Ultra-Sensitive Analysis of Aldosterone in Serum by HPLC-MS/MS

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INTRODUCTION

LC-MS/MS has become an important tool for the measurement of steroid hormones in clinical research studies. Historically, these analytes have been measured using GC-MS or immunoassays. However it is generally accepted that the measurement of steroids by immunoassay suffers from a lack of specificity due to cross-reactivity, resulting in overestimation of serum concentrations for these analytes. Furthermore, immunoassay measurements tend to exhibit high variability at low concentrations that can provide erroneous and misleading results. GC-MS methods for steroids analysis usually require extraction and purification steps, as well as derivatization prior to analysis, which is less convenient and more time-consuming.

The trend is to move towards LC-MS/MS for the analysis of steroid hormones due to its many advantages, including sensitivity, selectivity, and ease of sample preparation. Nevertheless, the measurement of aldosterone in serum by LC-MS/MS poses analytical challenges owing to the low concentrations of this compound, interferences caused by endogenous steroids, and the relatively poor intrinsic ionization efficiency of this compound. In the work presented here we have employed the SCIEX Triple Quad™ 6500 tandem mass spectrometer to improve the limit of quantitation (LOQ) for aldosterone in human serum, compared to current methods employing existing LC/MS/MS technology.

MATERIALS AND METHODS

Sample Preparation

The sample preparation consisted of a liquid-liquid extraction, using methyl tert-butyl ether (MTBE), followed by dry-down and reconstitution of the sample

- 500uL of each serum sample was measured into a 5mL polypropylene tube;
- 50uL of aldosterone-d7 internal standard solution (Isosciences, King of Prussia, Pennsylvania, USA) was added to each tube, which was then vortex mixed for 15 seconds;
- 2500uL of MTBE was added to each tube, which was vortex mixed for 15 seconds;
- The samples were centrifuged at 3,000 rpm for approximately 5 minutes;
- 2000uL of the supernatant was transferred into a clean 2.2mL microcentrifuge tube, and dried down under nitrogen gas at 35C;
- The dried sample was reconstituted using 125uL of 20:80 v/v methanol:water, and transferred to an HPLC vial.

HPLC Conditions

A Shimadzu Prominence HPLC system was used, with a Phenomenex Gemini-NX C18 (150 x 3.0mm, 5µm) analytical column maintained at 40ºC. A gradient elution was employed, consisting of water + 2mM ammonium acetate (mobile phase A) and methanol + 2mM ammonium acetate (mobile phase B), at a flow rate of 500µL/min. The total run-time for the method was 10 minutes, to ensure adequate separation of the aldosterone analyte from endogenous interferences. The injection volume was set to 50µL.

MS/MS Conditions

A SCIEX Triple Quad™ 6500 LC/MS/MS system equipped with IonDrive™ Turbo V source was used, in negative Electrospray Ionization (ESI) mode. Two MRM transitions were used to monitor the analyte aldosterone, and one MRM transition was used to monitor the deuterated internal standard, aldosterone-d7. The optimized MRM conditions for the analyte and internal standard are summarized in Table 1.

Analyte	Q1	Q3	DP	CE	CXP
Aldosterone 1 (quantifier)	359.2	189.0	-120	-24	-14
Aldosterone 2 (qualifier)	359.2	331.1	-120	-23	-22
Aldosterone-d7 (Internal Standard)	366.2	338.2	-120	-23	-22

Table 1. MRM transitions for aldosterone analyte and internal standard.

RESULTS

The method described here was used to analyze a series of human serum samples containing concentrations of aldosterone ranging from 14 pg/mL to 300 pg/mL. A representative chromatogram for a sample containing 14 pg/mL aldosterone is shown in Figure 1, with signal-to-noise (S/N) = 108. The LC/MS/MS method enabled quantification of aldosterone at concentrations as low as 1 pg/mL in human serum. Figure 4 displays a solvent blank (left) and the 1pg/mL calibrator (right) spiked into human serum. As can be seen, the chromatographic peak for the 1 pg/mL calibrator has signal-to-noise (S/N) = 18.

