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UPLC-MS/MS Analysis of Aldosterone in Plasma for Clinical Research

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Abstract

In this application note we describe a clinical research method utilizing an Oasis MAX µElution Plate for the extraction of aldosterone from plasma.

Benefits

- Analytical selectivity improves reproducibility through removal of interferences

- Analytical sensitivity enables the quantification of low physiological levels of aldosterone

- Facilitates high sample throughput utilizing automation of the multiwell plate format

Introduction

Aldosterone is a mineralocorticoid steroid hormone that plays a central role in the regulation of blood pressure. Traditionally, aldosterone has been analysed by radioimmunoassays. However, these methods involve the use of hazardous radioisotopes. In addition, they can suffer from a lack of specificity due to the cross reactivity of structurally similar steroid hormones and metabolites, which may result in greater imprecision and inaccuracy. To minimize specificity issues, radioimmunoassay methods employ time-

consuming manual extraction protocols. UPLC-MS/MS combined with automation of the sample preparation with sample tracking capabilities provides an alternative means of aldosterone analysis for clinical research. An integrated workflow solution enables selective and analytically sensitive characterisation of aldosterone with a reduction in sample handling time.

In this application note we describe a clinical research method utilizing an Oasis MAX µElution Plate for the extraction of aldosterone from plasma, which has been automated on the Tecan Freedom Evo 100/4 Liquid Handler. Chromatographic separation was performed on an ACQUITY UPLC I-Class System using a CORTECS UPLC C_{18} Column followed by detection on a Xevo TQ-S Tandem Quadrupole Mass Spectrometer (Figure 1).

Figure 1. The Waters ACQUITY UPLC I-Class System and Xevo TQ-S Mass Spectrometer.

Experimental

UPLC conditions

System:	ACQUITY UPLC I-Class
Needle:	30 µL
Column:	CORTECS UPLC C_{18}, 2.1 x 100 mm, 1.6 µm (Waters p/n 186007095)
Mobile phase A:	Water
Mobile phase B:	Methanol
Needle wash solvent:	80% methanol$_{(aq)}$
Purge solvent:	40% methanol$_{(aq)}$
Column temp.:	45°C
Injection volume:	25 µL
Flow rate:	0.40 mL/min
Gradient:	See Table 1
Run time:	4.0 minutes

Time (min)	Flow Rate (ml/min)	%A	%B	Curve
Initial	0.400	60	40	Initial
1	0.400	60	40	6
2	0.400	40	60	6
2.3	0.400	5	95	11
2.8	0.400	60	40	11

Table 1. Gradient table for the separation of aldosterone. Operating backpressure at the initial conditions is 12000 psi.

MS conditions

System: Xevo TQ-S

Resolution: MS1 (0.7 FWHM)

                                 MS2 (0.5 FWHM)

Acquistion mode: Multiple reaction monitoring (MRM) (see Table 2
                                 for details)

Polarity: ESI negative

Capillary: 2.40 kV

Source temp.:   150 °C

Desolvation temp.:   600 °C

Dwell time: 0.1 seconds

Inter-scan delay: 0.01 seconds

Inter-channel delay: 0.02 seconds

Analyte	Precursor ion (m/z)	Product ion (m/z)	Cone voltage	Collision energy
Aldosterone (Quan)	359.2	189.2	55	18
Aldosterone (Qual)	359.2	297.2	55	16
Aldosterone-^{4}H$_2$	363.2	190.2	55	18

Table 2. MRM parameters for both aldosterone quantifier and qualifier and its internal standard, aldosterone-^{4}H$_2$.

Data management

MassLynx v4.1 with TargetLynx Application Manager

Sample preparation

Aldosterone certified reference solution and its stable labeled internal standard ($^4H_2$) were purchased from Cerilliant (Round rock, TX) and IsoSciences (King of Prussia, PA), respectively. Calibrators were prepared in a surrogate matrix of MSG4000 stripped human serum purchased from Golden West Biologicals (Temecula, CA). The calibration range for aldosterone was 42-4161 pmol/L (15-1500 pg/mL). QC materials were prepared using pooled plasma purchased from SeraLab (Haywards Heath, UK) at 99, 500 and 2000 pmol/L (36, 180 and 720 pg/mL).

