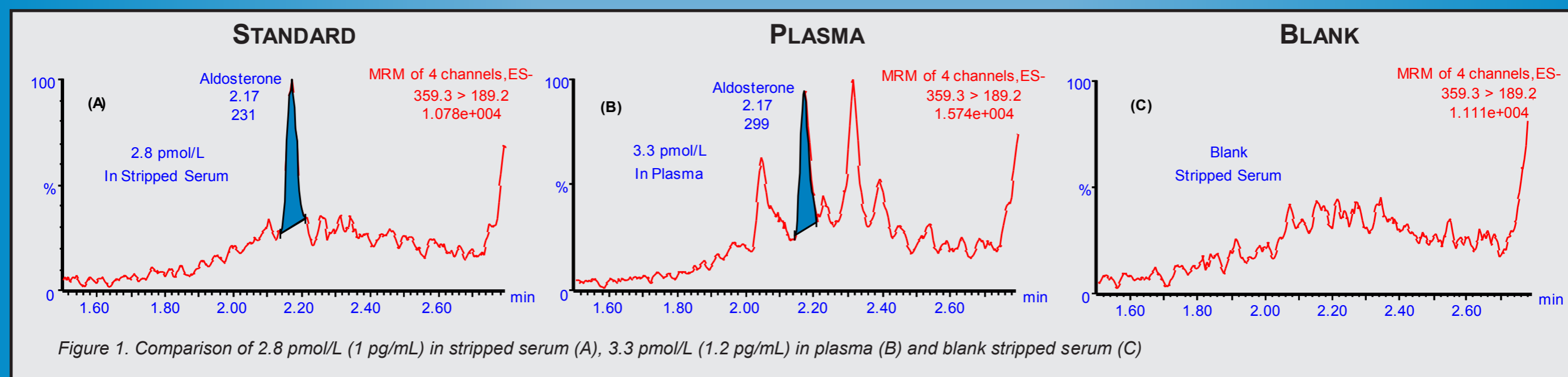


Dominic Foley, Gareth Hammond, Ben Dugas and Lisa J Calton
Waters Corporation, Stamford Avenue, Wilmslow, UK.

3 pmol/L ALDOSTERONE DETECTED IN PLASMA



METHODS

Materials

- Certified aldosterone reference material purchased from Merck (Poole, UK) was used to prepare calibrators MSG4000 Stripped Serum from Golden West Biologicals (CA, USA).
- QCs were created using the certified solution and pooled plasma purchased from SeraLab (Haywards Heath, UK).

Methods

- 200µL serum samples were pre-treated with internal standard, zinc sulfate in methanol and water. Samples were mixed and centrifuged.
- Sample supernatant was transferred to a Waters™ Oasis™ MAX µElution plate, washed with 1% formic acid in 10% acetonitrile, then 1% ammonia in 10% acetonitrile. Analytes were eluted with 60%_(aq) acetonitrile. Water was added prior to injection.
- Sample preparation was automated using the Tecan Freedom Evo 100 Liquid Handler.
- Using a Waters ACQUITY UPLC™ I-Class System, samples were injected onto a 2.1 x 100mm Waters CORTECS™ C₁₈ column with a CORTECS C₁₈, VanGuard™ pre-column using a methanol and 0.05mM ammonium fluoride gradient and analyzed with a Waters Xevo™ TQ-XS detector in negative ESI, using Multiple Reaction Monitoring (Table 1).
- The scan window was 1.5 – 3 minutes with the LC flow diverted to waste at all other times.
- The analysis time per sample was approximately 4.3 minutes injection to injection.

Analyte	MRM Transition (m/z)	Cone Voltage (kv)	Collision Energy (eV)
Aldosterone	359.3 > 189.2 (297.3, 331.3)	45	18 (15)
Aldosterone- ² H ₃	363.3 > 190.2	45	18

Table 1. MRM parameters for the analysis of aldosterone (Qualifier ion parameters)

Linearity, Analytical Sensitivity and Carryover

- Calibration was performed from 8 – 4162 pmol/L for aldosterone. Calibration lines were linear with $r^2 > 0.995$ (n=5) for all analytes.
- The S/N ratios for the lowest calibrator at 8 pmol/L for spiked serum were >10:1 over five separate occasions. Aldosterone peaks were detected down to 3.3 pmol/L in plasma (Figure 1).
- No significant carryover was observed from high concentration samples above the calibration range into subsequent blank injections.

