

Analysis of Aldosterone in Plasma for Clinical Research using the ACQUITY UPLC I-Class/Xevo TQ-S micro System

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This is an Application Brief and does not contain a detailed Experimental section.

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Abstract

To demonstrate the capability of the ACQUITY™ UPLC™ I-Class System and Xevo™ TQ-S micro Mass Spectrometer to quantify low levels of aldosterone in plasma for clinical research using a highly selective sample preparation technique.

Benefits

- Analytically sensitive analysis of aldosterone in plasma (low calibrator 42 pmol/L).
- Incorporation of automated sample preparation optimizes analytical sensitivity, reduces sample handling time, and alleviates the potential for operator error.
- Total precision $\leq 7.2\%$ across the calibration range for plasma QC samples over five days (n=25).

Introduction

Aldosterone is a mineralocorticoid steroid hormone, that is assessed in clinical research studies to help understand the pharmacological mechanism of aldosterone synthase inhibitors (ASIs).¹ Circulating levels of aldosterone in blood are typically found at low concentrations (<100 pmol/L), which makes its analysis particularly challenging. Successfully quantifying these low levels necessitate the use of a mass spectrometer with high analytical sensitivity in conjunction with highly selective sample preparation techniques.

Here we introduce the Xevo TQ-S micro utilizing innovative technology such as the Stepwave™ ion source technology to improve method robustness and reduce background noise, which enables accurate and precise quantification of low level analytes such as aldosterone.

Experimental

The Solution

In this technology brief, a UPLC-MS/MS method for the analysis of plasma aldosterone was successfully employed using the Xevo TQ-S micro in conjunction with automated selective solid phase extraction sample preparation. The sample preparation was automated on a Tecan® Freedom EVO100/4 using 96-well Oasis™ MAX μElution SPE plates followed by analysis on the MassLynx™ 4.1 controlled UPLC-MS/MS as outlined in application note [720005262](#).



*Waters ACQUITY UPLC I-Class/Xevo TQ-S
micro System.*

Sample Preparation and UPLC-MS/MS Analysis

Using the Tecan Freedom EVO100/4, plasma samples were diluted with internal standard, zinc sulphate, methanol, and phosphoric acid. Following centrifugation, sample supernatant was loaded onto the 96-well Oasis MAX μ Elution SPE plate (p/n:186001829 <<https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186001829-oasis-max-96-well--elution-plate-2-mg-sorbent-per-well-30--m-1-p.html>>) following conditioning and equilibration. Consecutive washes with phosphoric acid, ammonia in 10% methanol, and water were performed. Samples were eluted with 70% aqueous methanol followed by water.

30 μ L of each extracted sample was injected on an ACQUITY UPLC I-Class System/Xevo TQ-S micro System utilizing a water/methanol gradient and a CORTECS™ UPLC C₁₈ Column (p/n: 186007095 <<https://www.waters.com/nextgen/global/shop/columns/186007095-cortecs-c18-column-90a-16--m-21-mm-x-100-mm-1-pk.html>>). The MRM parameters used in this analysis are shown in Table 1.

Analyte	Precursor (m/z)	Product (m/z)	Cone (V)	Collision (eV)
Aldosterone (Quan)	359.2	189.2	38	14
Aldosterone (Qual)	359.2	297.2	38	10
Aldosterone- ² H ₄	363.2	190.2	38	14

Table 1. MRM parameters for both aldosterone quantifier and qualifier and its internal standard, aldosterone-²H₄.

	Calibration		Cal 1 (42pmol/L)	
	r ²	Slope (m)	Peak area	S/N
Mean	0.9988	0.00262	73	37
%RSD	0.1%	1.3%	8.6%	19.1%

Table 2. Mean values obtained for the calibration curve and the lowest calibrator (Cal 1) on the Xevo TQ-S micro over five days. S/N is calculated on the raw data using peak to peak at ± 1 SD.

Results and Discussion

Calibration curves, quality control (QC) samples, and plasma samples were extracted and analyzed over five separate days. The S/N for the low calibrator at 42 pmol/L was >25:1 over the five days, achieved through low background noise on the instrument, selectivity of the MRM trace and a clean SPE sample extract. In addition, the correlation coefficient demonstrates excellent linearity across a range of 42–4161 pmol/L.

Note: To convert SI units to conventional mass units divide by 2.774 for aldosterone (pmol/L to pg/mL).

