# HOW LOW CAN WE GO? ANALYSIS OF ALDOSTERONE USING A HIGHLY ANALYTICALLY SENSITIVE TANDEM QUADRUPOLE MASS SPECTROMETER FOR CLINICAL RESEARCH

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## OVERVIEW

**Aldosterone is a mineralocorticoid steroid hormone produced in the zona glomerulosa of the adrenal cortex. The production of aldosterone is assessed during pharmacological clinical research studies of aldosterone synthase (CYP11B2) inhibitors, where low level detection of aldosterone is required.**

**Radioimmunoassay (RIA) is traditionally used to analyse aldosterone. However, RIA methods can suffer from a lack of analytical selectivity due to the cross reactivity of structurally similar steroid hormones. LC-MS/MS can reduce analytical selectivity issues associated with aldosterone analysis using immunoassays, while providing analytical sensitivity to measure low levels of plasma aldosterone for clinical research. In this investigation, the performance of UPLC coupled to a new tandem quadrupole mass spectrometer, has been shown to provide analytical sensitivity down to 2pg/mL aldosterone using only 200µL of sample.**

## INTRODUCTION

Aldosterone plays a central role in the regulation of blood pressure through maintenance of the $Na^+/K^+$ balance in the the nephron in the kidneys.

Steroid pathways associated with aldosterone consist of many structurally similar steroid hormones (Figure 1). There is underlying complexity within this pathway with hundreds of different metabolites and structurally similar species. Therefore, care must be taken when developing an analytical method, to limit interferences which affect accuracy and precision. Tandem mass spectrometry (MS/MS) provides a way to differentiate between these hormones, while the LC dimension provides separation of isobaric species.

Aldosterone also requires highly sensitive instrumentation to enable quantification at low levels in plasma. This requirement makes aldosterone an ideal candidate for evaluating the capabilities of a new, highly analytically sensitive mass spectrometer, the Waters® Xevo® TQ-XS (Figure 2).
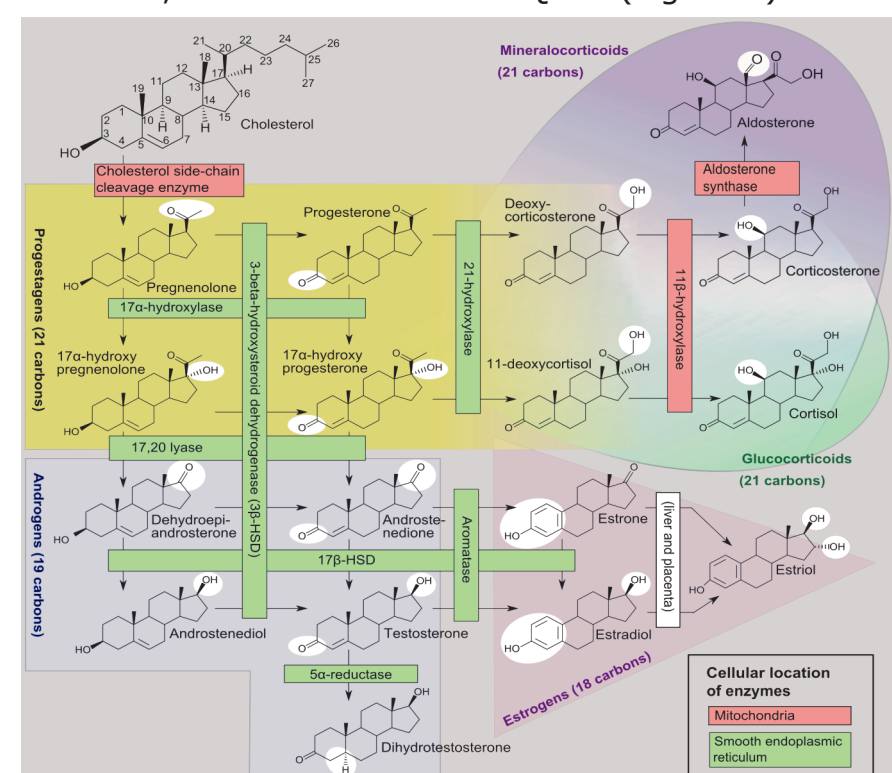


Figure 1. Human steroidogenesis; Enzymes, their cellular location, their substrates and products[1].

