonia $_{(aq)}$.

Solid Phase Extraction of aldosterone and angiotensin I: An Oasis MAX µElution plate was conditioned and equilibrated with methanol and 1% (w/v) ammonia $_{(aq)}$, respectively. 650 µL of supernatant was loaded on to the SPE plate. Samples were washed with 1% (w/v) ammonia in 20% methanol$_{(aq)}$. Aldosterone was eluted with 30% acetonitrile$_{(aq)}$ into the 1 mL 96-well collection plate, followed by elution of angiotensin I into the same plate using 0.5% (v/v) formic acid in 5% acetonitrile$_{(aq)}$. The samples were sealed, mixed, and centrifuged prior to injection onto the LC-MS/MS system.

LC-MS/MS analysis: Injection was performed in partial loop mode using an ACQUITY UPLC I-Class FL System. Aldosterone and angiotensin I were separated using a XBridge™ Premier Peptide BEH C$_{18}$ Column, 130 Å, 2.5 µm, 2.1 x 50 mm, with mobile phases of 0.2 mM 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.

Results and Discussion

Chromatographic separation was performed using the XBridge Premier Peptide BEH C$_{18}$ Column, with aldosterone and angiotensin I eluting within 2 minutes of injection (Figure 2).

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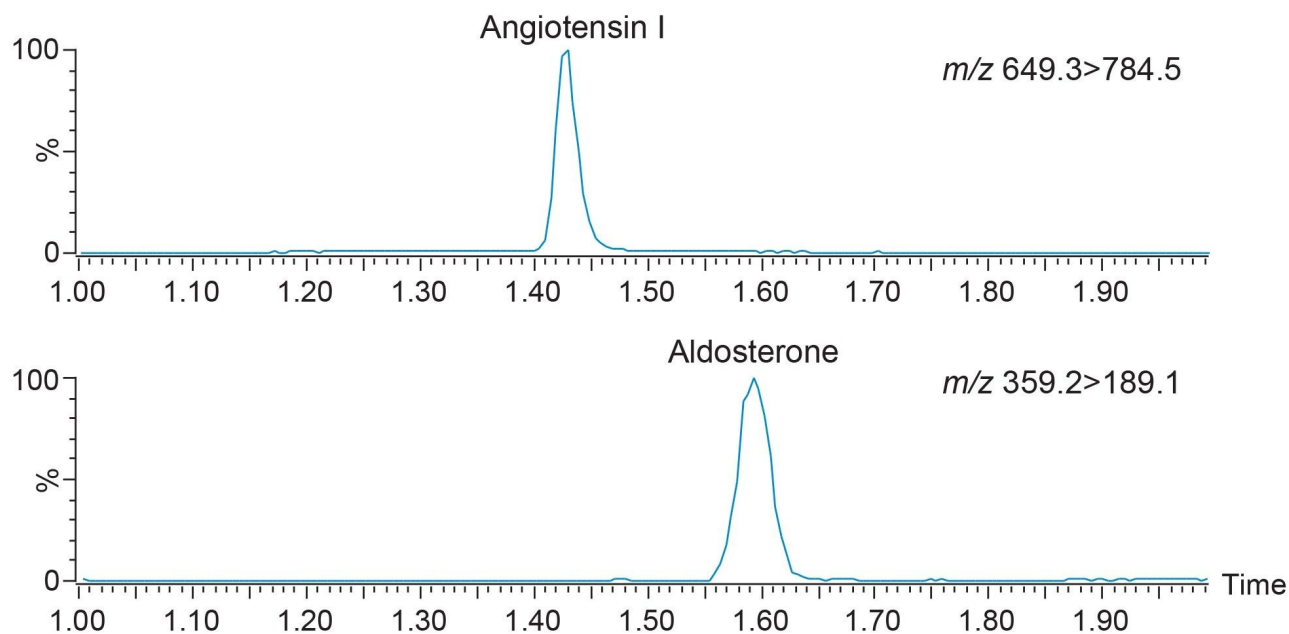
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Figure 2. Separation of aldosterone and angiotensin I using the XBridge Premier Peptide BEH C$_{18}$ Column.

No significant system carryover (<20% of the lowest calibrator) was observed from high concentration samples into subsequent blank injections. A 1:5 dilution using 2% (w/v) BSA in PBS was successfully employed to analyse high concentration samples at 25,700 pmol/L and 73 nmol/L/hr for aldosterone and plasma renin activity, providing a mean accuracy of 93% and 94%, respectively.

