



Sensitive quantitation of aldosterone in human serum

In this technical note, the combination of a rapid sample preparation procedure consisting of a liquid-liquid extraction with a robust and sensitive LC-MS/MS method using the SCIEX QTRAP 6500+ system enabled pg/mL detection of aldosterone extracted from human serum. The method demonstrated excellent precision and accuracy at the lowest calibrator (1 pg/mL). Excellent linearity was observed across the calibration series (1-10000 pg/mL), with a r^2 value of 0.9960. Low-level sensitivity was achieved at the lowest calibrator (1pg/ mL) with a signal-to-noise ratio [S/N] of 4:1. In addition, the method showed excellent precision [8.8%] and accuracy [102.5%] at the lowest calibrator (1 pg/mL), demonstrating the quantitative performance of the assay.

Key benefits of aldosterone analysis from human serum using the QTRAP 6500+ system

Low-pg/mL level sensitivity and excellent quantitative performance:

Sensitive quantitation of testosterone was performed with excellent precision [8.8%] and accuracy [102.5%] at the lowest calibrator (1 pg/mL)

Rapid sample preparation:

Aldosterone was extracted from human serum samples using a liquid-liquid extraction (LLE), evaporation, and reconstitution

Excellent linearity: Calibration curves for the aldosterone showed an r^2 value of 0.9960 across the calibration range [1-10,000 pg/mL]

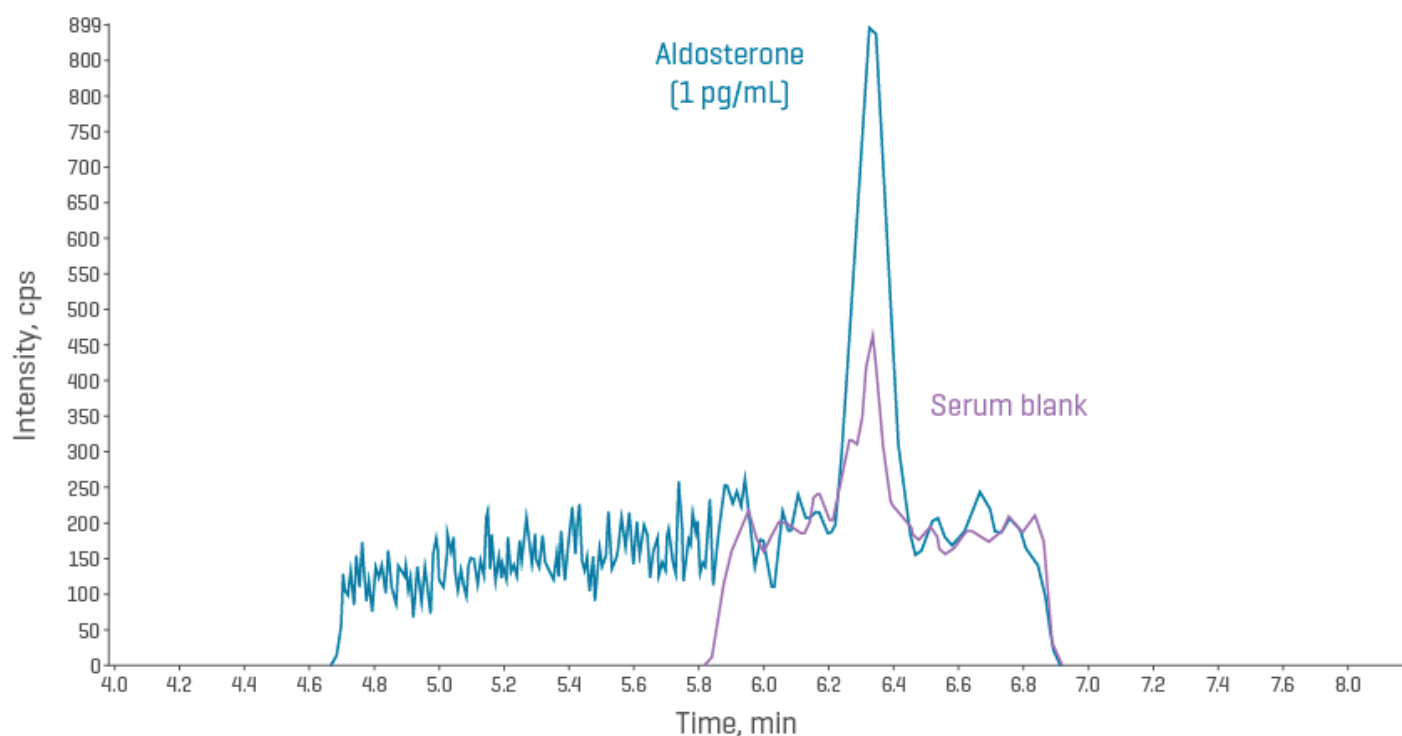


Figure 1. Analytical sensitivity of a 1 pg/mL [2.77 pmol/L] aldosterone standard extracted from serum matrix. Analysis of a 1 pg/mL aldosterone standard in matrix shows a S/N of 4:1 based on the peak-to-peak algorithm in SCIEX OS.

Introduction

Aldosterone, a mineralocorticoid hormone produced by the adrenal glands, plays a critical role in regulating blood pressure, electrolyte balance, and fluid homeostasis. Because aldosterone circulates at low concentrations and is structurally similar to other steroids, its accurate quantitation in biological samples is essential for clinical research studies investigating hormonal imbalances, cardiovascular risk, and the efficacy of therapeutic interventions.

