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

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A highly selective, sensitive research method utilizing automated sample preparation and UPLC-MS/MS for the analysis of aldosterone in plasma has been developed.

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

GOAL

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.

BACKGROUND

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.

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 720005262EN.

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 µElution SPE plate (P/N:186001829) 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 µL 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 C_{18} Column (P/N:186007095). 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-$^2H_4$	363.2	190.2	38	14

Table 1. MRM parameters for both aldosterone quantifier and qualifier and its internal standard, aldosterone-$^2H_4$.

RESULTS

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 ≤7.2% RSD as shown in Table 3.

	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.

	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.

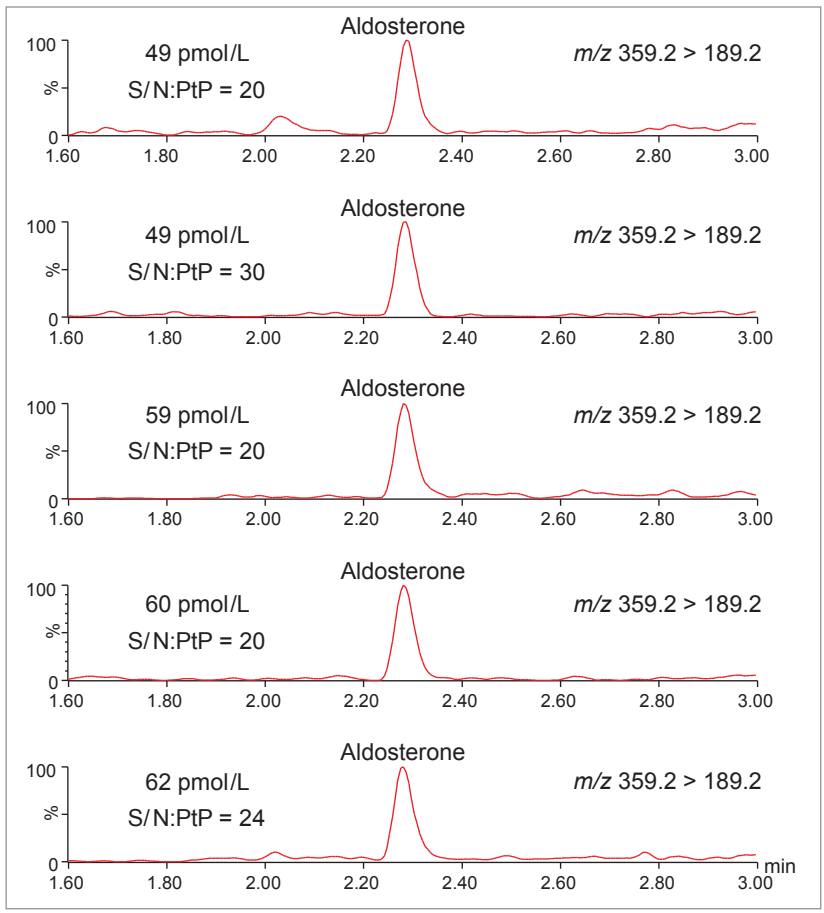

Figure 1. Extracted SPE samples with concentrations ranging from 49–62 pmol/L aldosterone in plasma using the Xevo TQ-S micro. S/N is calculated on the raw data using peak to peak at ±1 SD.

SUMMARY

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 µElution 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.

The benefits of this method include:

- 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 ≤7.2% across the calibration range for plasma QC samples over five days (n=25).

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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