Matrix Effects

- Matrix effect investigations for aldosterone was performed using six individual serum samples (BioIVT, UK).
- Normalized matrix factor calculations, based on the analyte:internal standard response ratio demonstrated that the internal standards compensated for any ion suppression observed, with a mean matrix factor of 1.01 (0.94 – 1.07) and RSD of 5.2%.

Precision

- Low, mid and high concentrations were 36, 286 and 2932 pmol/L for aldosterone with total precision and repeatability using the Tecan Freedom Evo 100 Liquid Handler $\leq 6.3\%$ for aldosterone (Table 2).

Accuracy

- EQA samples were analyzed for aldosterone (n=15). The results were compared to the mass spectrometry mean for each EQA sample.
- Deming regression and Altman-Bland agreement was performed for each of the analytes (Table 3).
- Altman-Bland agreement demonstrates a mean method bias within $\pm 5.6\%$ for aldosterone (Figure 2).

RESULTS

Analyte	Total Precision			Repeatability		
	Low	Mid	High	Low	Mid	High
Aldosterone	4.4%	6.3%	2.7%	4.4%	6.0%	2.6%

Table 2. Total precision and repeatability for the aldosterone in plasma at three QC concentrations

Analyte	Deming equation	Proportional bias (p)	Constant Bias (p)	Mean Method Bias
Aldosterone	0.99x - 2.31	0.263	0.695	+5.6%

Table 3. Deming regression and Altman-Bland analysis performed on analysed EQA samples for aldosterone, which were compared to the EQA MS mean values. P values <0.05 would indicate statistically significant bias

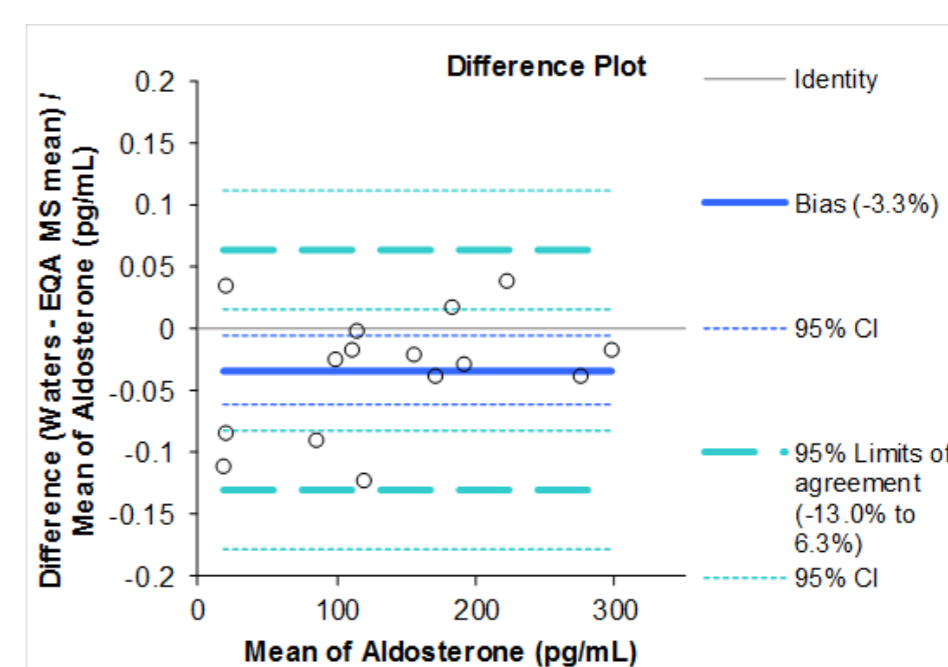


Figure 2. Altman-Bland agreement performed on analysed EQA samples for aldosterone, which were compared to the EQA MS mean

CONCLUSION

- A clinical research method to quantify aldosterone has been developed
- Through the use of offline automated Oasis MAX µElution sample preparation, an analytically sensitive UPLC-MS/MS method was developed using 200µL serum
- The method demonstrates excellent precision ($\leq 6.5\%$) and bias (-3.3%)

For Research Use Only. Not for use in diagnostic procedures.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS