A Versatile Workflow to Measure Plasma Renin Activity and Aldosterone for Clinical Research Using Automated On-line Extraction Coupled to LC-MS/MS

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INTRODUCTION

Researchers studying the renin-angiotensin-aldosterone system need efficient ways to measure renin activity and aldosterone concentration in plasma sample preparations. We report a workflow to accomplish this utilizing automated on-line extraction coupled to LC-MS/MS, which simplifies sample preparation.

METHODS

Plasma Renin Activity

In order to measure angiotensin I (Ang I), donor plasma specimens (100 µL), calibrators and Lyphochek hypertension markers controls (QCs, Bio-Rad Laboratories, Berkeley, CA) were prepared per Carter et al (1) and incubated at 37ºC in the autosampler tray of a Thermo Scientific™ Prelude™ SPLC system.

25 µL injections were made at the beginning of the incubation and between 12 and 17 hours later (overnight) into a Thermo Scientific™ Cyclone™ TurboFlow™ column (0.5 x 50 mm) on the same channel of the Prelude SPLC system.

Extracted Ang I and its internal standard were automatically transferred to a 50 x 2.1 mm analytical column – either a XBridge BEH C18, 5 µm (Waters Corp., Milford, CA) or a Thermo Scientific™ Accucore™ aQ, 2.6 µm column – heated to 30ºC for final isolation using a mobile phase gradient from water to methanol, both containing 0.2% formic acid (Figure 1).

Figure 1. Angiotensin I LC parameters.

Column 1	Cyclone 0.5x50mm							Column 2	Accucore aQ 2.6um, 50x2.1mm				
Loading Pump	Tertiary							Eluting Pump	Binary				
A	H2O + 0.2% Formic Acid							A	H2O + 0.2% Formic Acid				
B	MeOH + 0.2% Formic Acid							B	MeOH + 0.2% Formic Acid				
C	2:2:1 ACN:IPA:Acetone							C					
Step	Start	Sec	Flow	Grad	%A	%B	%C	Tee	Loop	Flow	Grad	%A	%B
1	0.00	30	1.50	Step	90.0	10.0	-	====	out	0.50	Step	90.0	10.0
2	0.50	60	0.20	Step	90.0	10.0	-	T	in	0.40	Step	90.0	10.0
3	1.50	15	1.00	Step	-	-	100.0	====	in	0.50	Ramp	70.0	30.0
4	1.75	15	0.50	Step	100.0	-	-	====	in	0.50	Ramp	50.0	50.0
5	2.00	15	1.00	Step	-	-	100.0	====	in	0.50	Ramp	20.0	80.0
6	2.25	15	0.50	Step	100.0	-	-	====	in	0.50	Ramp	5.0	95.0
7	2.50	30	1.00	Step	50.0	50.0	-	====	in	0.50	Step	5.0	95.0
8	3.00	30	1.00	Step	50.0	50.0	-	====	in	0.50	Step	90.0	10.0
9	3.50	45	0.70	Step	-	-	100.0	T	out	0.00	Step	90.0	10.0
10	4.25	15	0.50	Step	90.0	10.0	-	T	out	0.20	Step	90.0	10.0
11	4.50	60	1.25	Step	90.0	10.0	-	====	out	0.70	Step	90.0	10.0

The analytes were eluted into a heated electro-spray interface of a Thermo Scientific™ TSQ Endura™ mass spectrometer (MS) and subjected to positive-ion selected-reaction monitoring of their +3 charge states (Figure 2).

Figure 2. Angiotensin MS/MS parameters

SRM Table

Compound	Start Time (min)	End Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)
Ang I	0	1.4	Positive	433.2	619.4	20
Ang I	0	1.4	Positive	433.2	647.4	17
IS	0	1.4	Positive	437.3	631.4	22
IS	0	1.4	Positive	437.3	660.4	18
Electro-clean	1.4	1.5	Negative	437.3	660.4	18

Ang I amounts for each sample were measured using a ^{13}C, ^{15}N – labeled Ang I internal standard (AnaSpec Inc., Fremont, CA) and calibrators in each batch.

Plasma renin activity of each specimen and QC was expressed as the increase in Ang I formed from initial to final incubation (ng/mL/hr) using the date/time stamp of respective data files.