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

Sample extraction

Extraction was performed using a Tecan Freedom Evo 100/4 Liquid Handler. To 200 µL of sample; 25 µL of 4000 pmol/L internal standard, 200 µL 0.1 M zinc sulphate in 50% methanol and, 450 µL of 0.05% (v/v) phosphoric acid were added. The samples were mixed between each reagent addition. The samples were centrifuged for 5 minutes at 1000g.

The Oasis MAX µElution Plate (Waters p/n 186001829) was conditioned and equilibrated with 200 µL methanol and water, respectively. An aliquot of each of the pretreated samples (625 µL) was loaded into individual wells of the plate and slowly pulled through at low vacuum. Consecutive washes with 200 µL of 0.05% (v/v) phosphoric acid, 200 µL 0.1% ammonia in 10% methanol, and 200 µL water were performed. Aldosterone was eluted using 50 µL of 70% methanol$_{(aq)}$, followed by 40 µL water.

Method conditions

On instrument setup ensure the mobile phase lines are purged for a minimum of 10 minutes to remove additives, which could negatively impact sensitivity for aldosterone.

Results and Discussion

No interferences were observed at the retention time of aldosterone when nine structurally related compounds were examined (18-hydroxycorticosterone, cortisol, cortisone, 11-deoxycorticosterone, corticosterone, 11-deoxycortisol, 21-deoxycortisol, prednisolone, and prednisone). In Figure 2, the chromatographic selectivity of the column is demonstrated through the baseline resolution of aldosterone and its metabolic precursor, 18-hydroxycorticosterone. Separation of 18-hydroxycorticosterone is necessary due to the detection of its isotopic transition in the aldosterone-$^4H_2$ internal standard MRM trace, when present in the sample at 60 nmol/L. In addition, separation of prednisone was required due to ion suppression of aldosterone at prednisone concentrations of 1 µmol/L. This baseline resolution allows for the independent quantification of aldosterone.

Analytical sensitivity investigations reveal that the analytical sensitivity of this method would allow precise quantification (<20% RSD) at 42 pmol/L for aldosterone. Signal:noise (S/N) of the 42 pmol/L calibration standard was >10:1 on ten separate occasions.

