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



Link to Application Note

Giorgio Oliveiro¹, Rosilene Burgos¹, Dominic Foley², Good Bosh³ and Leanne Davey¹

1) Waters Technology, IDA Business Park, Drinagh, Wexford, Ireland, 2) Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, Cheshire, UK. 3) Waters S.A.S. Saint-Quentin en Yvelines Cedex, France.

INTRODUCTION

The Renin-Angiotensin-Aldosterone System (RAAS) is critical in Matrix Effects Evaluation 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



FIGURE 2. Raincloud plot analysis quantifies the impact of the matrices on the MS signal en-hancement. 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.



0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60 1.80 1.90 2.00 2.10 2.20 2.30 2.40 2.50 2.60 2.70 2.80 2.90 R T (min)





Method Comparison Aldosterone Plasma Renin Activity Passing-Bablok fit (y = -2.492 + 0.9618 x) Passing-Bablok fit (y = -0.01941 + 1.273 x) 10000 2 3 4 5 6 7 8 9 10 nt LC-MS/MS (pmol/L) ent LC-MS/MS (nmoL/L/hr

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



LC-MS/MS Injection

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

ŀ	Aldosterc	one	Manual	⋓	Automated		
Sample	TAR	GET	R^2 0.9	977	R^2 0.9987		
n=8	Nominal Conc (pmol/mL)	Nominal Conc (pg/mL)	Calculated Mean Conc (pg/mL)	Total (%CV)	Calculated Mean Conc png/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	
Cal_6	5548.0	2000	1995.5	3.0	2026.5	2.4	
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	
A	ngiotens	sin I	Manual	⋓	Automate	d 📕	
A	Angiotens TAR	sin I _{GET}	Manual R^2 0.99	9525	Automate R^2 0.99	d	
A Sample n=8	Angiotens TAR Nominal Conc (nmol/mL)	GET Nominal Conc (ng/mL)	Manual R^2 0.99 Calculated Mean Conc (ng/mL)	0525 Total (%CV)	Automate R^2 0.99 Calculated Mean Conc (ng/mL)	d for the second	
A Sample n=8 Cal_0	Angiotens TAR Nominal Conc (nmol/mL) 0	GET Nominal Conc (ng/mL) 0	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0	U525 Total (%CV) 0	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0	d 0625 Total (%CV) 0	
A Sample n=8 Cal_0 Cal_1	Angiotens TAR Nominal Conc (nmol/mL) 0 0.5	GET Nominal Conc (ng/mL) 0 0.6	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6	0 7.0	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6	d	
A Sample n=8 Cal_0 Cal_1 Cal_2	Angiotens TAR Nominal Conc (nmol/mL) 0 0.5 1.9	GET Nominal Conc (ng/mL) 0 0.6 2.4	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6	0 7.0 5.6	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6	d .6 0625 Total (%CV) 0 4.6 2.4	