Plasma Aldosterone

Donor plasma specimens (150 µL) as well as calibrators and the Lyphocheck QCs were mixed with 50 µL of internal standard solution (2 ng/mL water plus 10% methanol and 2% acetonitrile).

90 µL injections of each sample preparation were made into a Cyclone 0.5 x 50 mm TurboFLow column on the other channel of the Prelude SPLC with aqueous mobile phase (Figure 3.)

Plasma Aldosterone

Extracted aldosterone and its internal standard were automatically transferred to a 50 x 2.1 mm Accucore aQ, 2.6 µm column heated to 40ºC for final isolation using a mobile phase gradient from water containing 5% 2-propanol and 3.5 mM ammonium acetate to pure methanol (Figure 3),

Figure 3. Aldosterone LC parameters.

Column 1	Two Cyclone 0.5x50mm							Column 2	Accucore aQ 2.6um, 50x2.1mm				
Loading Pump	Tertiary							Eluting Pump	Binary				
A	H2O + 0.1% Formic Acid							A	H2O+5% IPA+ 0.02% HAc & AmOH				
B	MeOH pure							B	MeOH pure				
C	2:2:1 ACN:IPA:Acetone							C					
Step	Start	Sec	Flow	Grad	%A	%B	%C	Tee	Loop	Flow	Grad	%A	%B
1	0.00	30	1.50	Step	100.0	-	-	====	out	0.50	Step	100.0	-
2	0.50	60	0.20	Step	100.0	-	-	T	in	0.50	Step	100.0	-
3	1.50	15	1.00	Step	-	-	100.0	====	in	0.40	Ramp	70.0	30.0
4	1.75	15	0.50	Step	100.0	-	-	====	in	0.40	Ramp	65.0	35.0
5	2.00	15	1.00	Step	-	-	100.0	====	in	0.40	Ramp	60.0	40.0
6	2.25	15	0.50	Step	100.0	-	-	====	in	0.40	Ramp	50.0	50.0
7	2.50	30	1.00	Step	40.0	60.0	-	====	in	0.40	Ramp	20.0	80.0
8	3.00	30	1.00	Step	40.0	60.0	-	====	in	0.50	Step	100.0	-
9	3.50	75	0.90	Step	100.0	-	-	====	out	0.50	Step	100.0	-

The analytes were eluted into the heated electro-spray interface of the TSQ Endura MS and subjected to negative-ion selected-reaction monitoring (Figure 4). Mobile phase conditions enhanced ionization.

Figure 4. Aldosterone MS/MS parameters.

SRM Table

Compound	Start Time (min)	End Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Aldosterone	0	1.5	Negative	359.2	189.1	19	120
Aldosterone	0	1.5	Negative	359.2	331.05	17	120
IS	0	1.5	Negative	366.25	276.25	23	130
IS	0	1.5	Negative	366.25	338.25	16	130
Electro-clean	1.5	1.6	Positive	366.25	338.25	16	130

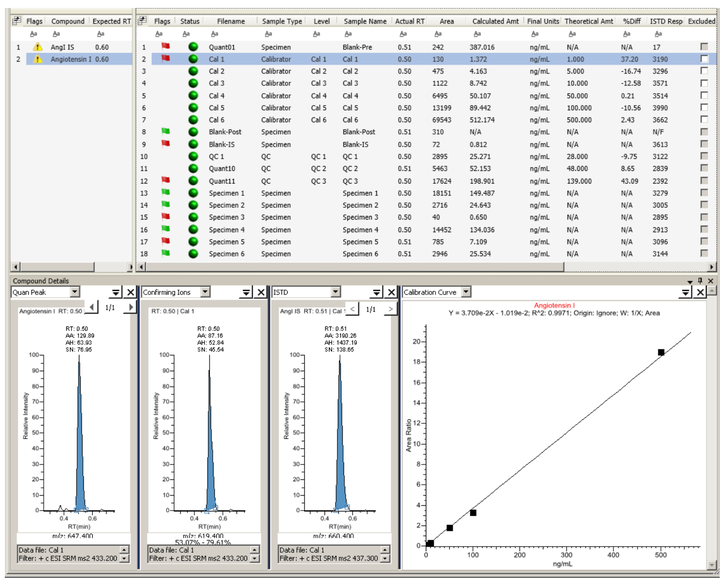
Aldosterone was quantitated using Aldosterone-D_4 internal standard (Cerilliant Corp., Round Rock, TX).