No significant interferences (recovery within ±15% bias) were observed at the retention time of aldosterone and angiotensin I when other structurally related compounds with similar polarities were individually examined and other high abundance endogenous compounds.

Analytical sensitivity investigations were performed using aldosterone and angiotensin I spiked samples in 2% (w/v) BSA in PBS over three occasions across and below the calibration range (n=30 at each concentration). The method would allow for precise quantification (<20% RSD, S/N(PtP) >10:1) at 28 pmol/L for aldosterone and 0.08 nmol/L/hr for plasma renin activity.

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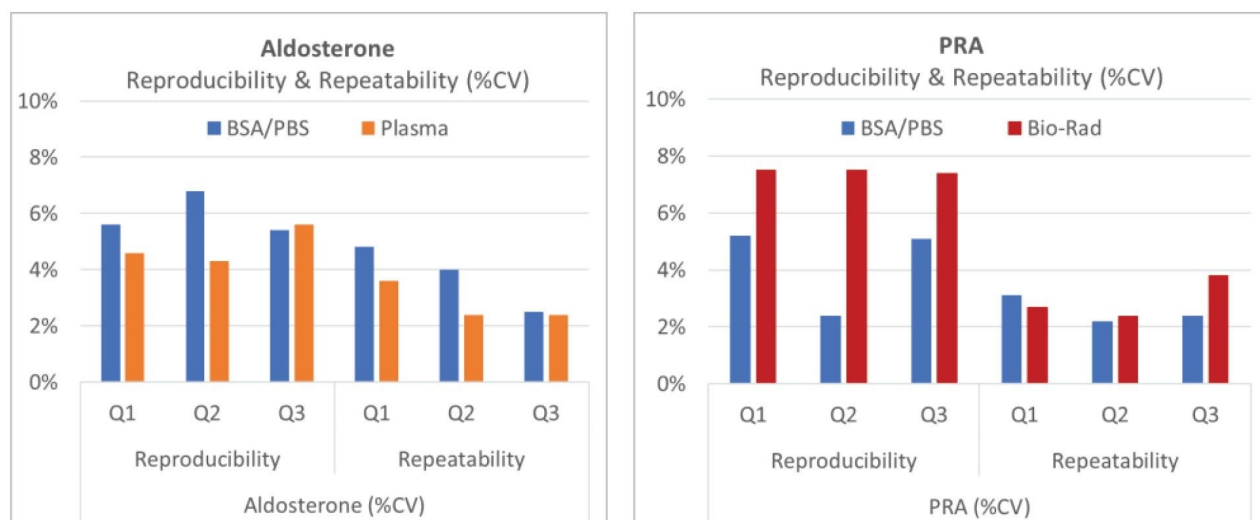
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Figure 3. Reproducibility and repeatability for the aldosterone and plasma renin activity in QCs prepared from 2% (w/v) BSA in PBS and human plasma (Bio-Rad QCs for PRA only).

Reproducibility and repeatability were determined by extracting and quantifying five replicates of three level QC material per run day over five separate occasions (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 plasma renin activity 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 plasma renin activity were ≤7.5% CV (Figure 3).

The method was shown to be linear for aldosterone (22–8328 pmol/L) and angiotensin I (plasma renin activity) 0.18–70 nmol/L (0.06–23 nmol/L/hr) when different ratios of high and low concentration pools of the analytes were combined and analyzed. In addition, calibration lines in 2% (w/v) BSA in PBS were linear with coefficient of determinations (r^2) >0.995 for all analyses.

Matrix effect investigations for aldosterone were performed using individual donor plasma. Normalized matrix factor calculations, based on the analyte:internal standard response ratio demonstrated that the internal standards compensated for any ion suppression observed with matrix factors ranging from 89–99% for aldosterone and 96–105% for plasma renin activity.

