Simultaneous quantitation of renin activity, aldosterone and angiotensin II in human plasma using rapid protein precipitation with the Citrine Triple Quad system from SCIEX

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ABSTRACT

Three compounds angiotensin I , aldosterone and angiotensin II were analyzed by Multiple Reaction Monitoring (MRM) acquisition using a method on the Citrine Triple Quad system from SCIEX. All 3 compounds had good reproducibility within the range of the standard curve (n=7). The stock solution for angiotensin I, aldosterone and angiotensin II was diluted to obtain the working solutions for aldosterone and angiotensin II at concentrations 0.015, 0.03, 0.05, 0.1, 0.5, 1, and 5 ng/mL, and for angiotensin I at concentrations 0.3, 0.6, 1, 2, 10, 20 and 100 ng/mL . The analyte peak area coefficient of variation (CV) of 500 consecutive injections was <5% for all 3 compounds (the plasma sample concentration was 1 ng/mL for aldosterone and angiotensin II and 20 ng/mL for angiotensin I). The lower limit of quantitation (LLOQ) was 0.3 ng/mL for angiotensin I and 0.015 ng/mL for aldosterone and angiotensin II, which met the clinical requirements.

The study investigated the effects of different mobile phase additives, different columns and the different kinds of precipitant on the sensitivity and peak shape of the analytes. Ammonium fluoride is often a good additive to increase the sensitivity of aldosterone, which was analyzed in negative MRM mode. The Phenomenex Kinetex C18 column provided very good peak shape while achieving good analyte separation. In addition, using 20% trichloroacetic acid (TCA) as precipitant provided a high degree of endogenous matrix precipitation while minimizing instrument background. Ammonium fluoride provided a suitable pH for the analyte to bind to the LC column.

Finally, the optimized mobile phases, precipitant and columns were established. The mobile phase system provided good peak shape and sensitivity while reducing instrument downtime. This enabled a fast and reliable method for studying the diagnosis of primary aldosteronism (PA).

INTRODUCTION

According to the guidelines on PA issued by the Endocrine Society, the plasma aldosterone-to-renin ratio (ARR) is recommended for detecting possible cases of PA [1].
Typically, solid phase extraction (SPE) is used to extract these compounds, which is more complex and time-consuming compared to protein precipitation techniques.

This poster presents a rapid, robust and cost-effective method that has been validated for the simultaneous measurement of ARR-related analytes in human plasma. This LC-MS/MS method uses MRM to detect renin activity, aldosterone and angiotensin II with limits of quantitation (LOQ) of 0.015 ng/mL for aldosterone and angiotensin II and 0.3 ng/mL for renin activity. Renin activity is measured by the ability of the plasma to generate angiotensin I from angiotensinogen. A Citrine Triple Quad system from SCIEX was used to separate and quantify the aldosterone, angiotensin II and angiotensin I. All LOQ values for these 3 compounds met the requirements of the clinical trials.

MATERIALS AND METHODS

Sample preparation:
A total of 400 µL of human plasma was incubated using an enzyme inhibitor solution. This solution contained 1 mmol/L phenylmethyl-sulfonyl fluoride (PMSF) and 0.3 mg/mL soybean serine trypsin inhibitor (SBIT), followed by the addition of 14 µL formic acid to stop the reaction process. Then, 150 µL of the sample was extracted using 20% TCA. Stable isotope labeled aldosterone-D_8, angiotensin I - [arginine $^{13}C_6$,$^{15}N_4$] and angiotensin II - [arginine $^{13}C_6$,$^{15}N_4$] were used as the internal standards for aldosterone, angiotensin I and angiotensin II, respectively. The mixture was vortexed for 2 min before being refrigerated at 4 ℃ for 30 min. After centrifugation at 14,000 rpm for 10 min, the mixture was transferred to a vial for instrumental analysis.

HPLC conditions:
Chromatography was performed using the Jasper system from SCIEX with a Phenomenex Kinetex C18 column (100 x 3.0 mm, 2.6 µm particle size) and column oven set to 50 ℃. Mobile phase A was water with 0.5 mM ammonium fluoride, and mobile phase B was methanol. The flow rate was 350 µL/min and the injection volume was 30 µL.

MS/MS conditions:
The mass spectrometer was Citrine Triple Quad system from SCIEX with a Turbo V ion source and an electrospray ionization (ESI) probe. Angiotensin I, aldosterone and angiotensin II were detected using 2 MRM transitions per compound to allow quantitation and identification based on the ratio of quantifier and qualifier transitions as defined by European Union (EU) regulation 2002/657/EC. Angiotensin I and angiotensin II were monitored in positive mode, and aldosterone was monitored in negative mode. The acquisition method was divided into 2 separate periods (Figure 1) : angiotensin I and II were in positive mode for the initial 2.8 min, and the second period monitored aldosterone in negative mode for the remaining 2.2 min.

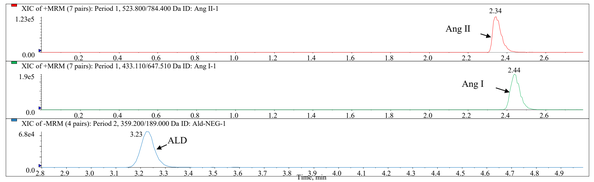

Figure 1. Two separate acquisition periods were used in the method. Angiotensin I (Ang I) and angiotensin II (Ang II) were monitored in positive ion scanning for the first 2.8 min while aldosterone (ALD) was monitored in negative mode for the remaining 2.2 min.

