

SIMULTANEOUS MEASUREMENT OF ALDOSTERONE AND PLASMA RENIN ACTIVITY IN HUMAN SERUM USING LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY (LC-MS/MS) FOR CLINICAL RESEARCH



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Link to Application Note

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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. Here we evaluate a single LC-MS/MS method for the combined measurement of aldosterone and PRA for clinical research purposes.

ANALYTICAL STRATEGY

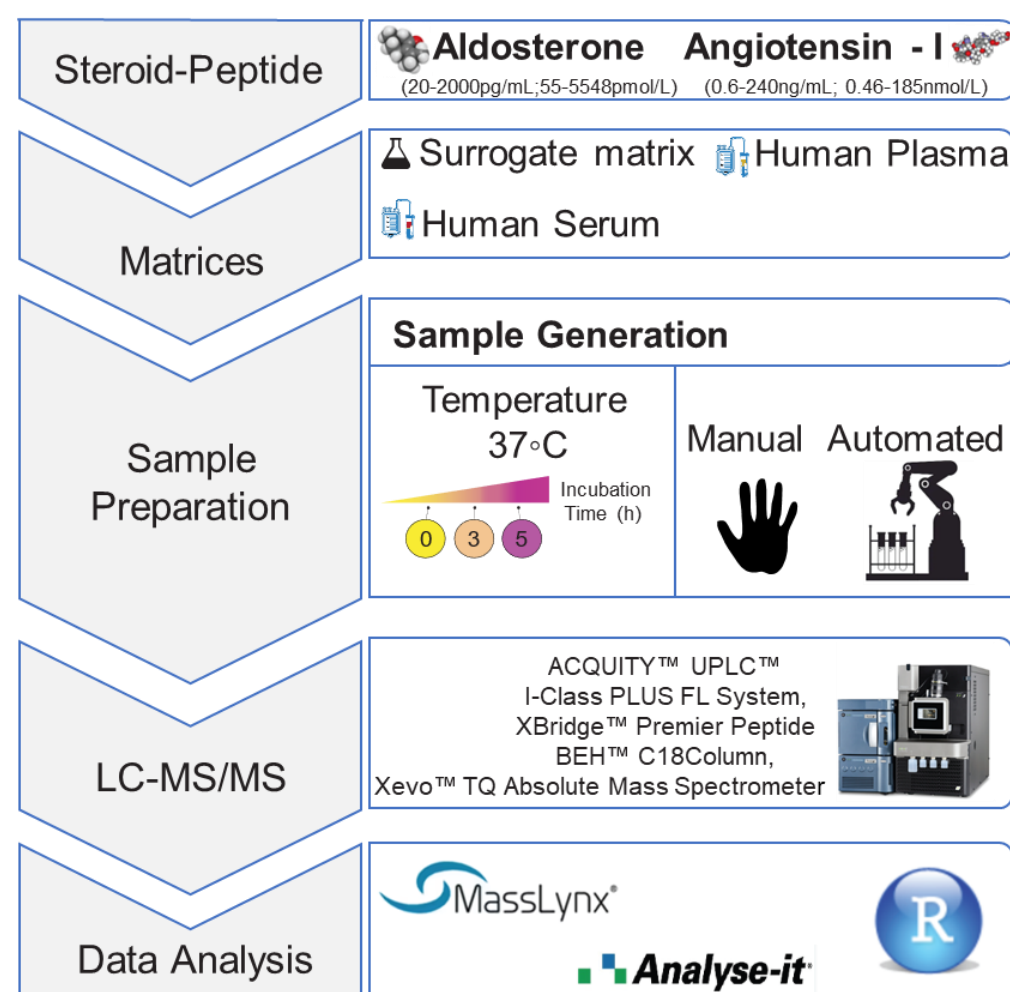
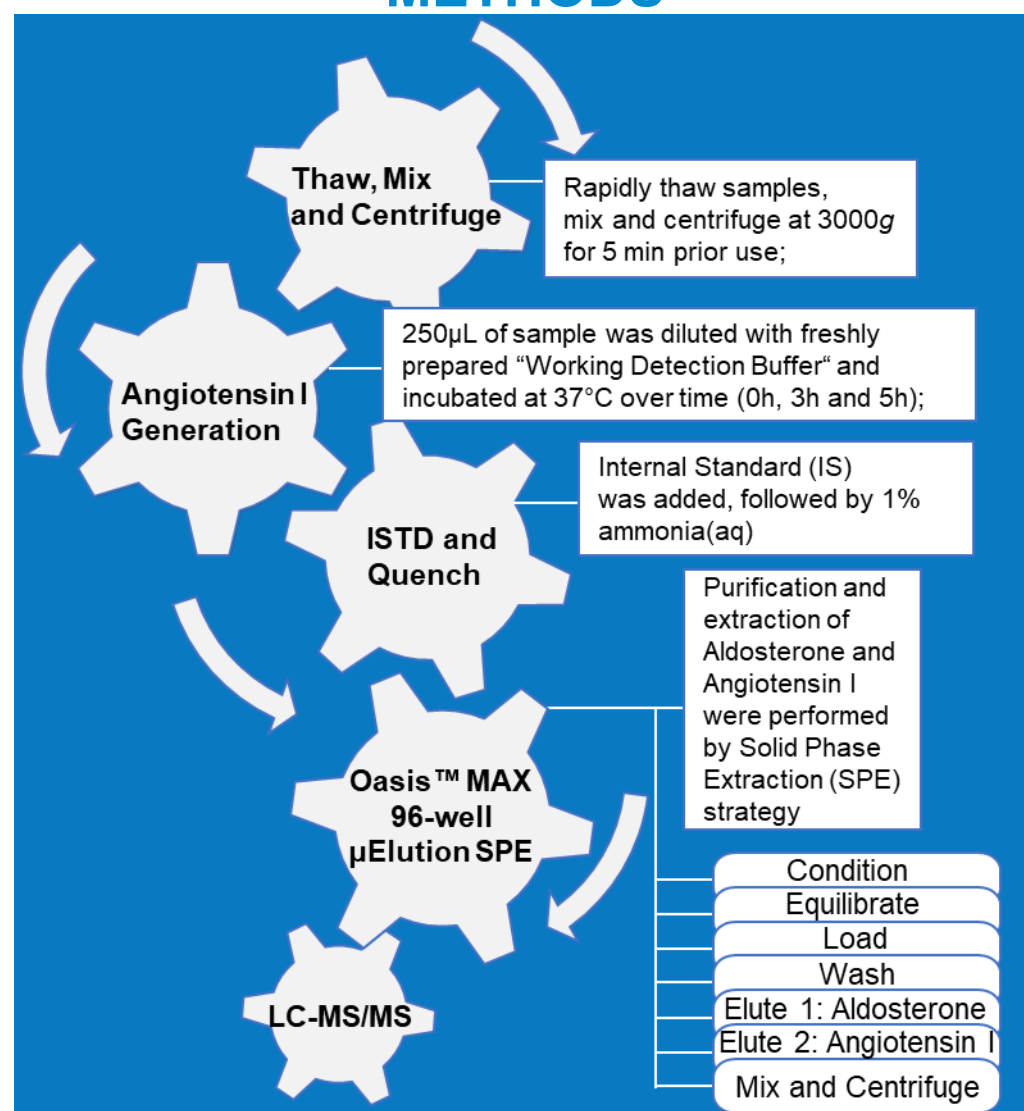