Analysis of 39 aldosterone EQA samples from UK NEQAS was performed to assess agreement to the scheme

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LC-MS mean, with data evaluated using both Deming fit and Altman-Bland agreement. Deming fit provided agreement of y=0.93x+2.14 and Altman-Bland agreement provided a mean method bias of -6.1%, demonstrating excellent agreement with the EQA LC-MS mean values for aldosterone (Figures 4a–b).
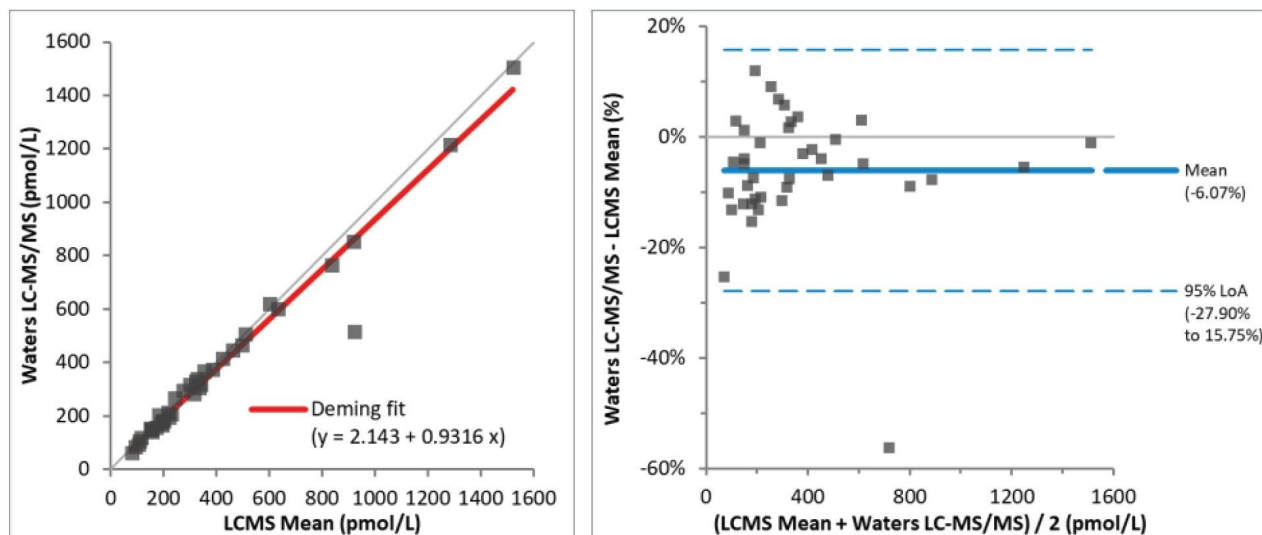

Figure 4. Comparison of the Waters LC-MS/MS method to the EQA scheme MS method mean for aldosterone using (a) Deming fit and (b) Altman-Bland agreement.

A method comparison was performed using samples previously analyszed by two separate independent LC-MS/MS methods for aldosterone and plasma renin activity. 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% (Figure 5a–b). Comparison of 61 plasma renin activity samples provided a Passing-Bablok fit of y=1.27x-0.02 with an Altman-Bland agreement mean method bias of 21.4% (Figure 5c–d).

