

Sensitive quantitation of aldosterone in human serum using MRM³

In this technical note, a rapid liquid-liquid extraction procedure, and MRM³ acquisition on the SCIEX QTRAP 6500+ system, achieved low-pg/mL detection of aldosterone in human serum. The use of MRM³ acquisition was shown to reduce matrix background, allowing for an LOQ of 2.5 pg/mL with excellent precision [6.6%] and accuracy [107.0%], demonstrating the quantitative performance of the method. Excellent linearity was observed within the calibration series [2.5-1000 pg/mL], with a r^2 value of 0.9961. Low-level sensitivity was achieved at the lowest calibrator [2.5 pg/mL] with a signal-to-noise ratio [S/N] of 8:1.

Key benefits of aldosterone analysis from human serum using MRM³ acquisition on the QTRAP 6500+ system

- **Low-pg/mL level sensitivity and excellent quantitative performance:** Sensitive quantitation of aldosterone was performed with excellent precision [6.6%] and accuracy [107%] at the lowest calibrator level [2.5 pg/mL]
- **Rapid sample preparation:** Aldosterone was extracted from human serum samples using a liquid-liquid extraction [LLE], evaporation, and reconstitution
- **Excellent linearity:** Calibration curve for the aldosterone showed an r^2 value of 0.9961 across the calibration range [2.5-1000 pg/mL]

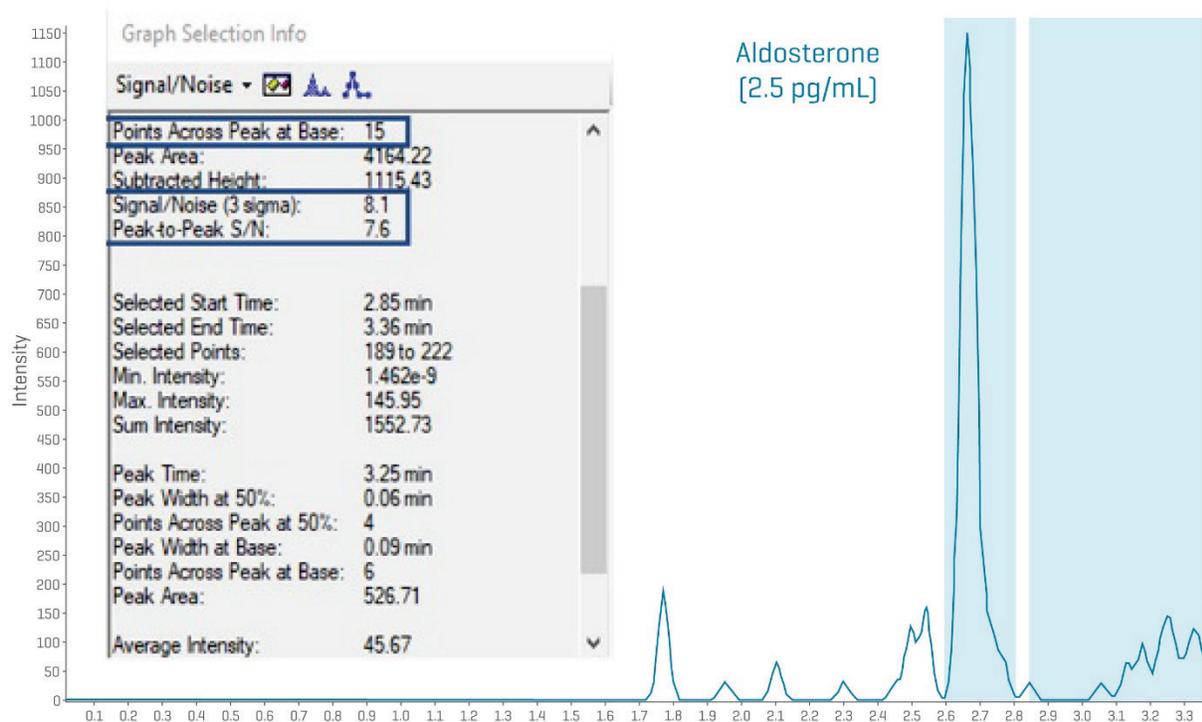


Figure 1. Analytical sensitivity of a 2.5 pg/mL [6.9 pmol/L] aldosterone standard extracted from serum matrix and analyzed using MRM³. Analysis of a 2.5 pg/mL standard in matrix shows a S/N of 8:1 based on a peak-to-peak algorithm.

Introduction

Aldosterone, a mineralocorticoid hormone synthesized by the adrenal cortex, plays a critical role in regulating blood pressure, electrolyte balance, and fluid homeostasis. Its dysregulation is implicated in various conditions. Given its low circulating concentrations and structural similarity to other steroids, accurate quantitation of aldosterone in biological matrices is essential for clinical research applications. MRM³ offers enhanced specificity and sensitivity for detecting aldosterone, supporting robust analysis in complex sample types.¹

Methods

Sample preparation: Serum samples (200 µL) were extracted by a liquid-liquid extraction (LLE) method. The organic layer was then separated and evaporated to dryness. Following reconstitution in 200 µL of mobile phase, 25 µL of the resulting solution was injected onto the LC-MS/MS system.

