Determination of Plasma Renin Activity by LC/MS/MS for Clinical Research

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Introduction

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is ideally suited for the rapid analysis of analytes in complex matrices. A highly sensitive and specific LC/MS/MS analytical method has been developed for the determination of plasma renin activity (PRA) for clinical research. An analytical method for quantifying Angiotensin I in plasma is applied for the determination of Plasma Renin Activity. Plasma samples are incubated for 3 hours at 37°C. A sample preparation procedure by solid phase extraction (SPE) allows efficient extraction of Angiotensin I in plasma. Plasma renin activity is calculated by subtracting Angiotensin I concentration in a blank plate.

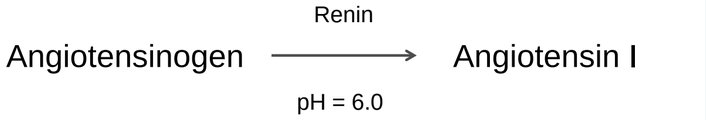


Figure 1. Renin-Angiotensin system

Plasma Renin Activity is then calculated by:

$$PRA = ([\text{Angiotensin I}]_{37oC} - [\text{Angiotensin I}]_{0oC})\ \Delta t$$

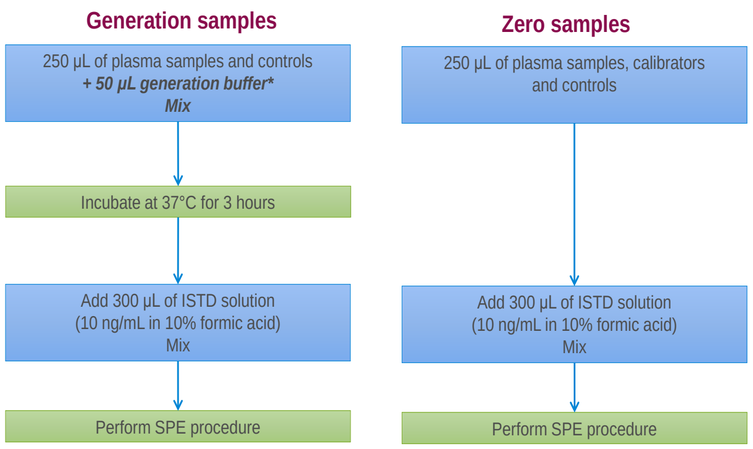
Calibrators were created by spiking a 1% bovine serum albumin buffer pH 6 solution with various concentrations of Angiotensin I. The chromatographic system consists of an Agilent Poroshell 120 SB-C18 column and a mobile phase comprised of methanol and water containing 0.2% formic acid. Quantifier and qualifier transitions were monitored and an isotopically labelled Internal Standard was included to ensure accurate and reproducible quantitation.

Experimental

Sample Preparation

Calibrators (Proteochem) are prepared with 1% bovine serum albumin buffer pH 6 solution. Isotopically labelled Internal standard (Anaspec) and Biorad plasma controls were used. Plasma samples should not exceed 4°C at any time until ready for incubation or extraction.

Step 1: Preparation of generation and zero samples



*: Generation buffer: 1M Tris Base+ 0.2M EDTA + 1 mM PMSF, pH 5.5 with acetic acid

Step 2 - Solid Phase Extraction (SPE)

Use preparation from step 1

1: Condition SPE cartridge (Agilent BondElut Plexa, 30 mg, 3 mL, PN: 12109303) with:
- 1 mL methanol
- 1 mL 5% formic acid in water

2: Add samples

3: Wash with:
- 1 mL 5% formic acid in water
- 1 mL 20% methanol in water
- Dry at full vacuum for 5 minutes

4: Elute with:
- 250 µL of methanol.
- Apply vacuum 5" Hg for 60 seconds

Note: use of plastic ware is recommended for optimum recoveries

Experimental

LC Analytical Method

Agilent 1290 HPLC binary pumps, well plate sampler with thermostat, temperature-controlled column compartment.

Parameter	Value
Analytical Column	Agilent Poroshell 120 SB-C18, 2.1 x 50 mm, 2.7 µm, PN: 689775-902
Guard Column	Agilent Poroshell 120 SB-C18, 2.1 x 5 mm, 2.7 µm, PN: 821725-912
Injection Volume	20 µl
Needle Wash	1:1:1:1 MeOH:ACN:IPA:H2O + 0.1% formic acid in Flush port for 20 seconds
Injector Temperature	4 °C
Mobile Phase A	Water + 0.2 % Formic Acid
Mobile Phase B	Methanol + 0.2 % Formic Acid
Flow rate	0.6 mL/min.
Pump Gradient	Time (min.) %B 0.0 10 0.5 10 1.5 95 3.5 95
Stop time	3.5 min.
Post Time	1.5 min.