FIGURE 1. Analytical workflow. LC-MS/MS quantification of Aldosterone and Renin Activity (Angiotensin I) in human matrices.

METHODS



RESULTS

Matrix Effects Evaluation

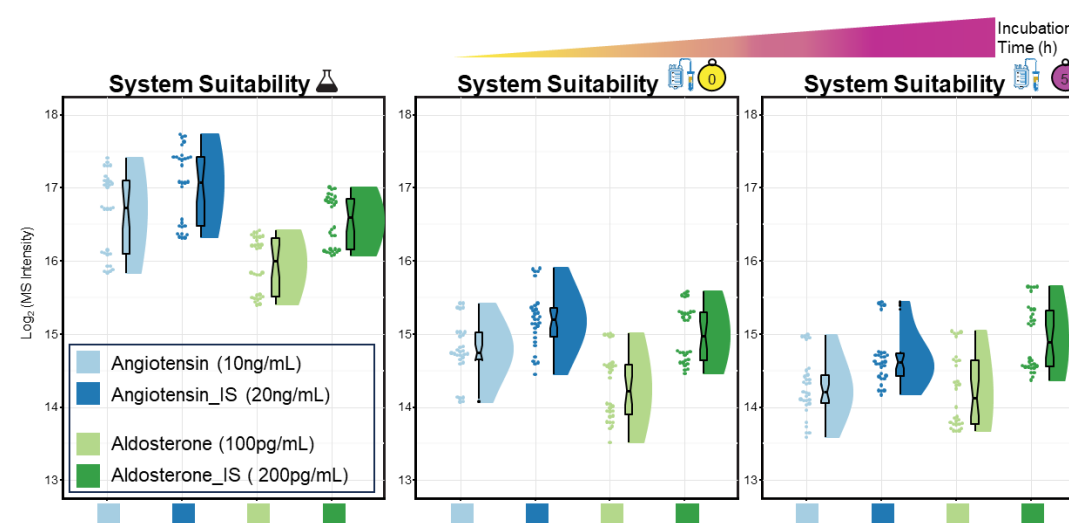


FIGURE 2. Raincloud plot analysis quantifies the impact of the matrices on the MS signal enhancement. Successful analytical reliability of the LC-MS/MS methodology, moving from surrogate matrices into human matrices was achieved.

Chromatograms of Angiotensin I and Aldosterone over time.

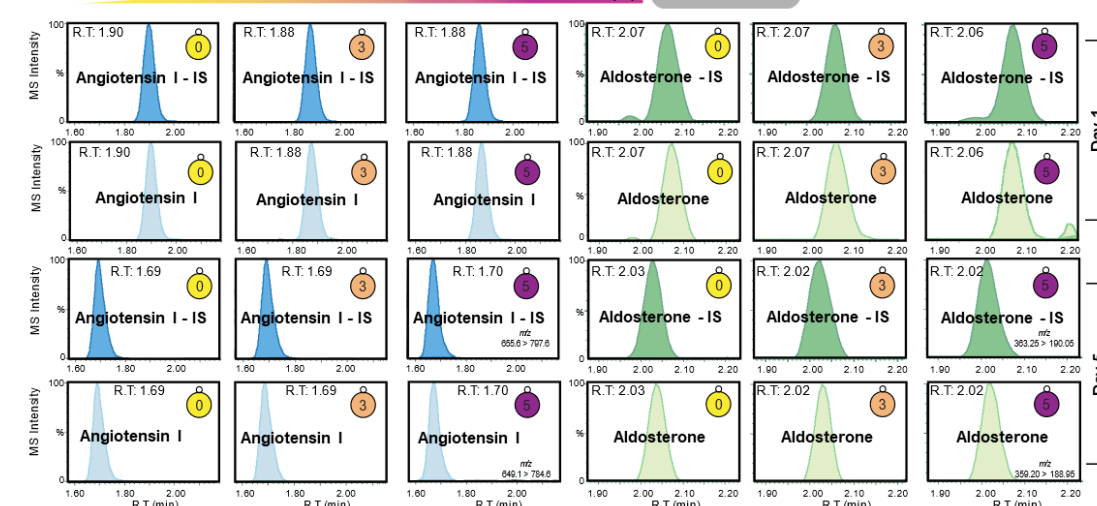
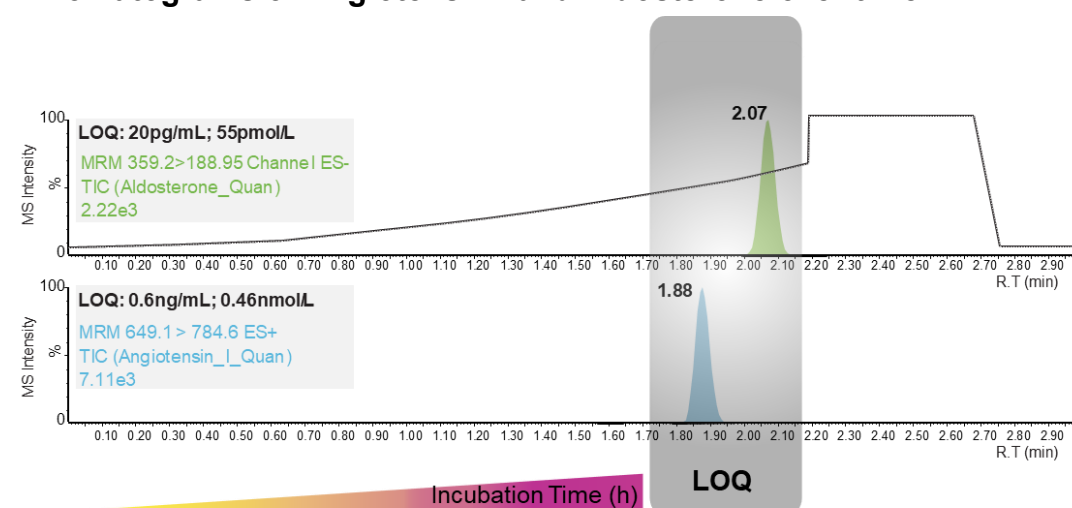


