Simultaneous quantification of angiotensin I, aldosterone and cortisol using a single sample preparation

Using the Citrine Triple Quad system

J. Grace van der Gugten[1], Daniel T. Holmes[1] and Dan Blake[2]
[1]St. Paul's Hospital, Canada; [2]SCIEX, UK

The renin–angiotensin system (RAS), or renin–angiotensin–aldosterone system (RAAS), is a hormone system that regulates blood pressure, fluid and electrolyte balance and systemic vascular resistance. When renal blood flow is reduced, the enzyme, renin, is secreted into the bloodstream and is responsible for the generation of angiotensin I, which is subsequently converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor that causes blood vessels to narrow, resulting in increased blood pressure. Angiotensin II also stimulates the adrenal secretion of aldosterone, a hormone that causes the reabsorption of sodium and water into the blood, increasing the intravascular volume which, in turn, increases blood pressure. Additionally, cortisol, generated by closely related pathways, supports blood pressure regulation and responses to various physiologic stresses.

Scientists involved in cardiovascular research, particularly those investigating the causes and effects of high blood pressure, require simple methodologies to monitor the levels of these peptides and hormones in human plasma. The use of mass spectrometry as a detection system allows such methods to be developed. Here we present a method for the simultaneous detection of angiotensin I, aldosterone and cortisol in human plasma, using a single sample preparation and rapid chromatography. Figure 1 shows an example chromatogram that shows peaks for these 3 compounds in an extracted sample.

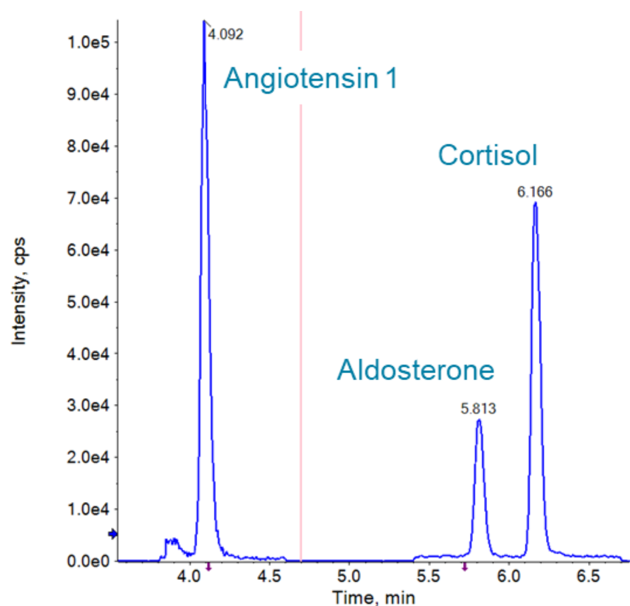

Figure 1. Combined chromatogram of all analytes using rapid polarity switching. Peaks are shown for the simultaneous analysis of angiotensin 1 in positive mode (4.04 minutes) and aldosterone (5.78 minutes) and cortisol (6.12 minutes) in negative mode**.**

Key features of the Citrine Triple Quad system for the simultaneous quantification of angiotensin I, aldosterone and cortisol

- Robust technology allows direct quantification of angiotensin I, aldosterone and cortisol using a single, automatable sample preparation and fast chromatography
- The high sensitivity of the Citrine Triple Quad system allows the required limits of detection to be achieved, particularly for aldosterone
- A targeted MRM workflow with rapid polarity switching allows sensitive detection with high selectivity
- The reduced matrix effects associated with a tandem mass spectrometry approach give higher confidence in results

Methods

Sample preparation: Sample preparation from 250 µL human plasma involved initial generation of angiotensin I via a buffered incubation process, followed by solid phase extraction. A summary of the method is as follows:

- Generation of angiotensin I, by
    - Addition of aldosterone (Aldo) and cortisol (Cort) stable isotope-labeled internal standards (IS) in buffer
    - Incubation at 37°C for 1 hour
    - Addition of angiotensin I (Ang-I) stable isotope-labeled internal standard in formic acid
- Solid Phase extraction using an Evolute Express 30 mg 96-well plate (Biotage) and a 2-stage elution process, consisting of an alkaline methanol/water elution for angiotensin I and an MTBE/ethyl acetate elution for aldosterone and cortisol, separated by a plate drying step
- Evaporation of combined elutes and reconstitution in mobile phase for injection

Chromatography: Chromatographic separation was achieved using a Gemini 3 µm, NX-C18 110A, 50 x 2 mm (Phenomenex, PN 00B-4453-B0) column. Mobile phase A was formic acid in water and mobile phase B was methanol. Mobile phase B was applied over a chromatographic runtime of 10.8 minutes.