A Sample n=8 Cal_0 Cal_1 Cal_2 Cal_3	Angiotens TAR Nominal Conc (nmol/mL) 0 0.5 1.9 9.3	GET Nominal Conc (ng/mL) 0 0.6 2.4 12.0	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 12.8	0 7.0 5.6 2.5	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 13.0	d .625 Total (%CV) 0 4.6 2.4 1.5	
Sample n=8 Cal_0 Cal_1 Cal_2 Cal_3 Cal_4	TAR Nominal Conc (nmol/mL) 0 0.5 1.9 9.3 37.0	GET Nominal Conc (ng/mL) 0 0.6 2.4 12.0 48.0	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 12.8 52.6	0 7.0 5.6 2.5 2.0	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 13.0 52.6	d	
Sample n=8 Cal_0 Cal_1 Cal_2 Cal_3 Cal_4 Cal_5	TAR Nominal Conc (nmol/mL) 0 0.5 1.9 9.3 37.0 92.5	GET Nominal Conc (ng/mL) 0 0.6 2.4 12.0 48.0 120.0	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 12.8 52.6 127.0	0 5.6 2.5 2.0 2.3	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 13.0 52.6 125.0	d	
Sample n=8 Cal_0 Cal_1 Cal_2 Cal_3 Cal_4 Cal_5 Cal_6	TAR Nominal Conc (nmol/mL) 0 0.5 1.9 9.3 37.0 92.5 185.0	GET Nominal Conc (ng/mL) 0 0.6 2.4 12.0 48.0 120.0 240.0	Manual R^2 0.98 Calculated Mean Conc (ng/mL) 0 0.6 2.6 12.8 52.6 127.0 228.0	0 525 Total (%CV) 0 7.0 5.6 2.5 2.0 2.3 1.9	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 13.0 52.6 125.0 229.1	d	
Sample n=8 Cal_0 Cal_1 Cal_2 Cal_3 Cal_4 Cal_5 Cal_6 QC_1	TAR Nominal Conc (nmol/mL) 0 0.5 1.9 9.3 37.0 92.5 185.0 1.5	GET Nominal Conc (ng/mL) 0 0.6 2.4 12.0 48.0 120.0 240.0 2.0	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 12.8 52.6 127.0 228.0 2.0	0 525 Total (%CV) 0 7.0 5.6 2.5 2.0 2.3 1.9 6.2	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 13.0 52.6 125.0 229.1 2.1	d	
Sample n=8 Cal_0 Cal_1 Cal_2 Cal_3 Cal_4 Cal_5 Cal_6 QC_1 QC_2	TAR Nominal Conc (nmol/mL) 0 0.5 1.9 9.3 37.0 92.5 185.0 1.5 3.9	GET Nominal Conc (ng/mL) 0 0.6 2.4 12.0 48.0 120.0 240.0 2.0 5.0	Manual R ² 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 12.8 52.6 127.0 228.0 2.0 5.2	0 525 Total (%CV) 0 7.0 5.6 2.5 2.0 2.3 1.9 6.2 3.1	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 13.0 52.6 125.0 229.1 2.1 4.8	d	
Sample n=8 Cal_0 Cal_1 Cal_2 Cal_3 Cal_4 Cal_5 Cal_6 QC_1 QC_2 QC_3	TAR Nominal Conc (nmol/mL) 0 0.5 1.9 9.3 37.0 92.5 185.0 1.5 3.9 19.3	GET Nominal Conc (ng/mL) 0 0.6 2.4 12.0 48.0 120.0 240.0 2.0 5.0 25.0	Manual R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 12.8 52.6 127.0 228.0 2.0 5.2 24.3	0 525 Total (%CV) 0 7.0 5.6 2.5 2.0 2.3 1.9 6.2 3.1 5.4	Automate R^2 0.99 Calculated Mean Conc (ng/mL) 0 0.6 2.6 13.0 52.6 125.0 229.1 2.1 4.8 26.8	d 0625 Total (%CV) 0 4.6 2.4 1.5 1.6 1.0 2.1 3.0 2.6 1.4	