Figure 3. FIGURE 3. Selected peaks correspond to the limit of Quantification (LOQ) of both analytes over different incubation time. A small and not significant shift in retentiontime (≤ 0.20 sec) was detected for Angiotensin I, while Aldosterone chromatographic reproducibility was not impacted.

Accuracy, Reproducibility and Precision

Incubation (h)	Aldosterone Calibration Range (20 - 2000pg/mL; 55 - 5548 pmol/L)				Angiotensin I Calibration Range (0.6 - 240 ng/mL; 0.5 - 185 nmol/L/h)			
	Nominal Conc (pg/mL)	Nominal Conc (pmol/L)	Calc. Mean Conc (pg/mL)	Total (%CV) <15%	Nominal Conc (ng/mL)	Nominal Conc (nmol/L/h)	Calc. Mean Conc (ng/mL)	Total (%CV) <15%
0	55	20	20.6	10.2 (-)	0.5	0.6	0.6	6.1 (+)
3	277	100	102.0	7.5 (-)	1.9	2.4	2.5	5.6 (+)
5	832	300	285.4	5.9 (-)	9.3	12.0	13.2	2.2 (+)
0	2774	1000	1007.0	4.3 (-)	37.0	48.0	52.3	2.3 (+)
3	5548	2000	2030.5	5.9 (-)	93.0	120.0	126.1	1.9 (+)
5	55	20	21.0	6.1 (-)	185.0	240.0	228.7	1.5 (+)
3	111	40	40.9	9.4 (-)	0.2	0.6	0.6	5.4 (+)
5	277	100	98.2	7.7 (-)	0.1	12.0	13.3	1.8 (+)
0	832	300	284.1	7.8 (-)	12.0	48.0	52.4	2.0 (+)
3	2774	1000	1014.5	7.4 (-)	0.4	2.4	2.6	3.6 (+)
5	5548	2000	2013.3	7.1 (-)	1.9	12.0	13.9	2.5 (+)
0	55	20	20.0	6.6 (-)	7.4	48.0	53.3	2.2 (+)
3	111	40	40.1	12.9 (-)	37.0	240.0	228.3	2.2 (+)
5	277	100	97.4	6.0 (-)	0.2	0.6	0.6	5.4 (+)
0	832	300	284.2	6.7 (-)	0.1	0.6	0.6	5.3 (+)
3	2774	1000	1021.7	3.6 (-)	0.4	2.4	2.6	3.6 (+)
5	5548	2000	2032.3	6.7 (-)	19.0	120.0	125.5	1.4 (+)