Total precision was determined by extracting and quantifying three replicates of tri-level QC material on two occasions per day over five separate days (n = 30). Repeatability was assessed by analyzing three replicates at each QC level. The results of these experiments are shown in Table 3, where total precision and repeatability at the low (99 pmol/L), mid (500 pmol/L), and high (2000 pmol/L) concentrations is ≤9.8% RSD for aldosterone.
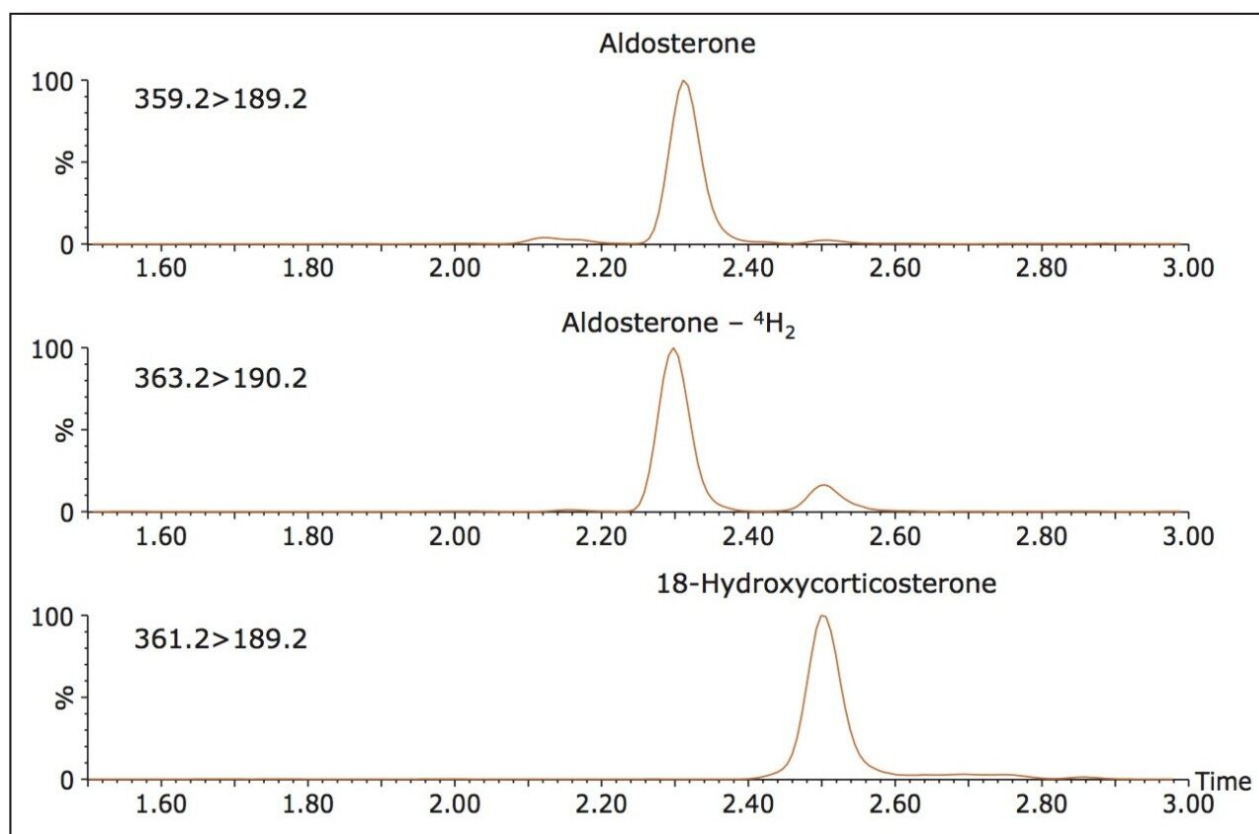

Figure 2. UPLC separation of aldosterone, aldosterone-$^4H_2$, and 18-hydroxycorticosterone using a CORTECS UPLC C$_{18}$ Column.

Compound	Total QC precision (RSD)			QC repeatability (RSD)		
	Low	Mid	High	Low	Mid	High
Aldosterone	9.8%	7.1%	4.8%	8.2%	7.0%	3.9%

Table 3. Total precision and repeatability for the analysis of aldosterone.

The method was shown to be linear over the range of 42–4161 pmol/L when different ratios of high and low concentration pools of aldosterone were combined and analysed. In addition, calibration lines in spiked serum were linear with coefficient of determinations (r^2) >0.996 on 10 separate occasions.

No system carryover was observed from high concentration samples at 10400 pmol/L into subsequent blank injections. A 1:2 dilution was successfully performed on an over-range sample with a concentration of 7606 pmol/L, providing a mean accuracy of 99% with an RSD of 4.4%.

Matrix effects were evaluated as the peak area of extracted post spiked aldosterone plasma samples taken as a percentage of extraction solvent samples spiked to equivalent concentrations. Mean (range) matrix effects were 1.10 (1.03–1.20) with RSD of 5.9% for aldosterone. Calculations using analyte:internal standard response ratio indicated compensation for signal enhancement by the internal standard, providing a mean (range) net matrix effect of 1.00 (0.90–1.10) with RSD of 7.7% for aldosterone.