Mass spectrometry: Mass spectrometry analysis was performed on the Citrine Triple Quad system, operated in both positive and negative electrospray ionization modes within individual time windows that depended on retention time. Compound-dependent parameters were optimized for all compounds and their corresponding internal standards. 3 MRM transitions were analyzed for angiotensin I, 2 MRM transitions were analyzed for both the aldosterone and cortisol ions and 1 MRM transition was analyzed for each of the 3 internal standards used in the method (Tables 1 and 2).

Table 1. Mass spectrometry parameters for target analytes, positive ESI window (0 – 4.7 minutes).

Analyte	Q1 (Da)	Q3 (Da)	DP	CE	CXP
Ang-I 1	*433.2*	*647.5*	*100*	*25*	*30*
Ang-I 2	*433.2*	*619.3*	*100*	*25*	*30*
Ang-I 3	*433.2*	*110.0*	*100*	*30*	*30*
Ang-I IS	*436.6*	*657.5*	*100*	*25*	*30*

Table 2. Mass spectrometry parameters for target analytes, negative ESI window (4.7 – 10.8 minutes).

Analyte	Q1 (Da)	Q3 (Da)	DP	CE	CXP
Aldo 1	*359.2*	*189.0*	*-80*	*-26*	*-15*
Aldo 2	*359.2*	*331.3*	*-65*	*-18*	*-15*
Aldo IS 1	*366.2*	*338.3*	*-80*	*-20*	*-15*
Aldo IS 2	*366.2*	*194.0*	*-80*	*-26*	*-15*
Cort 1	*361.3*	*282.1*	*-100*	*-35*	*-15*
Cort 2	*361.3*	*297.1*	*-100*	*-35*	*-15*
Cort IS	*365.3*	*127.8*	*-100*	*-35*	*-15*

Data processing: Quantitation was carried out using Analyst MD software, version 1.6.3. Standard curves were generated using linear fits with a 1/x weighting.

Analytical sensitivity

Analytical sensitivity was investigated with a series of calibration standards prepared in matrix and processed using the procedure outlined above. Figure 2 shows the chromatograms generated from the lowest combined matrix calibrator, with signal/noise calculations based on peak-to-peak parameters.

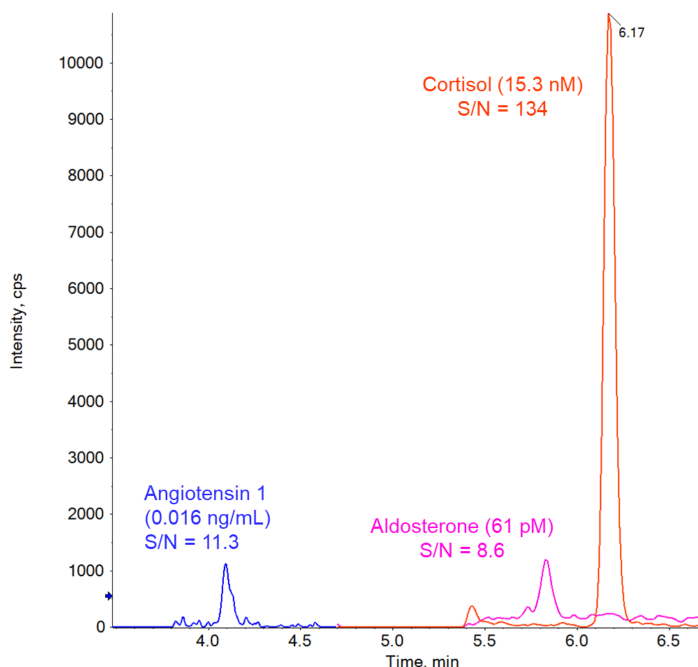

Figure 2. Chromatogram of the lowest combined matrix calibrator. MRM traces and peak-to-peak signal/noise values shown for angiotensin-1, aldosterone and cortisol.