Incubation (h)	Aldosterone: QC range (35 - 1250 ng/mL; 97 - 3468 pmol/L) - Spec <15%				Angiotensin I QC range (2 - 125 ng/mL) - Spec <15%			
	QC1	QC2	QC3	QC4	QC1	QC2	QC3	QC4
0	35	100	250	1250	35	100	250	1250
3	13.4%	13.1%	8.9%	2.8%	3.3%	3.2%	2.9%	0.8%
5	13.1%	10.2%	10.5%	7.6%	12.2%	9.1%	9.8%	7.6%

Incubation (h)	Plasma Renin Activity (PRA) Range				Total Precision (% CV)				Total Repeatability (% CV)			
	nmol/L/h	2 (ng/mL)	5 (ng/mL)	25 (ng/mL)	QC1	QC2	QC3	QC4	QC1	QC2	QC3	QC4
0	1.5-96.4	13.0%	8.9%	2.8%	3.3%	3.2%	2.9%	0.8%	0.8%	0.6%	0.6%	
3	0.5-32.1	9.1%	6.3%	4.0%	3.2%	2.1%	2.3%	1.7%	1.7%	1.5%	1.5%	
5	0.3-19.3	7.0%	4.9%	2.6%	2.7%	2.1%	1.4%	0.9%	0.9%	0.7%	0.7%	

Table 1. The sensitivity of the tests was demonstrated by extracting and quantifying from two replicate serum samples on two occasions per day over five separate days and across different incubation time: (n=20x0h, n=20x3h, n=20x5h). High reproducibility, repeatability were determined by extracting four levels of QC material over five separate days and performed across several incubation times, indicating overall a total precision and repeatability of $\leq 15\%$ CV for both analytes.

Method Comparison

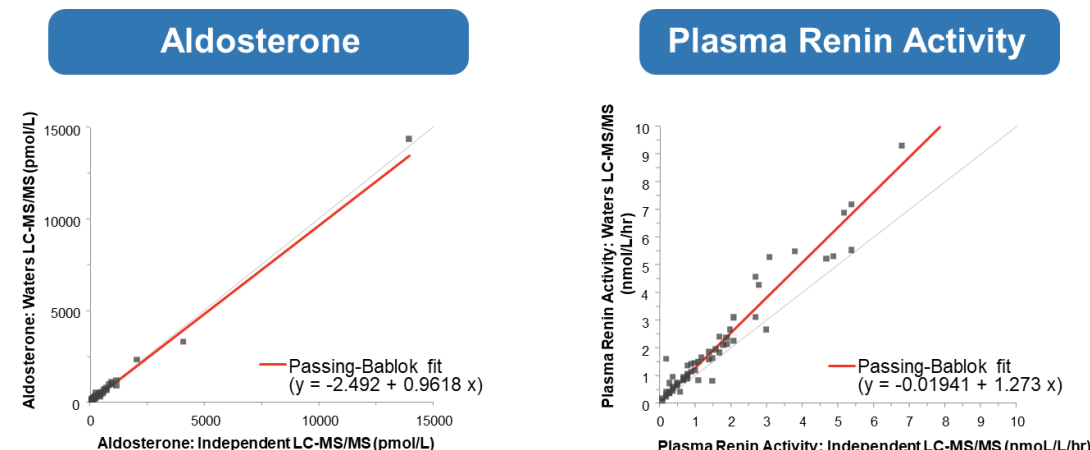


Figure 4. Comparison of 58 Aldosterone samples provided a Passing-Bablok fit of $y=0.96x-2.49$ with an Altman-Bland agreement mean method bias of -6.0%; Comparison of 61 Plasma Renin Activity samples provided a Passing-Bablok fit of $y=1.26x-0.02$ with an Altman-Bland agreement mean method bias 21.4%;