Table 1. LC Parameters

MS Analytical Method

Agilent 6460 QQQ LC/MS with Agilent JetStream Technology	
Ion Mode	Agilent JetStream Electrospray, positive ionization
Drying gas	350°C, 7 L/min
Nebulizer gas	40 psi
Sheath gas	400°C, 12 L/min
Capillary voltage	4000 V
Nozzle voltage	0 V
Q1/Q3 resolution	0.7 unit
Δ EMV	200 V
Scan rate	3 spectra/sec

Table 2. MS Parameters

Compound	Prec Ion	Prod Ion	Frag (V)	CE (V)	CAV (V)
Angiotensin I (Quant)	152.1	107	115	16	5
Angiotensin I (Qualfier)	176.1	111.1	65	24	5
Angiotensin I - ISTD	190.1	172.1	70	8	5

Table 3: MRM Transitions table

Results and Discussion

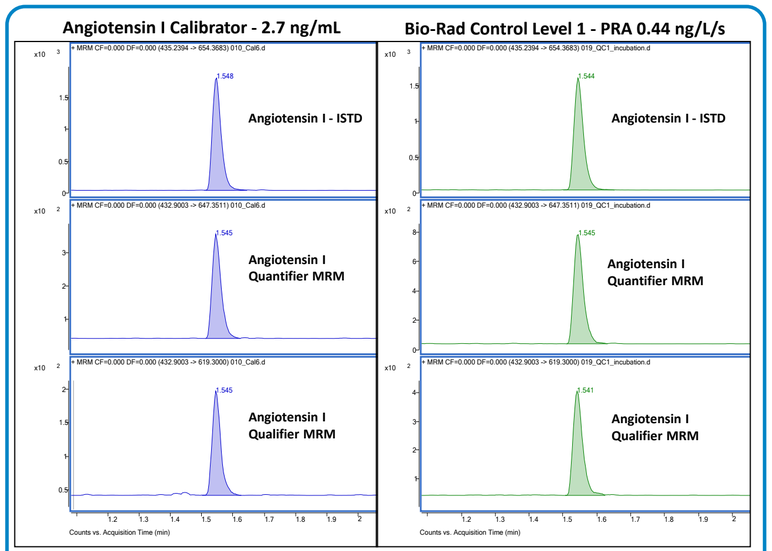


Figure 2. Chromatography for Angiotensin I Calibrator – 2.7 bg/mL and Bio-Rad Lyphocheck Control Level 1

Results and Discussion

Accuracy and Reproducibility

Bio-Rad Lyphocheck hypertension markers controls were used to test the accuracy and reproducibility of this analytical method. Measurements were repeated in triplicates to assess intra-day and on three separate day to assess inter-day reproducibility and CVs were found to be below 6%, see table 4. All measurements are in ng/L/s.

	QC Level	Measured Value Intra-day (n=3)	Intra-day CV% (n=3)	Measured Value Inter-day (n=3)	Inter-day CV% (n=3)
Plasma Renin Activity ng/L/s	1	0.404	5.0	0.443	5.4
	2	0.933	2.7	0.956	4.4
	3	3.223	5.8	3.573	4.4

Table 4. Results of Bio-Rad Lyphocheck hypertension markers controls by LC-MS/MS

Calibration curve

Preparation of Angiotensin I calibrants was done in 1% bovine serum albumin and were prepared as zero samples. The procedure was repeated on three separate day to assess inter-day reproducibility and CVs were found to be below 6%. Accuracies at all levels are within acceptance criteria, see table 5.

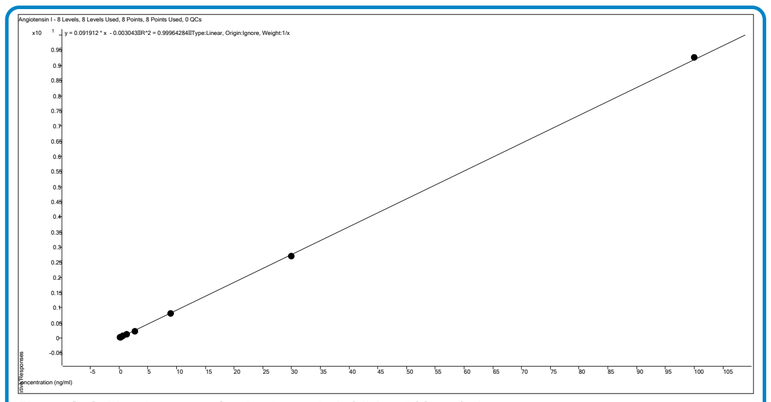


Figure 3. Calibration curve for Angiotensin I, 0.17 to 100 ng/mL

Compound	R^2 n = 3	Concentration (ng/mL)	Accuracy (%) n = 3	Inter-day CV (%) n = 3
Angiotensin I	0.9996	0.1688	114.3	3.6
		0.3375	101.0	3.8
		0.675	96.9	5.3
		1.35	98.6	3.2
		2.7	93.2	5.7
		9	99.2	0.8
		30	98.4	1.7
		100	100.8	0.7

Table 5: Summary of analyte performance.

Conclusions

A robust analytical method for quantifying Angiotensin I in plasma by LC/MS/MS which is applied for the determination of Plasma Renin Activity has been developed. Typical analytical method performance results are well within acceptable criteria.

References:

J Grace Van Der Gugten, Daniel T Holmes, St.Paul's Hospital, University of British Columbia, Vancouver, Canada. "Plasma Renin Activity by Tandem Mass Spectrometry Employing Analyte Immunoprotection". ASMS 2012 Vancouver Poster.

Bystrom, Cory E. "Plasma Renin Activity by LC-MS/MS: Development of a Prototypical Clinical Assay Reveals a Subpopulation of Human Plasma Samples with Substantial Peptidase Activity". Clinical Chemistry 56:10 1561–1569 (2010). DOI: 10.1373/clinchem.2010.146449