RESULTS

A method for quantitation and identification of 3 PA-related analytes—ALD, Ang I and Ang II—was developed, and the MRM transitions are shown in Table 1, where the "*" denotes in the table was the quantifier mass transition. The stable isotope labeled Aldosterone-D_8, Angiotensin I - [arginine $^{13}C_6$,$^{15}N_4$] and Angiotensin II - [arginine $^{13}C_6$,$^{15}N_4$] were used as the internal standard for ALD, Ang I and Ang II, respectively. The LOQ and linear range of the analytes are shown in Table 2.

Analyte	Q1	Q3	DP	CE
Period1 (ESI+, 2.8 min)				
Angiotensin I	433.1	647.5*	130	13
	433.1	619.4	130	15
Angiotensin I - [arginine $^{13}C_6$,$^{15}N_4$]	436.4	657.5	130	27
	436.4	629.4	130	27
Angiotensin II	523.8	784.4*	100	28
	523.8	263.2	100	30
Angiotensin II - [arginine $^{13}C_6$,$^{15}N_4$]	528.8	263.2	100	31
Period2 (ESI-, 2.2 min)				
Aldosterone	359.1	189.1*	-100	-24
	359.1	331.1	-100	-21
Aldosterone-D_8	367.2	194	-110	-27
	367.2	339.3	-110	-25

Table 1. MS parameters for MRM transitions of ALD, Ang I and Ang II by LC-MS/MS.

Analyte	LOQ (ng/mL)	ULOQ (ng/mL)	Linear range (ng/mL)
Ang I	0.3	100	0.3-100
Ang II	0.015	5	0.015-5
ALD	0.015	5	0.015-5

Table 2. LOQ and linear range of detected compounds.

According to the document C62-A from the Clinical and Laboratory Standards Institute (CLSI)[2] and the Chinese Guidance for Liquid Chromatography and Mass Spectrometry Clinical Application, we have validated the method. The recovery data are shown in Table 3, demonstrating the accuracy of the assay at low, medium and high concentrations spiked into human plasma (n=5). In addition, an actual clinical sample was analyzed using the developed method. The chromatogram is shown in Figure 2.

Analyte	Ang I			Ang II			ALD		
Spike concentration (ng/mL)	1	10	50	0.05	0.5	3	0.05	0.5	3.0
Accuracy (%)	93.8	101.3	103	109.9	98.6	101.2	92.9	97	96.7

Table 3. Recovery accuracy (%) of low, medium and high concentration spikes into human plasma for angiotensin I, angiotensin II and aldosterone.

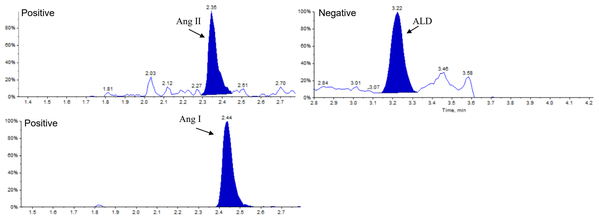

Figure 2. A clinical typical chromatogram for Ang I (2.44 min), Ang II (2.35 min) and ALD (3.22 min). Ang I and Ang II were detected in positive ion mode while ALD was detected in negative ion mode.

Method robustness test:
In this method, TCA was used as precipitant. To test the robustness of the method and whether the high concentration of acid contained in the sample could have adverse effects on the HPLC column, a spiked sample was prepared and 500 needles were injected continuously to observe the peak area changes of the 3 substances to be detected. The total injection time was >50 hours. The specific data change trend can be seen in Figure 3. The y-axis is the peak area of the 3 analytes and the x-axis is the injection time. The results show that the analyte peak area CV of 500 consecutive injections was <5% for all 3 compounds, which also means the method has a very stable performance.

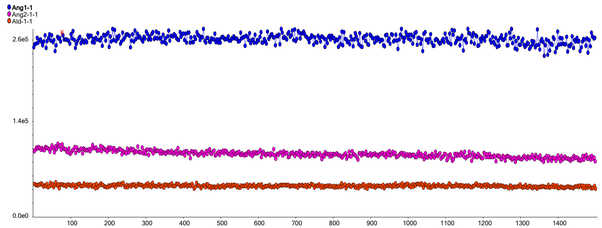

Figure 3. Peak areas of angiotensin I, angiotensin II and aldosterone during 500 continuous injections.

CONCLUSIONS

A fast, robust and reliable method for the detection of 3 PA-related analytes—angiotensin I, angiotensin II and aldosterone—was developed and validated. A direct protein precipitation sample preparation method was used, resulting in time and cost savings. Chromatographic separation using a C18 column was combined with the high sensitivity detection of the Citrine Triple Quad system. The method was demonstrated to be accurate through spiking the analytes into human plasma. The method was also shown to be robust through the 500 continuous injections. In addition, using separate acquisition periods for positive and negative polarity ensured the high sensitivity and robustness of the method.

Although not shown in this poster, the SPE method[3] and protein precipitation methods were compared and demonstrated comparable results between the 2 methods. LOQs of 0.015–5 ng/mL were found for angiotensin II and aldosterone and 0.3-100 ng/mL for angiotensin I. All LOQ values met the clinical requirements.

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