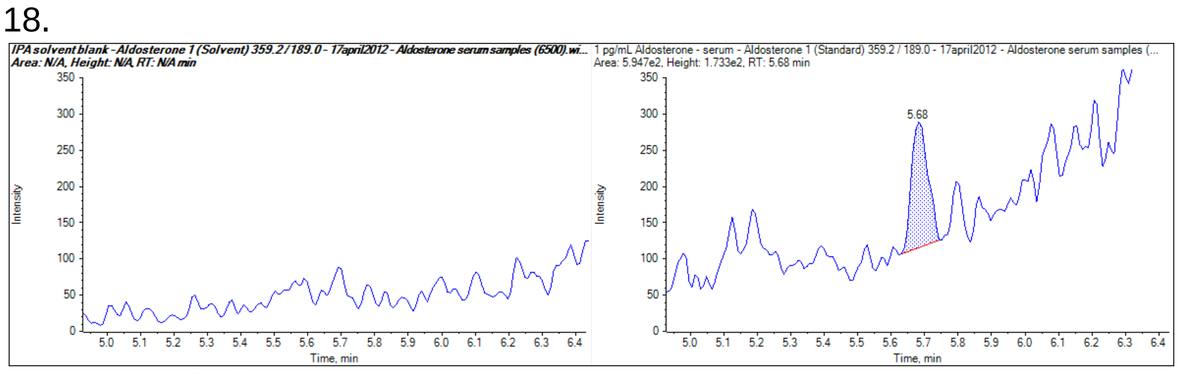

Figure 1. The LC/MS/MS method described here enabled quantification of aldosterone in human serum at 1pg/mL. A solvent blank (left) and 1 pg/mL aldosterone calibrator spiked in human serum (right) are displayed.

The method displayed excellent linearity over the concentration range from 1-1000 pg/mL (r = 0.99971), as shown in Figure 1. The accuracies range from 89-118% over the entire concentration range from 1-1000 pg/mL of aldosterone, and the CV% ranges from 0.5-9.1%. The accuracy and CV% for the lowest calibrator, at 1pg/mL, were 100% and 8.7%, respectively.

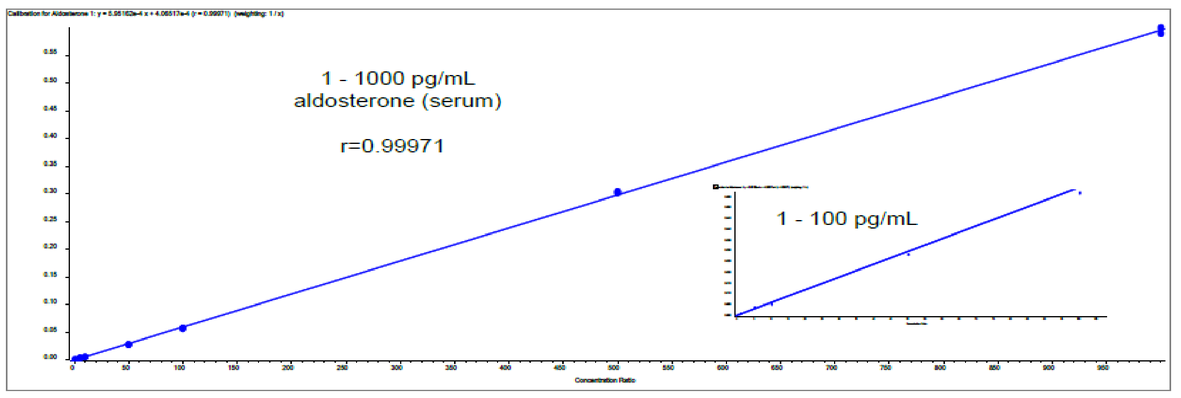

Figure 2. Calibration curve for aldosterone in human serum, from 1 pg/mL to 1000 pg/mL. The method displayed excellent linearity over the concentration range, with r = 0.99971.

Actual Concentration	Calculated Concentration (pg/mL)	Accuracy (%)	CV (%)
1 pg/mL Aldosterone	1.0	100	8.7
5 pg/mL Aldosterone	5.9	118	2.2
10 pg/mL Aldosterone	8.9	89	9.1
50 pg/mL Aldosterone	47.3	95	0.4
100 pg/mL Aldosterone	95.4	95	0.8
500 pg/mL Aldosterone	509.0	102	0.5
1000 pg/mL Aldosterone	998.4	100	1.2

Table 2. Statistics for the analysis of aldosterone using the AB SCIEX Triple Quad™ 6500 system.

CONCLUSIONS

A sensitive, robust and reliable method has been demonstrated for the analysis of aldosterone in serum, using a simple liquid-liquid extraction sample preparation.

The use of the SCIEX Triple Quad™ 6500 system, featuring IonDrive™ technology, has enabled improved limits of quantitation (LLOQ = 1 pg/mL), and provided larger dynamic range compared to earlier high performance MS/MS systems. Plotting raw peak areas, with no internal standard correction, 6 orders of magnitude of linear dynamic range were observed, which will permit the analyst to measure both serum and urine levels of aldosterone using the same method.

ACKNOWLEDGEMENTS

The authors wish to thank St Paul's Hospital in Vancouver, Canada, for the generous donation of serum samples.