Analysis of Aldosterone in Plasma for Clinical Research using Automated Extraction

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Background: Here we evaluate a newly developed UPLC-MS/MS method for the measurement of plasma aldosterone for clinical research purposes. An analytically sensitive method was developed using a mixed-mode Solid Phase Extraction (SPE) sorbent in 96-well plate format. Automated extraction was employed, enabling high throughput of samples. Analysis was performed using an ACQUITY UPLC® I-Class system, with samples injected onto a 2.1 x 100mm Waters CORTECS UPLC C_{18} column, separated using a water/methanol gradient and quantified with a Waters Xevo® TQ-S mass spectrometer to obtain quantitative measurement of aldosterone at high sensitivity.

Methods: Aldosterone certified reference material purchased from Cerilliant (Round Rock, TX) was used to create calibrators in stripped pooled serum purchased from Golden West Biologicals (Temecula, CA). QC material was prepared in K_2EDTA plasma purchased from SeraLab (Haywards Heath, UK). Plasma samples were analyzed using the newly developed method and the quantified results were compared to an independent LC-MS/MS method for aldosterone. All samples were pre-treated with zinc sulphate, methanol and phosphoric acid. SPE was carried out with a Waters Oasis® MAX µElution 96 well plate to reduce ion suppression and concentrate the samples without the need for evaporation. Automated extraction was performed using the Tecan Freedom Evo 100/4 Liquid Handler. Using an ACQUITY UPLC I-Class system, samples were injected onto a 2.1 x 100mm Waters CORTECS UPLC C_{18} column using a water/methanol gradient elution profile and quantified with a Waters Xevo TQ-S mass spectrometer.

Results: The method demonstrated no significant carryover or matrix effects and was shown to be linear from 15 – 1500 pg/mL for aldosterone. Analytical sensitivity investigations indicate the analytical sensitivity of this method would allow precise quantification (<20%) at 15 pg/mL. Coefficients of variation (CV) for total precision and repeatability on 5 separate days for low (36 pg/mL), mid (180 pg/mL) and high (720 pg/mL) QC material were all < 10% (n = 30) for

aldosterone using automated extraction. Comparison with samples (n=59) previously analyzed by an independent LC-MS/MS method demonstrated good agreement, showing no significant constant or proportional bias ($p > 0.05$).

Conclusions: We have successfully quantified aldosterone in plasma using an automated SPE protocol with UPLC-MS/MS analysis, for clinical research purposes. The method demonstrates excellent linearity and precision, with minimal matrix effects.

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