Liquid chromatography: Chromatographic separation was achieved on a Phenomenex Kinetex C18 column (50 x 3 mm, 2.6 µm, 00B-4462-Y0). Mobile phase A was ammonium fluoride in water and mobile phase B was ammonium fluoride in methanol. The total run time was 4 minutes at a flow rate of 600 µL/min.

Mass spectrometry: Data was collected using a [QTRAP 6500+ system](#) with an IonDrive Turbo V ion source and operated in electrospray ionization (ESI) positive mode using the MRM³ scan function. The MRM³ experiment used a scan speed of 10,000 Da/s, fixed fill time of 250 ms, excitation time of 20 ms and the auxiliary frequency 2 (AF2) was set to 0.2. 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 aldosterone in a control human serum sample at final concentrations of 2.5-1000 pg/mL. The extracted ion chromatogram showed a signal-to-noise [S/N] ratio of 8:1 at the lowest matrix calibrator concentration [2.5 pg/mL], as calculated using a peak-to-peak algorithm.

The quantitative performance of the method was investigated by injecting a series of 3 replicate samples spiked at each of the 9 calibrator levels ranging between 2.5-1000 pg/mL. Linearity and precision were assessed across the calibration range. **Figure 2** shows the calibration curve for aldosterone. The plot shows an excellent linear response across the calibration series, with an r^2 value of 0.9961.

The accuracy and precision values were calculated from 3 replicate injections of the lowest matrix calibrator analyzed [2.5 pg/mL]. The % accuracy was 107% and precision [%CV] was 6.6%.

Conclusions

A fast and sensitive LC-MS/MS method using MRM³ acquisition for the detection of aldosterone in human serum samples was developed. The method demonstrated:

- Fast sample preparation which consisted of a liquid-liquid extraction using only 200 µL of sample volume
- Reduced chromatographic background and interferences using MRM³ acquisition, resulting in increased sensitivity and selectivity
- Excellent linear responses across the calibration series, with an r^2 value of 0.9961
- Good sensitivity resulting in S/N of 8:1 at the lowest calibrator level [2.5 pg/mL]
- High quantitation performance of the method, resulting in excellent precision [6.6%] and accuracy [107%] at the lowest calibrator level [2.5 pg/mL]

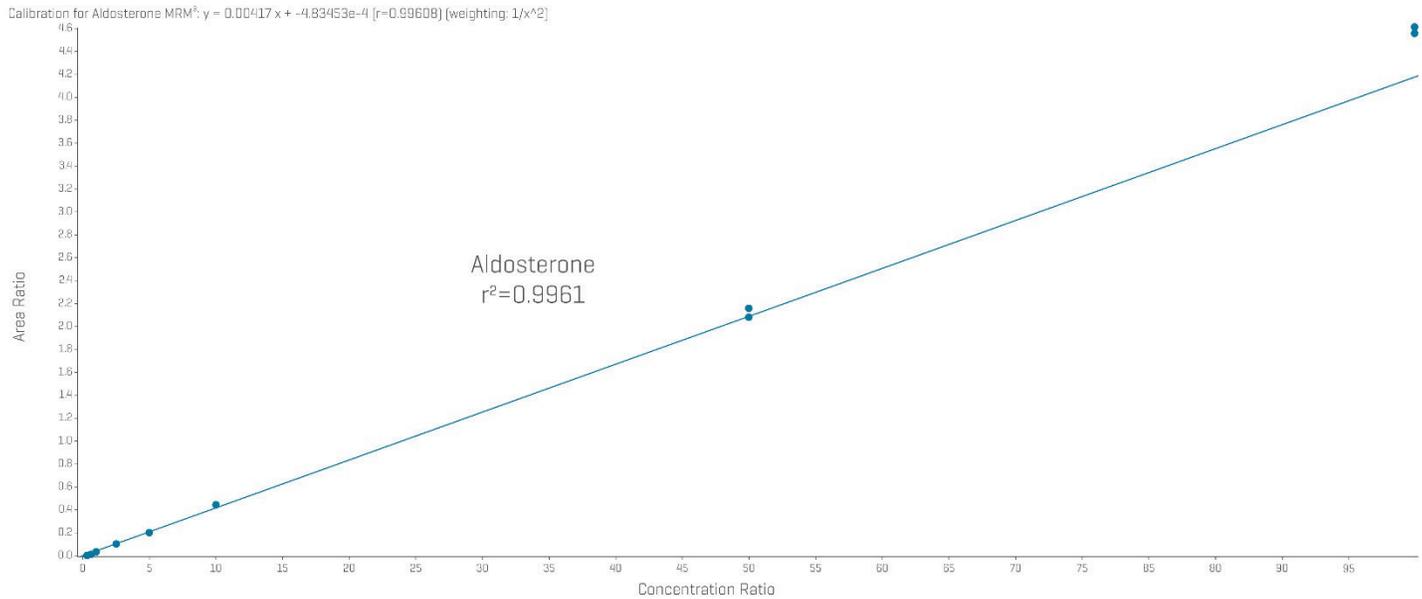


Figure 2. Linear calibration curve for aldosterone extracted from serum matrix and analyzed using MRM3. The calibration curve was run in triplicate across the range of 2.5–1000 pg/mL [6.9–2778 pmol/L]. The curve was generated using linear regression and $1/x^2$ weighting, resulting in a r^2 value of 0.9961.

References

1. MRM3 quantitation for highest selectivity in complex matrices. [SCIEX technical note, RUO-MKT-02-2739-A.](#)

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