The additional selectivity provided by the Oasis MAX chemistry provides a clean sample extract for ESI MS analysis of aldosterone. This is observed in the extraction and quantification of 49 pmol/L of aldosterone in plasma using the Xevo TQ-S micro (Figure 1).

Reproducibility of the method was assessed by extracting and quantifying plasma samples using six replicates at low (128 pmol/L), mid (1011 pmol/L), and high (2926 pmol/L) concentrations. All results were $\leq 7.2\%$ RSD as shown in Table 3.

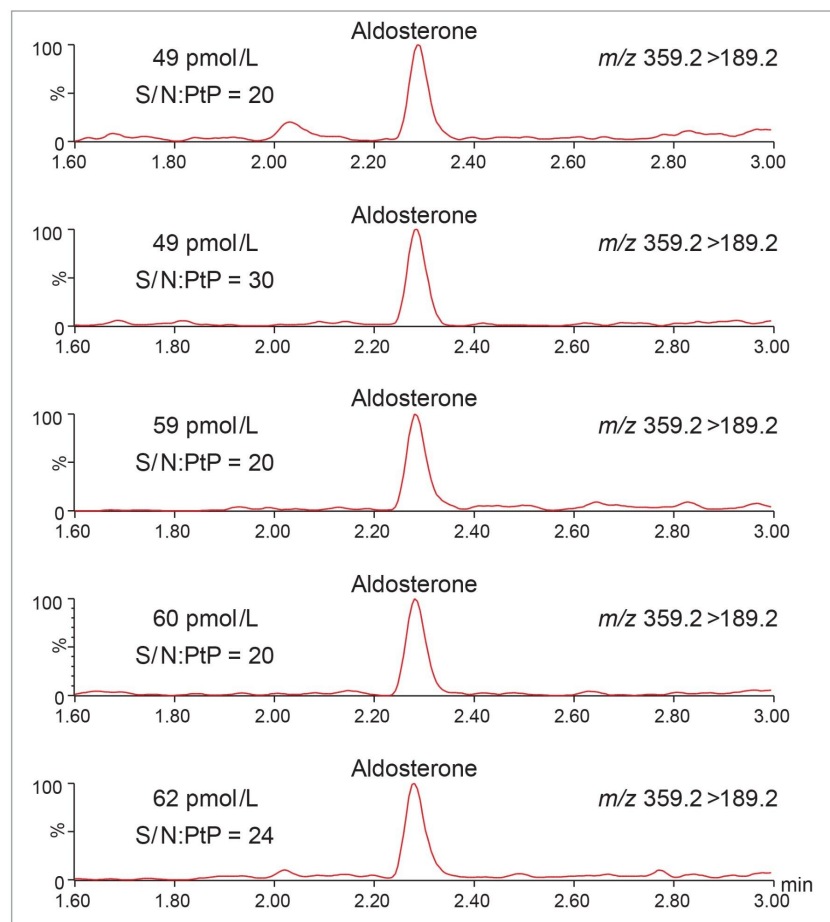


Figure 1. Extracted SPE samples with concentrations ranging from 49–62 pmol/L aldosterone in plasma using the Xevo TQ-S micro. S/N is calculated on the raw data using peak to peak at ± 1 SD.

	Xevo TQ-S micro (RSD%)		
	Low	Mid	High
Total	6.0%	7.2%	3.8%
Repeatability	5.3%	7.0%	3.0%

Table 3. Total precision and repeatability assessment for the analysis of aldosterone in plasma on the Xevo TQ-S micro analyzing five replicates at three concentrations over five days.

Conclusion

A UPLC-MS/MS method for the analysis of plasma aldosterone for clinical research has been developed using an ACQUITY UPLC I-Class/Xevo TQ-S micro System. This method incorporates highly efficient automated sample preparation using a Freedom EVO 100/4 and 96-well Oasis MAX μ Elution SPE plates. The robust and analytically sensitive Xevo TQ-S micro has been shown to provide excellent precision and linearity of response across all plasma aldosterone levels that were tested.

References

1. Schumacher CD *et al.* Aldosterone Synthase Inhibition for the Treatment of Hypertension and the Derived Mechanistic Requirements for a New Therapeutic Strategy. *J Hypertens.* Oct 2013; 31(10): 2085–2093.

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