## METHODS

**Materials**

- Certified aldosterone reference material (Cerilliant, Round Rock, TX) was used to prepare calibrators (15—1500 pg/mL; 42-4161pmol/L) in MSG4000 pooled human serum (Golden West Biologicals, CA). QC samples were prepared in pooled human plasma (Seralab, UK)
- Total precision was determined by extracting and quantifying ten replicates of quad-level QC material per day over three consecutive days (n=30). Repeatability was determined by analyzing ten replicates at each QC level.
- A comparison was performed between the Xevo TQ-S and Xevo TQ-XS using anonymized aldosterone plasma samples (n=45).
- A comparison was also performed using 1pg/mL aldosterone solution and 2pg/mL aldosterone in serum.

**Methods**

- Using a Tecan® Freedom EVO® 100 Liquid Handler, samples were pre-treated with internal standard, zinc sulfate in methanol and 0.05% phosphoric acid.
- Following centrifugation, sample supernatant was transferred to a Waters Oasis® MAX µElution plate, washed with 0.05% phosphoric acid, 0.1% ammonia in 10% methanol, water and eluted with methanol.
- Using a Waters ACQUITY UPLC® I-Class System, samples were injected onto a 2.1x100mm CORTECS® UPLC $C_{18}$ column using a water/methanol gradient at 0.3mL/min and analyzed on both Waters Xevo TQ-S and Xevo TQ-XS detectors using MRM transitions seen in Table 1.

| Compound | MRM Transition (m/z) | Cone (V) | Collision (eV) |
|---|---|---|---|
| Aldosterone | 359.2>189.2 (297.2) | 55 | 18 (16) |
| Aldosterone—$^2H_4$ | 363.2>190.2 | 55 | 18 |

Table 1. MRM parameters used for the analysis of aldosterone and its internal standard. Qualifier ion parameters are shown in parentheses.



Figure 2. Xevo TQ-XS Detector with ACQUITY UPLC I-Class

- ESI and UniSpray™ (Figure 3) were also evaluated for the analysis of aldosterone.
- UniSpray is a novel ionization technique, developed to provide ionization of the widest range of compounds in a single analysis. It has a simplified probe design, reducing maintenance and downtime.
- Ionization using UniSpray occurs when a high velocity nebulized spray impacts a polished, stainless steel target rod, typically held at 1kV.
- Gas flow follows the curvature of the target surface and is directed towards the inlet orifice (Coanda effect).
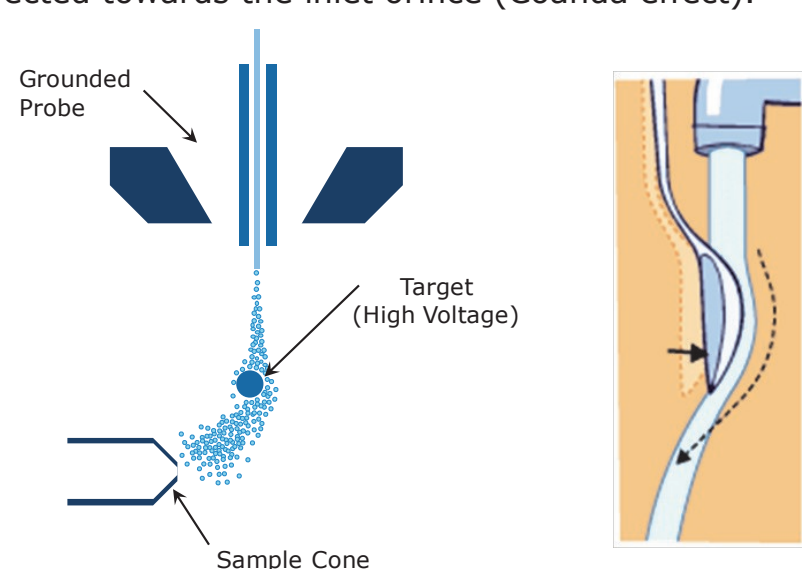


Figure 3. UniSpray configuration, demonstrating the Coanda effect, whereby the flow follows the curvature of the target pin towards the inlet orifice.