RESULTS



Figure analy sec) v not in	e 3. I tes o vas o npaci	FIGUR over difi detecte ted.	E 3. So ferent l ed for A	electec incuba \ngiote	l peak tion tin nsin I,	s cor ne. A whil	rrespo A sma le Ala	ond all ar loste	to the nd not erone o	limit o signific chroma	f Quan cant sl atograj	tification (LOQ) of both nift in retentiontime (≤0.20 ohic reproducibility was
Αссι	irac	y, Re	prod	ucibi	lity a	nd F	Prec	isio	on			
	Aldo (20 - 2	sterone Ca 000pg/mL:	55 - 5548 g	ange mol/L)			Angio (0.6 -)	tensin 240 na/	mL: 0.5 – 1	on Range 185 nmol/L	/h)	Table 1 The sensitivity
ncubation (h)	Nomina Conc (pmol/L	I Nominal Conc (pg/mL)	Calc.Mean Conc (pg/mL)	Total (%CV) Spec r <15%	ESI Inc	ubation (h)	Nominal Conc (nmol/L/h	Nomir Con (ng/m	al Calc.Me c Conc iL) (ng/m	ean Total (%CV) (Spec <15%	ESI Mode	of the tests was demon- strated by extracting and
0	55 111 277 832 2774 5548	20 40 100 300 1000 2000	20.6 39.6 102.0 285.4 1007.0 2030.5	10.2 7.1 7.5 5.9 4.3 5.9	(-) (-) (-) (-) (-)	0	0.5 1.9 9.3 37.0 93.0 185.0	0.6 2.4 12.0 48.0 120. 240.	0.6 2.5 0 13.2 0 52.3 0 126.1 0 228.7	6.1 5.6 2.2 2.3 1.9 7 1.5	(+) (+) (+) (+) (+) (+)	quantifying from two rep- licate serum samples on two occasions per day over five separate days
3	55 111 277 832 2774 5548	20 40 100 300 1000 2000	21.0 40.9 98.2 294.1 1014.5 2013.3	6.1 9.4 7.7 7.8 7.4 7.1	(-) (-) (-) (-) (-)	3	0.2 0.6 3.1 12.0 31.0 62.0	0.6 2.4 12.0 48.0 120. 240.	0.6 2.7 0 13.3 0 52.4 0 124.7 0 229.0	5.4 1.9 1.8 2.0 7 1.7 9 2.0	(+) (+) (+) (+) (+) (+) (+)	and across different incubation time: (n=20x0h, n=20x3h, n=20x5h) High repro
5	55 111 277 832 2774 5548	20 40 100 300 1000 2000	20.0 40.1 97.4 284.2 1021.7 2022.3	6.6 12.9 6.0 6.7 3.6 6.7	(-) (-) (-) (-) (-) (-)	5	0.1 0.4 1.9 7.4 19.0 37.0	0.6 2.4 12.0 48.0 120. 240.	0.6 2.6 0 13.9 0 53.3 0 125.5 0 228.3	5.3 3.6 2.5 2.2 1.4 2.2	(+) (+) (+) (+) (+) (+) (+)	ducibility, repeatability were determined by ex- tracting four levels of QC
	Aldosterone QC range (35 - 1250 pg/mL; 97 - 3468 pmol/L) - Spec <15% III/alterial over tive sepa-											
	(h)		001			.v) L OC	· A C	001				across several incuba-
		35 (pg/mL)	100 (pg/mL)	250 (pg/mL)	125 (pg/n	50 nL) (pg	35 g/mL)	100 (pg/mL)	250 (pg/mL)	1250 (pg/mL)	tion times, indicating overall a total precision	
0			13.4% 14.2%	13.1% 10.9%	12.4%	11.0)% 12 % 10	2.4% 0.5%	11.9% 9.6%	11.4% 10.7%	7.4% 7.9%	and repeatability of
5		13.1%	10.2%	10.5%	7.6	% 12	2.2%	9.1%	9.8%	7.6%	≤15% CV for both ana-	
Incubation (b)			Angioter	ISIN I QC I	ange (2 – ision (% C	2 – 125 ng/mL) - Spec < // CV)			3% al Repeatability (% C\/)		CV()	lytes.
	(F	Plasma Renin Activity PRA) Rang	QC1	QC2	QC3	QC	:4 0	2C1	QC2	QC3	QC4	
		nmol/L/h	2 (ng/mL)	5 (ng/mL)	25 (ng/mL)	12 (ng/n	5 nL) (ng	2 g/mL)	5 (ng/mL)	25 (ng/mL)	125 (ng/mL)	
0		1.5-96.4	13.0%	8.9%	2.8%	3.3	% 3	.2%	2.9%	0.8%	0.6%	
3		0.5-32.1	9.1%	6.3%	4.0%	3.2	% 2 % 2	.1%	2.3%	1.7%	1.5%	
J		0.0-10.0	1.070	4.370	2.070	2.1	70 Z	. 170	1.470	0.370	0.7 70	i i i i i i i i i i i i i i i i i i i

	Table 1. The sensitivity	
ESI	of the tests was demon-	
wode	strated by extracting and	
(+)	quantifying from two rep-	
(+)	licate serum samples on	
(+)	two occasions per dav	
(+)	over five separate davs	
(+)	and across different	
(+)	incubation time:	
(+)	(n=20x0h n=20x3h	
(+)	(11-20x011, 11-20x011, 11-20x01	
(+)	n=20x5n). High repro-	
(+)	ducibility, repeatability	
(+)	were determined by ex-	
(+)	tracting four levels of QC	5
(')	material over five sepa-	
<i>'</i>)	rate days and performed	
QC4	across several incuba-	
1250	tion times, indicating	
og/mL)	overall a total precision	
7.4%	and repeatability of	
7.9%	<15% CV for both ana-	
1.070		

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 manufactory 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.

For Research use Only. Not for use in diagnostic procedures.

©2024 Waters Corporation Waters, Xevo, ACQUITY, UPLC, XBridge are trademarks of Waters Technologies Corporation.