Samples were selected (n = 59) for comparison against an independently developed LC-MS/MS method for aldosterone. A selection of samples from this comparison having quantified values <100 pmol/L are shown in Figure 3. These chromatograms demonstrate the selectivity of the extraction protocol and the sensitivity of the analytical method.

Comparison data were processed using Analyse-it Software v2.3. The comparison between the two independent LC-MS/MS methods yielded a Deming regression of y = 1.07x - 22.94 (Figure 4A), which showed no significant proportional or constant bias (p>0.05). Altman-Bland agreement between the methods demonstrates a mean negative bias of -4.9% (95% CI -10.3 to 0.5%) (Figure 4B) for the UPLC-MS/MS method described herein.
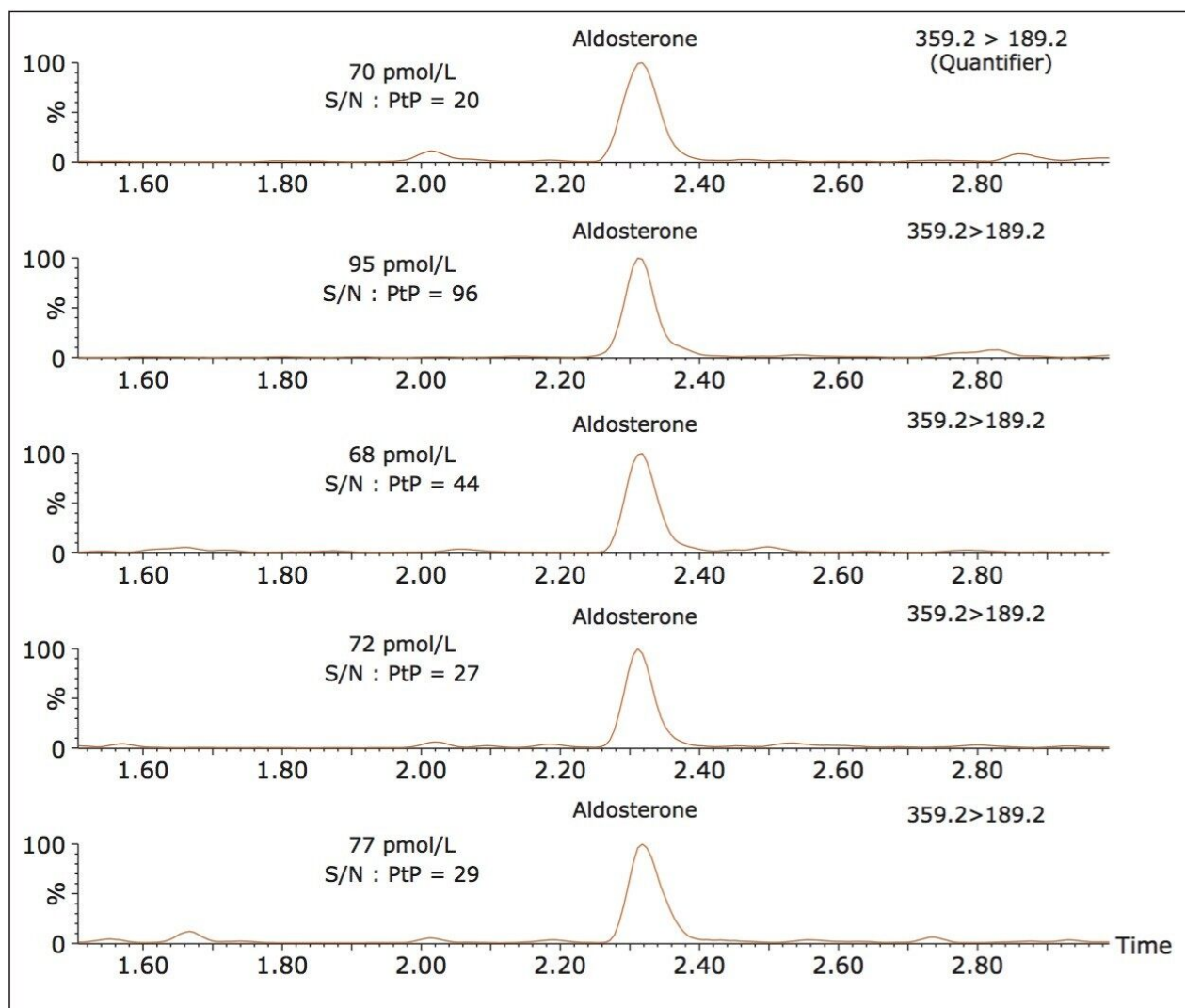
Figure 3. Five representative chromatograms from plasma samples having quantified aldosterone values <100 pmol/L. The signal-to-noise ratio was calculated on the raw data using peak to peak at ± 2 SD.
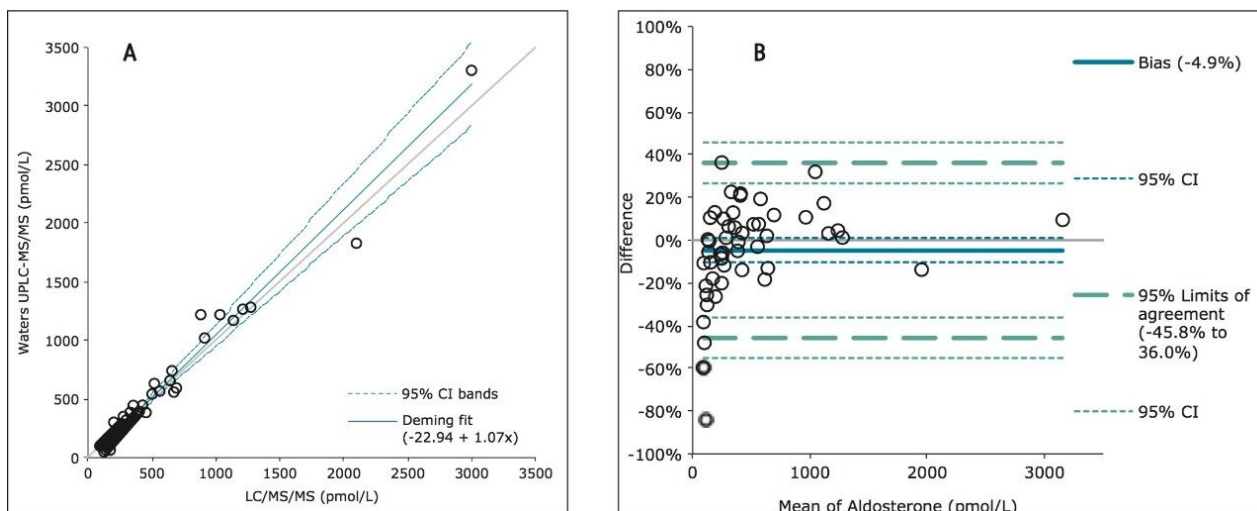
Figure 4. A) Deming regression comparing the Waters UPLC-MS/MS method to another LC-MS/MS method for aldosterone analysis, B) Altman-Bland agreement showing the % difference between the Waters UPLC-MS/MS method and another LC-MS/MS method for aldosterone analysis.

Conclusion

An analytically sensitive and selective clinical research method has been developed for the analysis of plasma aldosterone.

Using only 200 µL sample volume, this analytical method provides sufficient analytical sensitivity to analyze low physiological levels of aldosterone (42 pmol/L). Selective chromatography and SPE using anion exchange to remove interferences provides clean chromatograms, which enables accurate and precise quantification of aldosterone across the concentration range. Automation of the analytical method in combination with sample tracking capabilities improves laboratory workflow and reduces sample handling, which alleviates the potential for operator error.

Acknowledgement

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MassLynx MS Software <https://www.waters.com/513662>

Xevo TQ-S <https://www.waters.com/10160596>

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

TargetLynx <https://www.waters.com/513791>

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