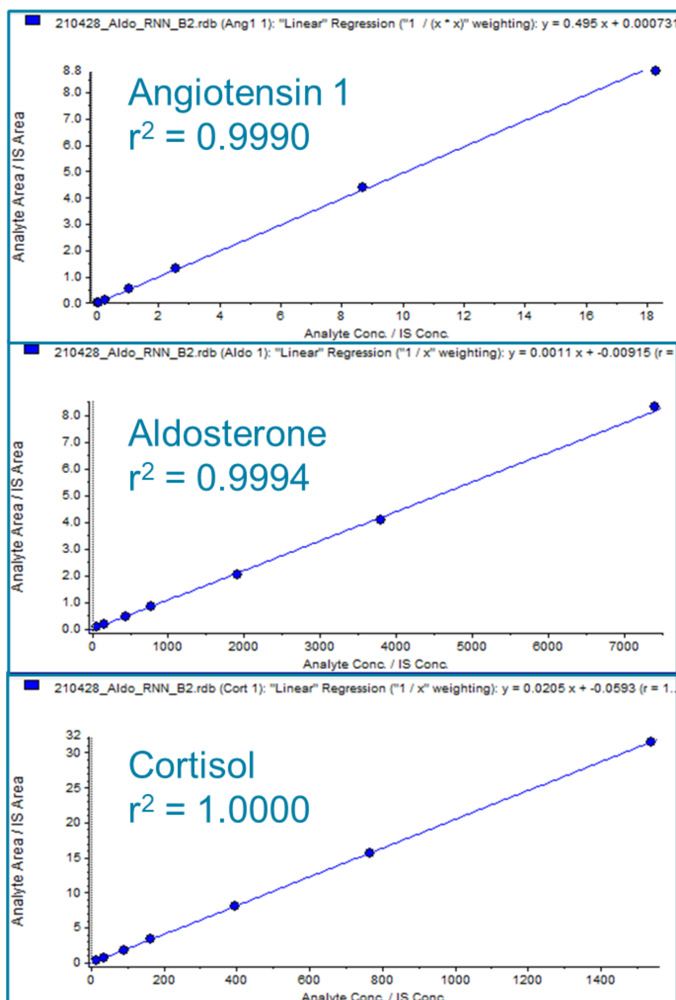

Figure 3. Calibration curve of the primary MRM transition of the each of the compounds reported. The calibration curve using the matrix calibrators was analyzed across established concentration ranges and all linearity (R^2) values were >0.999

Analytical linearity

A series of calibrators were prepared in matrix to assess linearity over the proposed analytical range required for the quantification of all analytes. The calibration ranges used for angiotensin 1, aldosterone and cortisol were 0.016–18 ng/mL, 60–7410 pmol/L and 15.3–1540 nmol/L, respectively. Figure 3 shows a calibration curve for the primary MRM transition of each of these analytes, analyzed as described.

Precision and accuracy

Within-run precision was demonstrated using replicates (n=5) of 3 pools of serum at separate low, medium and high concentrations across the range investigated, processed and quantified, as described. Table 3 shows the combined mean and %CV of each of the pools of serum for each analytes quantified.

Accuracy was demonstrated using value-assigned external quality control samples (German Society for Clinical Chemistry, dgkl.de) that were previously analyzed by GC-MS for steroids and antibody-based methods for angiotensin I. Angiotensin I concentration is expressed as plasma renin activity (PRA) in units of ng/L/s, using a conversion factor that accounts for the incubation time used in the generation stage of the sample preparation. Tables 4-6 show the individual and mean accuracy values across all concentrations analyzed, for aldosterone, cortisol and angiotensin I/PRA, respectively.

Table 3. Precision.

	Mean	SD	%CV
Angiotensin I			
Low pool	*0.36 ng/mL*	*0.037*	*10.3*
Medium pool	*1.14ng/mL*	*0.075*	*6.6*
High pool	*10.4 ng/mL*	*1.015*	*9.7*
Aldosterone			
Low pool	*58.6 pmol/L*	*5.75*	*9.8*
Medium pool	*195.3 pmol/L*	*12.8*	*6.5*
High pool	*1054 pmol/L*	*41.7*	*4.0*
Cortisol			
Low pool	*286.2 nmol/L*	*14.9*	*5.2*
Medium pool	*145.2 nmol/L*	*5.91*	*4.1*
High pool	*872.0 nmol/L*	*29.4*	*3.4*

Table 4. Aldosterone accuracy.