Endurance and Stability

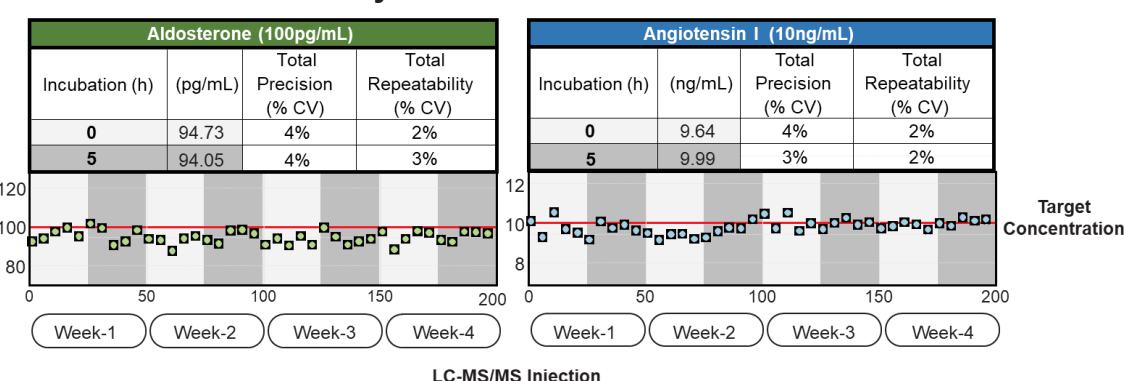


Figure 5 and Table 2. Evaluation of SST samples freeze/thaw cycles over 20 days at different incubation times. Each dot correspond to an average of five biological replicates

Comparison Sample Preparation: Automated v Manual

Sample	Aldosterone		Manual		Automated	
	Nominal Conc (pmol/L)	Nominal Conc (pg/mL)	Calculated Mean Conc (pg/mL)	Total (%CV)	Calculated Mean Conc (pg/mL)	Total (%CV)
Cal 0	0	0	0	0	0	0
Cal 1	55.5	20	19.9	7.7	20.2	3.4
Cal 2	111.0	40	40.7	8.4	41.7	3.5
Cal 3	277.4	100	102.0	5.6	98.8	2.8
Cal 4	832.2	300	300.0	5.0	290.9	2.0
Cal 5	2774.0	1000	998.2	5.6	975.1	2.1
QC 1	97.1	35	36.1	8.3	36.7	3.5
QC 2	277.4	100	99.6	7.7	98.5	3.2
QC 3	693.5	250	247.0	5.2	252.4	2.8
QC 4	3467.5	1250	1229.4	3.9	1234.4	2.2

Sample	Angiotensin I		Manual		Automated	
	Nominal Conc (nmol/L)	Nominal Conc (ng/mL)	Calculated Mean Conc (ng/mL)	Total (%CV)	Calculated Mean Conc (ng/mL)	Total (%CV)
Cal 0	0	0	0	0	0	0
Cal 1	0.5	0.6	0.6	7.0	0.6	4.6
Cal 2	1.9	2.4	2.6	5.6	2.6	2.4
Cal 3	9.3	12.0	12.8	2.5	13.0	1.5
Cal 4	37.0	48.0	52.6	2.0	52.6	1.6
Cal 5	92.5	120.0	127.0	2.3	125.0	1.0
Cal 6	185.0	240.0	228.0	1.9	229.1	2.1
QC 1	1.5	2.0	2.0	6.2	2.1	3.0
QC 2	3.9	5.0	5.2	3.1	4.8	2.6
QC 3	19.3	25.0	24.3	5.4	26.8	1.4
QC 4	96.4	125.0	128.7	2.0	129.0	1.2

Table 3. High reproducibility, repeatability and accuracy in both sample preparation strategies showed excellent method robustness. The successful automated method on the Hamilton MicroLab Star reduced %CV and improved the manufacturing process.

CONCLUSION

- An effective LC-MS/MS method for the quantification of the main components of the RAAS pathway is achieved.
- A single test that improves instrument utilization, reducing cost and allowing the user to expand their existing test menu on the same platform.

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