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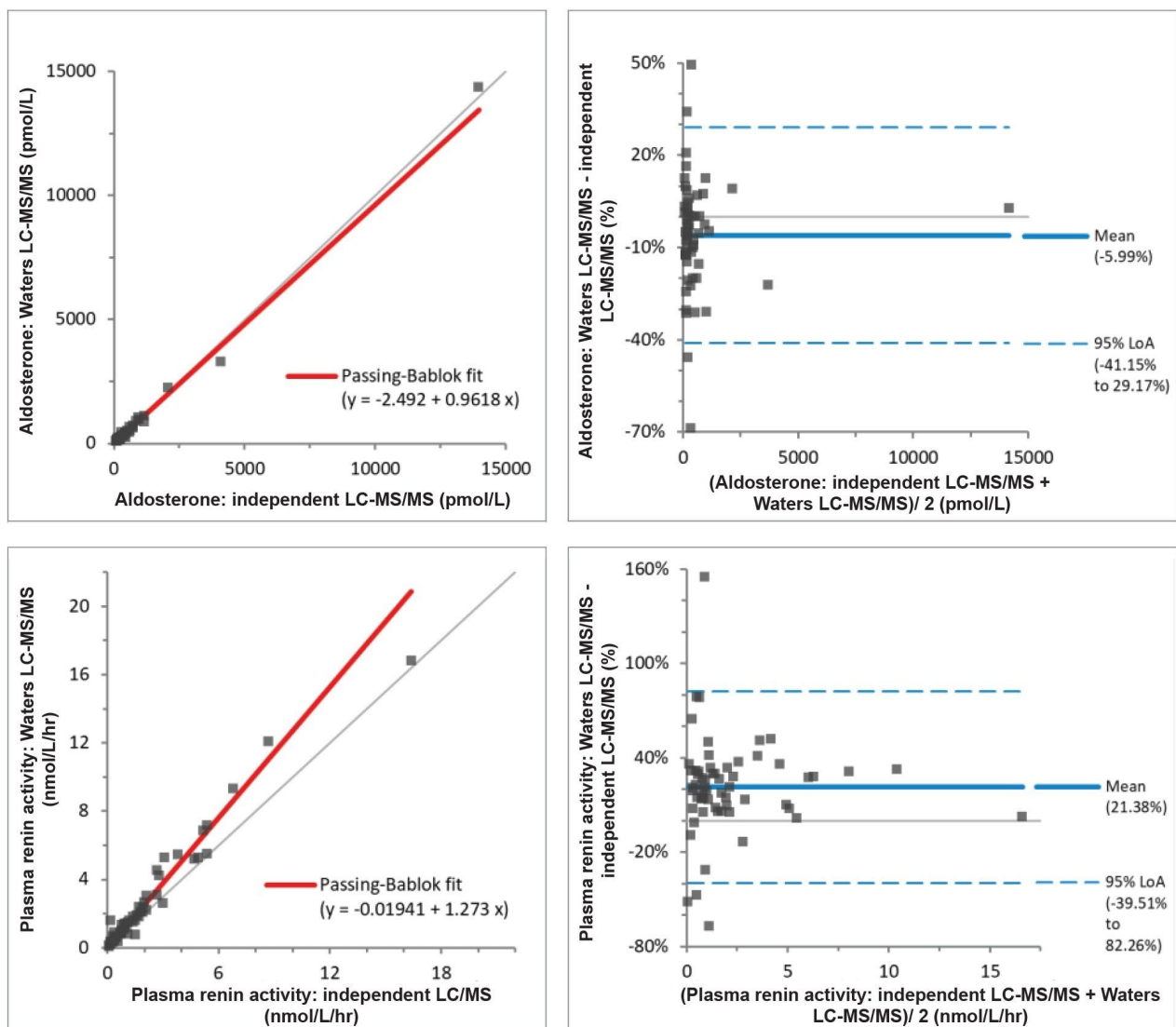
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Figure 5. Comparison of the Waters LC-MS/MS method to an independent LC-MS/MS method for aldosterone using (a) Passing-Bablok fit and (b) Altman-Bland agreement, and plasma renin activity using (c) Passing-Bablok fit and (d) Altman-Bland agreement.

A new benefit to the Xevo TQ Absolute Mass Spectrometer is the ability to use either argon or nitrogen as the collision gas. A direct comparison between a set of samples using the different gases, provided similar levels of analytical sensitivity. In addition, aldosterone comparison of 28 plasma samples provided a Deming fit of y=0.90x+7.87, with an Altman-Bland mean method bias of -8.2% using nitrogen. PRA comparison of 34 plasma

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samples provided a Deming fit of y=0.94x+0.09, with an Altman-Bland mean method bias of 1.7% using nitrogen.

Conclusion

An analytically sensitive and selective clinical research method has been developed for the analysis of aldosterone and plasma renin activity using the Xevo TQ Absolute Mass Spectrometer.

The Xevo TQ Absolute Mass Spectrometer enables the simultaneous analysis of physiologically low levels of aldosterone and plasma renin activity, 28 pmol/L and 0.8 nmol/L/hr respectively, using only 125 μL sample volume. Excellent precision performance across the calibration range have been demonstrated. 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. In addition, equivalent performance has been demonstrated between the use of argon and nitrogen as collision gas, which provides labs with an additional option during the installation of the Xevo TQ Absolute Mass Spectrometer for analysis of aldosterone and plasma renin activity.

Acknowledgements

Featured Products

MassTrak ACQUITY UPLC I-Class PLUS/Xevo TQ Absolute IVD System < https://www.waters.com/nextgen/global/products/mass-spectrometry-systems/masstrak-acquity-uplc-i-class-plus-xevo-tq-absolute-ivd-system.html>

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ Absolute Triple Quadrupole Mass Spectrometry < https://www.waters.com/nextgen/global/products/mass-spectrometry-systems/xevo-tq-absolute.html>

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MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

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