Analysis of Aldosterone in Plasma for Clinical Research using the Xevo TQ-S micro

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GOAL

To demonstrate the capability of Waters® 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 typically necessitate the use of a mass spectrometer with high analytical sensitivity in conjunction with highly selective sample preparation techniques.

The Xevo TQ-S micro utilizes innovative StepWave™ ion source technology to improve method robustness and reduce background noise, which enable accurate and precise quantification of low level analytes such as aldosterone.

THE SOLUTION

The method for the analysis of plasma aldosterone was successfully employed using automated selective solid phase extraction sample preparation followed by LC-MS/MS analysis. The sample preparation was automated on a Tecan Freedom EVO100/4 using 96-well Oasis® MAX µElution Plates followed by analysis on the ACQUITY UPLC® I-Class System with Xevo TQ-S micro and MassLynx® Software (v4.1).

Low levels of aldosterone were successfully quantified by using automated sample preparation and the Xevo TQ-S micro.

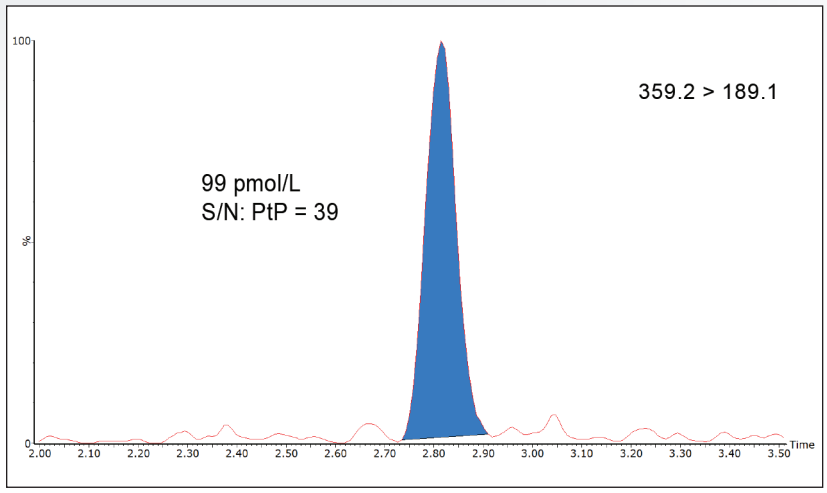

Figure 1. An extracted SPE sample of 99 pmol/L aldosterone in plasma on the Xevo TQ-S micro. S/N is calculated on the raw data using peak to peak at ±1 SD.

Sample preparation and LC-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 Plate (PN: 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/Xevo TQ-S micro system utilizing a water/methanol gradient and an ACQUITY UPLC BEH Phenyl Column (PN: 186002884). The MRM parameters used in this analysis are shown in table 1.

Analyte	Precursor ion (*m/z*)	Product ion (m/z)	Cone voltage (V)	Collision energy (V)
Aldosterone (Quan)	359.2	189.1	38	14
Aldosterone (Qual)	359.2	297.2	38	10
Aldosterone-d4 (IS)	363.2	190.0	38	14

Table 1. MRM parameters for both aldosterone quantifier (Quan) and qualifier (Qual) and its internal standard (IS), aldosterone-d4.

Results

As shown in table 2, a relatively low peak area at the LLOQ is obtained on the Xevo TQ-S micro using SPE extracted plasma samples. However, the signal:noise (S/N) is >20:1, which is an indication of the low background noise on the instrument resulting from the selectivity of the MRM trace and a clean SPE sample extract. In addition, the correlation coefficient demonstrates excellent linearity across a range of 28-2776 pmol/L.

MS system	Calibration curve	LLOQ (28pmol/L)	
	Correlation coefficient (r^2)	Peak area	S/N
Xevo TQ-S micro	0.9995	58	23

Table 2. Calibration curve and LLOQ performance characteristics on the Xevo TQ-S micro. S/N is calculated on the raw data using peak to peak at ±1 SD.

The additional selectivity provided by the Oasis MAX µElution Plate provides a clean sample extract for ESI MS analysis of aldosterone. This is observed in the extraction and quantification of 99 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 (99), Mid (500), and High (2000 pmol/L) concentrations. All results were ≤5.5% RSD as shown in table 3.

	Xevo TQ-S micro Repeatability (RSD%)		
Compound	Low	Mid	High
Aldosterone	5.5%	4.0%	4.7%

Table 3. Repeatability assessment for the analysis of aldosterone in plasma on the Xevo TQ-S micro.

SUMMARY

An LC-MS/MS method for the analysis of plasma aldosterone for clinical research has been developed. This method demonstrates highly efficient automated sample preparation with the Tecan Freedom EVO 100/4 and 96-well Oasis MAX µElution SPE Plates. Automated sample preparation optimizes analytical sensitivity and increases laboratory efficiency with reduced sample handling, alleviating the potential of operator error. The ACQUITY UPLC I-Class System, when combined with the robust and analytically sensitive Xevo TQ-S micro, has been shown to provide excellent repeatability (n = 6) and linearity of response across all aldosterone levels that were tested. Furthermore, the results from this clinical research method are acceptable to measure aldosterone at physiological levels.

References

1. Schumacher CD, Steele RE, Brunner HR. 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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