Sample	Result (pmol/L)	Target (pmol/L)	% Accuracy
DKGL HM 1-21 A	*249*	*280*	*88.9*
DKGL HM 1-21 B	*1400*	*1410*	*99.3*
DKGL HM 2-20 A	*1130*	*1029*	*109.8*
DKGL HM 2-20 B	*1570*	*1697*	*92.5*
DKGL HM 2-21 A	*1000*	*983*	*101.7*
DKGL HM 2-21 B	*233*	*251*	*92.8*
DKGL HM 3-20 A	*388*	*456*	*85.1*
DKGL HM 3-20 B	*413*	*468*	*88.2*
DKGL HM 4-20 A	*241*	*265*	*90.9*
DKGL HM 4-20 B	*661*	*677*	*97.6*
Mean accuracy			***94.7***

Table 5. Cortisol accuracy.

Sample	Result (pmol/L)	Target (pmol/L)	% Accuracy
DKGL HM 1-21 A	*309*	*312*	*99.0*
DKGL HM 1-21 B	*899*	*926*	*97.1*
DKGL HM 2-20 A	*949*	*1030*	*92.1*
DKGL HM 2-20 B	*296*	*283*	*104.6*
DKGL HM 2-21 A	*590*	*587*	*100.5*
DKGL HM 2-21 B	*688*	*732*	*94.0*
DKGL HM 3-20 A	*463*	*480*	*96.5*
DKGL HM 3-20 B	*358*	*356*	*100.6*
DKGL HM 4-20 A	*739*	*732*	*101.0*
DKGL HM 4-20 B	*331*	*333*	*99.4*
Mean accuracy			***98.4***

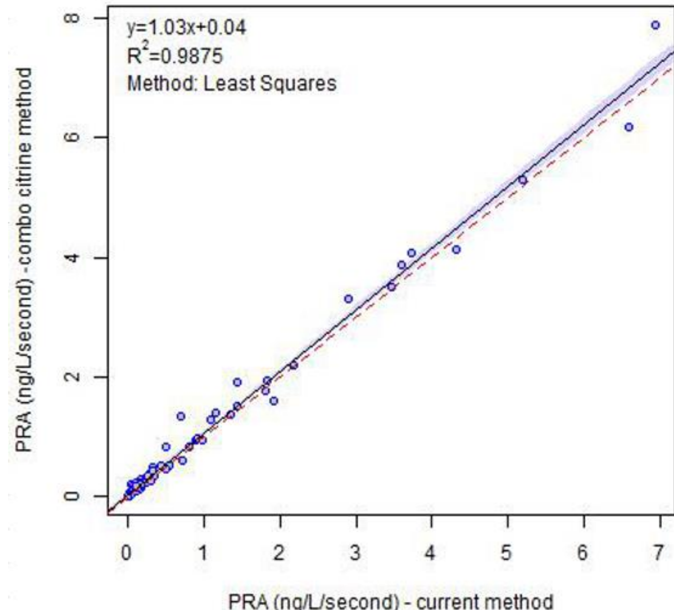

Figure 4. Least squares regression plot of angiotensin I (expressed as PRA, ng/L/s). Results shown across all concentrations analyzed, R^2=0.9875.

Table 6. Angiotensin I, expressed as PRA, accuracy.

Sample	Result (ng/L/s)	Target (ng/L/s)	% Accuracy
DKGL HP 1-21 A	*0.753*	*0.861*	*87.4*
DKGL HP 1-21 B	*1.208*	*1.225*	*98.6*
DKGL HP 2-21 A	*0.806*	*0.761*	*105.8*
DKGL HP 2-21 B	*0.839*	*0.972*	*86.3*
Mean accuracy			***94.5***

Comparison with existing MS-based assays

To show acceptable performance when compared with established assays, a series (n=87) of pre-analyzed samples were processed by the proposed methodology. The comparison between results generated by established[1] and proposed methodologies are displayed as ordinary least squares regression plots in Figures 4–6 for each of the compounds analyzed.