Methods

Sample preparation: Aldosterone was extracted from human serum using a liquid-liquid extraction (LLE), evaporation, and reconstitution.

Liquid chromatography: Chromatographic separation was achieved using a [Phenomenex Kinetex C8 column](#) (50 x 2.1 mm, 2.6 μ m, 00B-4497-AN). Mobile phase A was ammonium fluoride in water and mobile phase B was ammonium fluoride in methanol. The total run time was 12 minutes.

Mass spectrometry: Data was collected using a [QTRAP 6500+ system](#) with an IonDrive Turbo V ion source and operated in electrospray ionization (ESI) negative mode. The Scheduled MRM algorithm was used in [SCIEX OS software](#) (version 3.1.6) to collect 10-12 data points for quantifiable data. Compound-dependent parameters were optimized by infusion.

Data processing: Data processing was performed using [SCIEX OS software](#) (version 3.1.6). Peak integration was achieved using the MQ4 algorithm. Quantitative analysis was conducted in the Analytics module of SCIEX OS, where calibration curves, concentration calculations, and assay precision statistics were automatically generated.

Results and discussion

Figure 1 shows the chromatographic separation of aldosterone into a control human serum sample at final concentrations of 1-10,000 pg/mL for aldosterone. The extracted ion chromatogram showed a signal-to-noise [S/N] ratio of 4:1 at the lowest matrix

calibrator concentration (1 pg/mL), as calculated using the peak-to-peak algorithm in SCIEX OS.

The quantitative performance of the method was investigated by injecting a series of 3 calibrator samples spiked at concentrations from 1-10000 pg/mL. Linearity and precision were assessed across the calibration range. **Figure 2** shows the calibration curve for aldosterone over the analytes' respective calibration ranges. The plot shows an excellent linear response across the calibration series, with an r^2 value of 0.9960

The accuracy and precision values were calculated from 3 replicate injections of the lowest matrix calibrators analyzed. The accuracy was 102.5% and precision (%CV) was 8.8%.

Conclusions

A fast and sensitive LC-MS/MS method for the detection of aldosterone extracted from human serum samples was developed. The method demonstrated:

- Fast sample preparation which consisted of a liquid-liquid extraction
- Excellent linear responses across the calibration series, with an r^2 value of 0.9960
- Good sensitivity resulting in S/N of 4:1 at the lowest calibrator level (1 pg/mL)
- High quantitation performance of the method, resulting in excellent precision (8.8%) and accuracy (102.5%) at the lowest calibrator level (1 pg/mL)

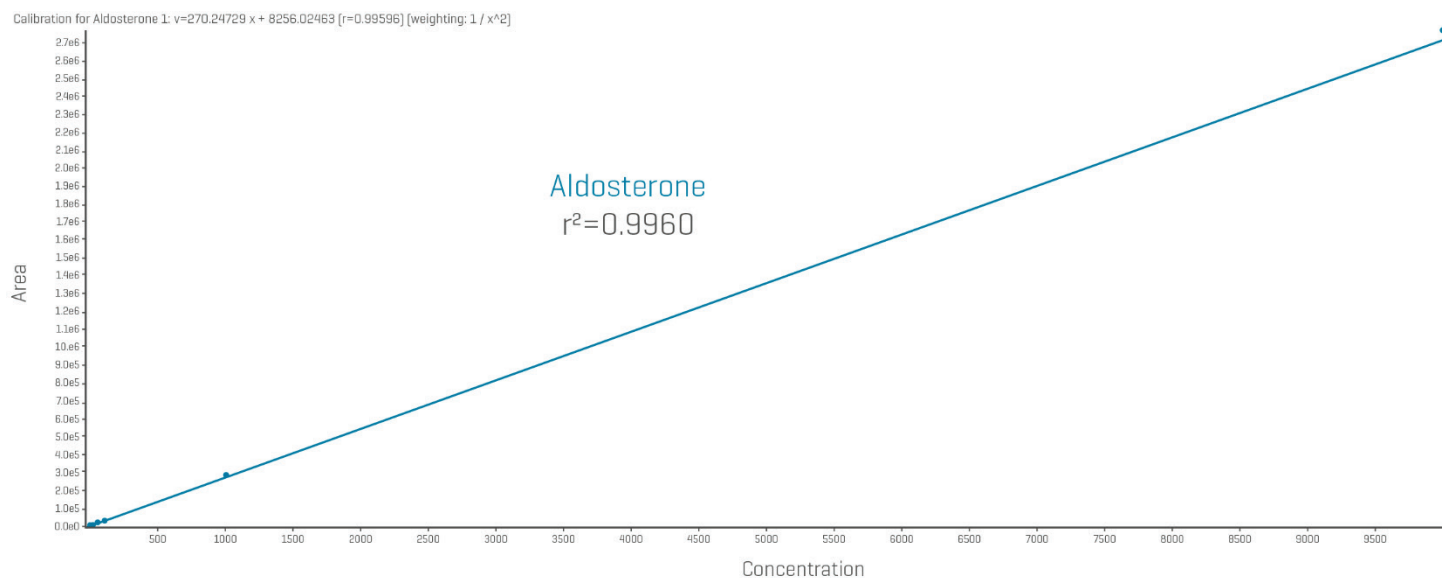


Figure 2. Linear calibration curve for aldosterone extracted from serum matrix using the primary MRM transition. The calibration curve using the matrix calibrators was analyzed across the concentration range [1-10000 pg/mL]. The curve was generated using linear regression and $1/x^2$ weighting, resulting in a r^2 value of 0.9960

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