## RESULTS

**Xevo TQ-XS Analytical Sensitivity**

- An aldosterone solvent standard was prepared at 1pg/mL (2.8pmol/L), injected in triplicate and analyzed on both the Xevo TQ-S and Xevo TQ-XS systems (Figure 4)
- There was a >5x improvement in S/N using the Xevo TQ-XS, with S/N being greater than the Limit of Quantification (S/N = 10) at 1pg/mL.
- There was a >6x improvement in peak area observed using the Xevo TQ-XS, with imprecision <2% RSD.
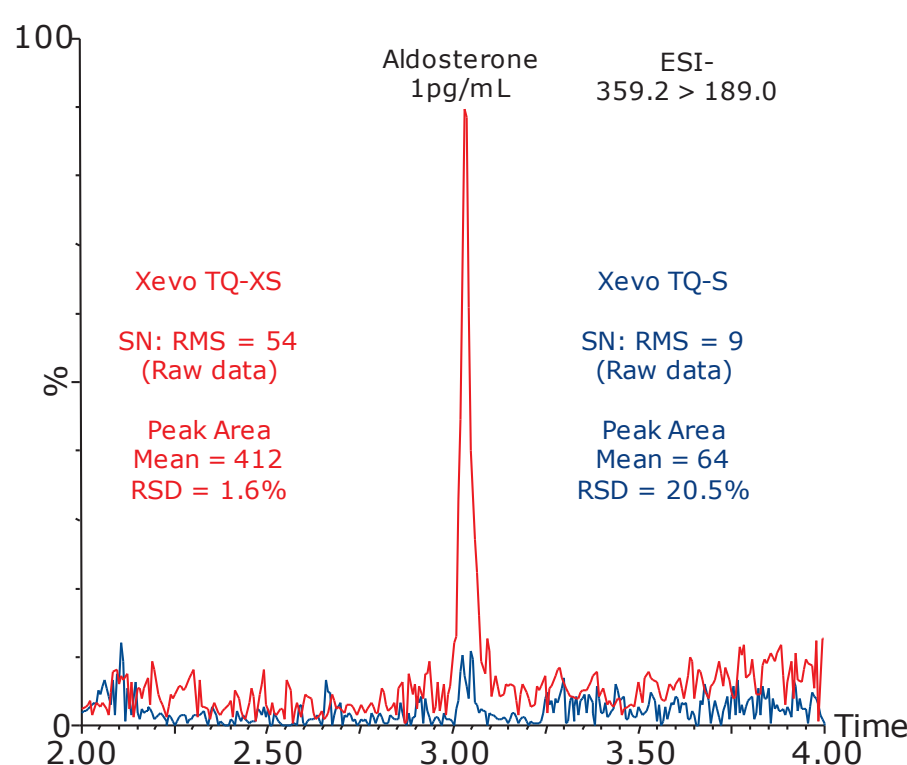


Figure 4. Injection of a 1pg/mL solution (n=3, 50fg on column) of aldosterone on the Xevo TQ-XS and Xevo TQ-S. S/N performed using RMS on raw data.

**Aldosterone in Plasma**

- An extracted plasma sample for aldosterone was injected on both the Xevo TQ-S and Xevo TQ-XS (Figure 5).
- There was >3x increase in observed S/N using the Xevo TQ-XS, providing enough analytical sensitivity for quantification of very low levels of aldosterone in plasma.
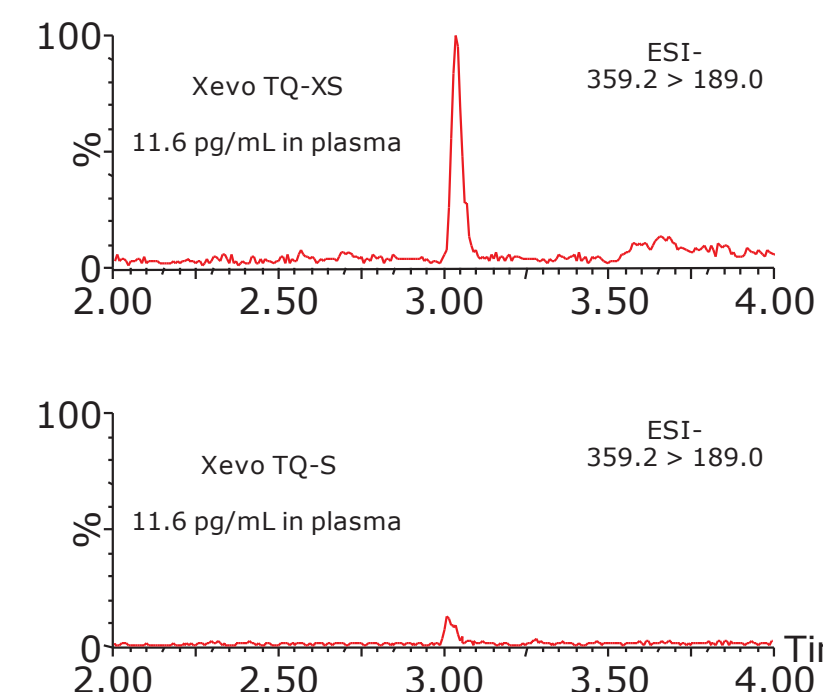