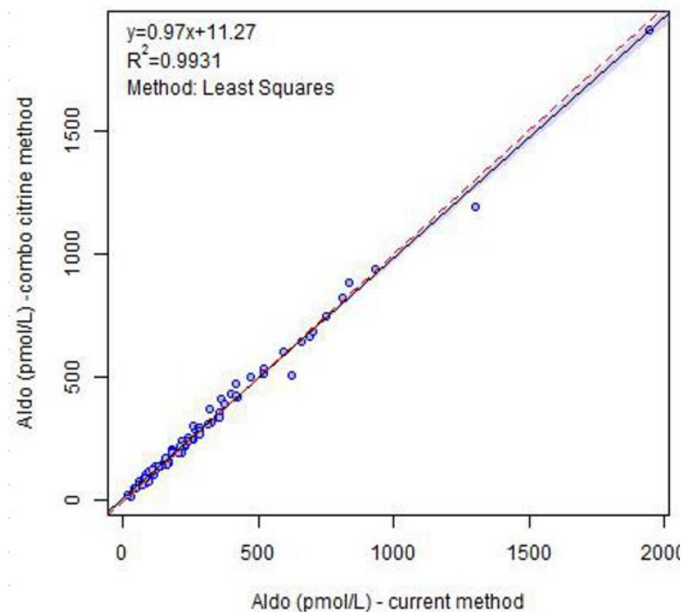

Figure 5. Least squares regression plot of aldosterone (pmol/L). Results shown across all concentrations analyzed, R^2=0.9931.

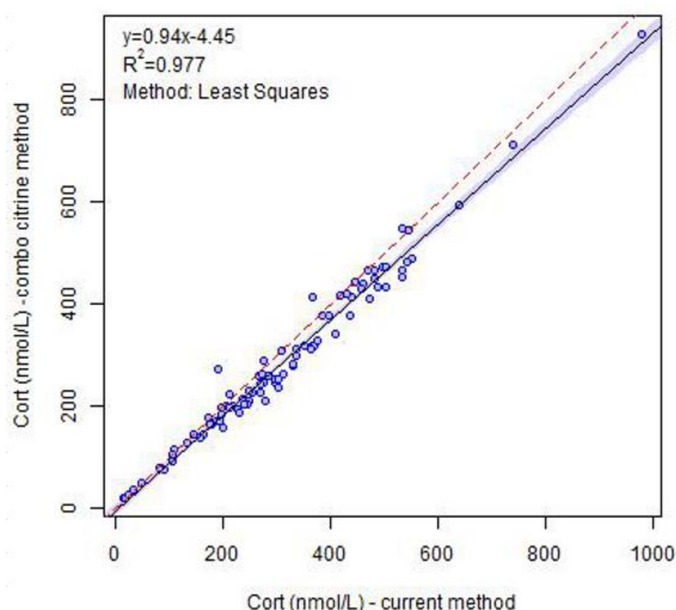

Figure 6. Least squares regression plot of cortisol (nmol/L). Results shown across all concentrations analyzed, R^2=0.977.

Conclusions

The data shown here were generated from a method developed for the sensitive and simultaneous quantification of angiotensin I, aldosterone and cortisol in human plasma. The workflow consisted of 3 main automatable steps for the generation of angiotensin I by incubation, solid phase extraction and evaporation and reconstitution of combined elutes, followed by a short (<11 min) polarity switched LC-MS/MS analysis.

The assay demonstrated the following performance parameters:

- Good sensitivity, evidenced by signal/noise ratios of 11.3, 8.6 and 134 for 0.016 ng/mL, 60 pmol/L and 15.3 nmol/L samples of angiotensin I, aldosterone and cortisol, respectively
- Linearity was defined over an appropriate, analyte-specific dynamic range and all analytes returned R^2 values of at least 0.999
- Precision, calculated as %CV using pooled serum at 3 concentrations, ranged from 3.4–10.3% for all analytes across all concentrations analyzed
- Accuracy, calculated using external quality control samples previously analyzed by alternative methods, ranged from 85.1–109.8% across all concentrations and analytes, with means of 94.5%, 94.7% and 98.4% for angiotensin I, aldosterone and cortisol, respectively
- Comparisons of previously analyzed samples between this and established MS-based assays for the quantification of aldosterone, cortisol and angiotensin I show good agreement across all concentration ranges analyzed

References

1. Van Der Gugten J.G., Holmes D.T. (2016) Quantitation of Plasma Renin Activity in Plasma Using Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). In: Garg U. (eds) Clinical Applications of Mass Spectrometry in Biomolecular Analysis. Methods in Molecular Biology, vol 1378. https://doi.org/10.1007/978-1-4939-3182-8_26

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