RESULTS

Plasma Renin Activity

The Accucore aQ and XBridge BHE columns produced virtually identical results. Figure 5 results involved the Accucore column.

Figure 5. Typical Angiotensin I quantitation results



The 5.5 minute method had a throughput of 10 samples per hour. and a linear (1/X weighting) analytical range of 1 to 500 ng/mL, with less than 0.5% carryover. Internal standard (IS) peak areas among calibrators varied no more than 7% in each batch. Matrix interferences among plasma samples presumably caused IS peak area reductions of 20 to 40%.

Overnight incubation provided reliable plasma renin activity (PRA) results (Table 1).

Table 1. Plasma Renin Activity (PRA) of QCs

	QC 1	QC 2	QC 3
Initial Ang I (ng/mL)	26	52	198
Final Ang I (ng/mL)	144	270	831
Difference (ng/mL)	118	218	633
PRA (ng/mL/hr)	7.3	13.4	39.0

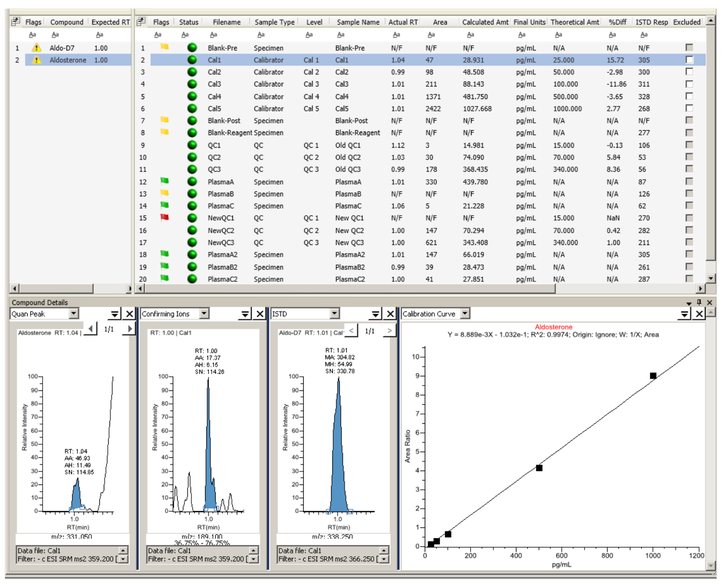
PRA results for donor plasma samples have not yet been compared to those from a reference lab.

RESULTS

Plasma Aldosterone

The 4.75 minute method for aldosterone had a throughput of 12 samples per hour. As shown in Figure 6, we achieved a linear (1/X weighting) analytical range of 25 to 1000 pg/mL, with no measurable carryover.

Figure 6. Typical Aldosterone quantitation results



Internal standard (IS) peak areas among calibrators varied no more than 12% in each batch. Matrix interferences among plasma samples presumably caused IS peak area reductions of 25 to 40%.

Aldosterone results for all 3 QC levels were within +/- 10% of expected values in each batch. Aldosterone results for donor plasma samples have not yet been compared to those from a reference lab.

CONCLUSIONS

Both Ang I and aldosterone research methods provided the desired analytical performance and throughput as they ran independently on a two-channel HPLC system equipped with on-line extraction. Sample preparations were easily accomplished in a matter of minutes. Setting the autosampler tray holder to 37ºC conveniently allowed running initial and final-incubation batches to quantitate Ang I and calculate plasma renin activity (PRA). Aldosterone batches conveniently ran before or after Ang I batches. PRA and aldosterone results for each donor specimen were efficiently acquired and reported using this versatile workflow.

REFERENCES

1. S Carter, LJ Owen, MN Kerstens, RPF Dullaart and BG Keevil. A liquid chromatography tandem mass spectrometry assay for plasma renin activity using online solid-phase extraction. Ann Clin Biochem 2012; 49: 570–579.

TRADEMARKS/LICENSING

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