Figure 5. Injection of an extracted aldosterone plasma sample on the Xevo TQ-XS and Xevo TQ-S, quantified at 11.6pg/mL (350fg on column).

**Linearity and Analytical Sensitivity**

- Calibration lines (15-1500pg/mL, 42-4161pmol/L) in extracted samples were linear with $r^2 > 0.999$ (n=3) for aldosterone.
- The S/N ratios for the lowest calibrator (15pg/mL, 42pmol/L) for spiked serum were >25:1 over 3 separate occasions.

Compound name: Aldosterone
Correlation coefficient: r = 0.999940, r^2 = 0.999880
Calibration curve: 0.00266993 * x + 0.00252306
Response type: Internal Std ( Ref 2 ), Area * ( IS Conc. / IS Area )
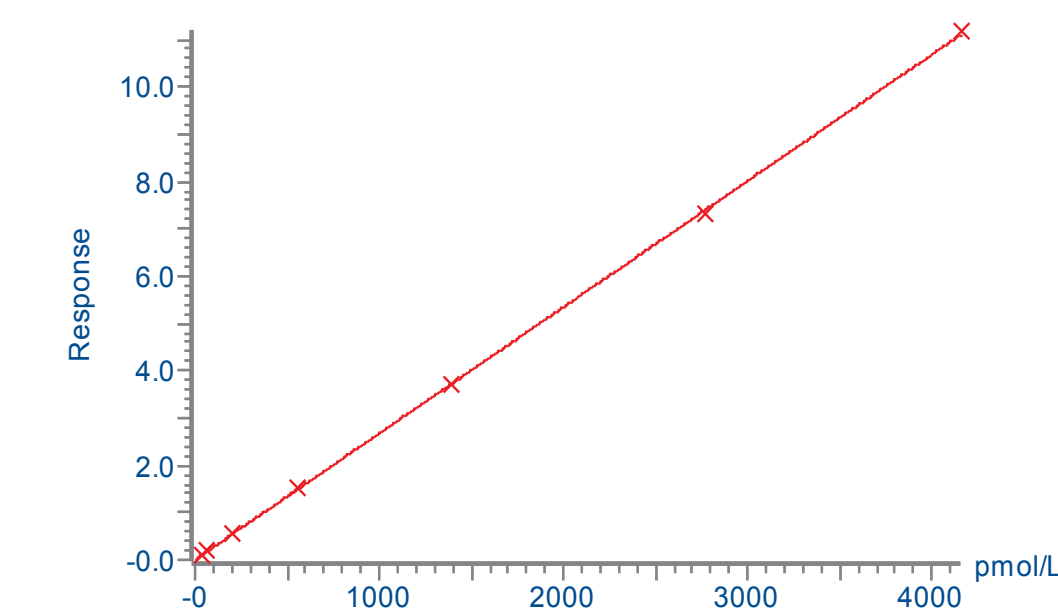Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Figure 6. An extracted aldosterone calibration line on the Xevo TQ-XS.

**Imprecision**

- QC1-4 concentrations in plasma were 21, 42, 174 and 669 pg/mL (58, 117, 482 and 1856 pmol/L) for aldosterone.
- Total precision and repeatability using the Liquid Handler were ≤ 8.2% on the Xevo TQ-S and ≤ 7.9% on the Xevo TQ-XS
- The Xevo TQ-XS has been shown to reduce imprecision of the method.

| MS system | Total QC Imprecision | | | |
|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 |
| Xevo TQ-XS | 7.9% | 5.6% | 3.9% | 5.4% |
| Xevo TQ-S | 8.3% | 7.2% | 5.0% | 5.4% |

Table 2. Total imprecision observed for the plasma QC samples on the Xevo TQ-XS and Xevo TQ-S.

**Comparison**

- A system comparison was performed using unadulterated plasma samples (n=45) that span the physiological range of aldosterone.
- Statistical analysis was performed on the results using Deming regression, Altman-Bland agreement and Linear Regression.
- Deming regression (Figure 7) for the system comparison was y = 0.98x—5.90, demonstrating no statistically significant proportional or constant bias (p>0.05).
- Altman Bland agreement demonstrates a mean bias of −4.2% for aldosterone.
- Linear regression ($r^2$) between the methods was 0.999.
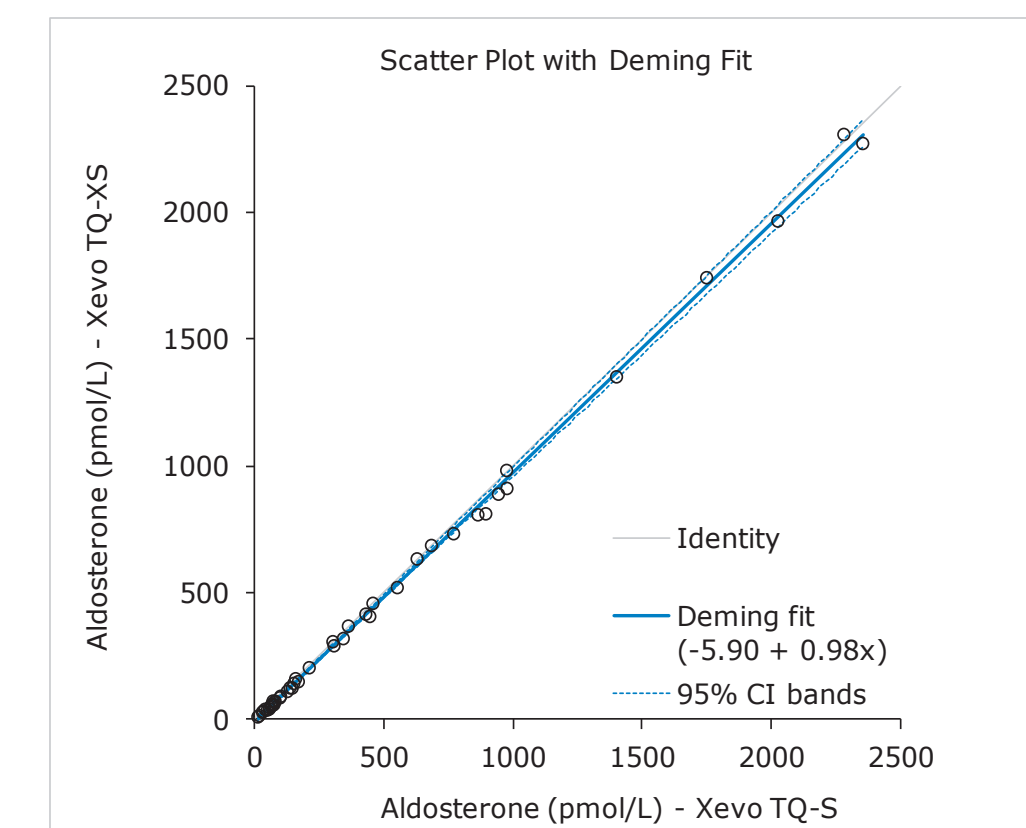- These results indicate that the Xevo TQ-XS is suitable for analysing aldosterone in plasma



Figure 7. Deming regression demonstrating excellent agreement between plasma aldosterone concentrations obtained on the Xevo TQ-S and Xevo TQ-XS.

**ESI vs UniSpray**

- Both ESI and UniSpray ionization techniques were compared on the Xevo TQ-XS by analyzing extracted serum aldosterone samples (n=3; 2pg/mL, 5.4pmol/L)
- Both a peak area and S/N improvement was observed using UniSpray (Figure 8).
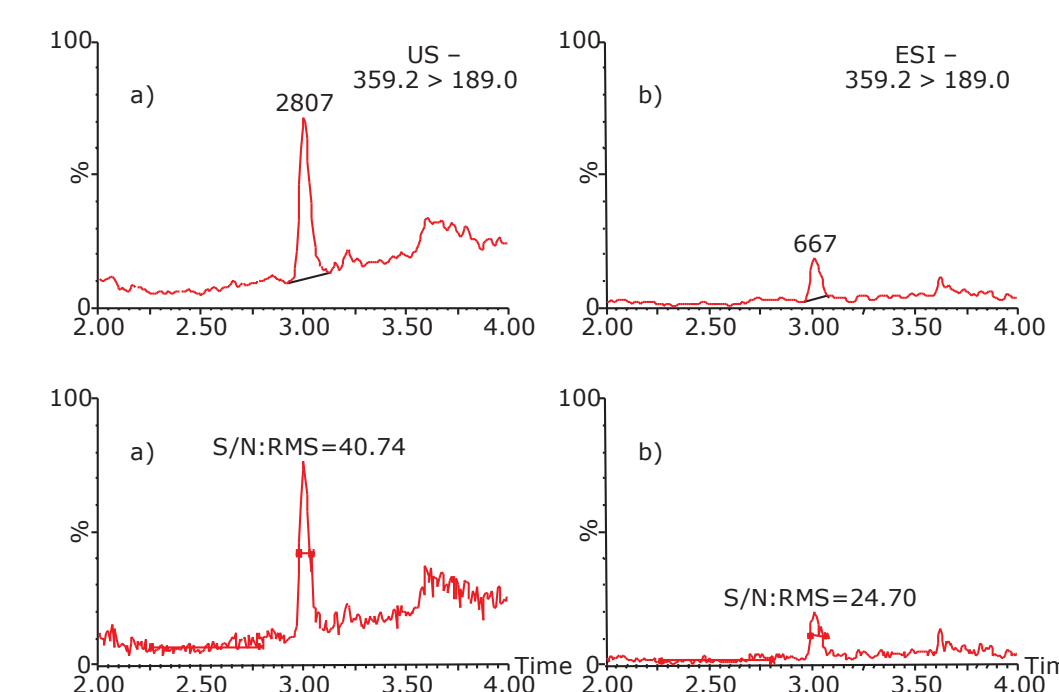


Figure 8. Comparison of UniSpray (a) and ESI (b) on the Xevo TQ-XS for an extracted aldosterone sample at 2pg/mL (60fg on column), showing peak area and S/N RMS values

- The quantification of the peak area at this concentration was shown to be reproducible (<20% RSD)(Table 3).

| Replicate | UniSpray | | ESI | |
|---|---|---|---|---|
| | Peak Area | S/N | Peak Area | S/N |
| 1 | 2807 | 40.7 | 667 | 24.7 |
| 2 | 3223 | 41.3 | 528 | 26.6 |
| 3 | 2855 | 46.0 | 567 | 20.7 |
| Mean | 2962 | 42.7 | 587 | 24.0 |
| SD | 227 | 2.9 | 72 | 3.0 |
| RSD | 7.7% | 6.8% | 12.2% | 12.5% |

Table 3. Analysis of extracted 2pg/mL aldosterone sample (n=3) using UniSpray and ESI on the Xevo TQ-XS

## CONCLUSION

- **The Xevo TQ-XS provides analytical sensitivity for the analysis of plasma aldosterone for clinical research purposes**
- **The improved analytical sensitivity and imprecision performance on the Xevo TQ-XS compared to the Xevo TQ-S, provides greater confidence in data collected at low concentrations**
- **UniSpray may provide additional analytical sensitivity, however, further evaluation is required to confirm performance for plasma aldosterone**

**References**

1. Häggström M, Richfield D (2014). "Diagram of the pathways of human steroidogenesis". Wikiversity Journal of Medicine **1** (1).

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