RAPID UPLC-MS/MS DRIED BLOOD SPOT ANALYSIS OF STEROID HORMONES USING THE XEVO TQ-S MICRO FOR CLINICAL RESEARCH

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RAPID ANALYSIS OF STEROIDS IN DBS

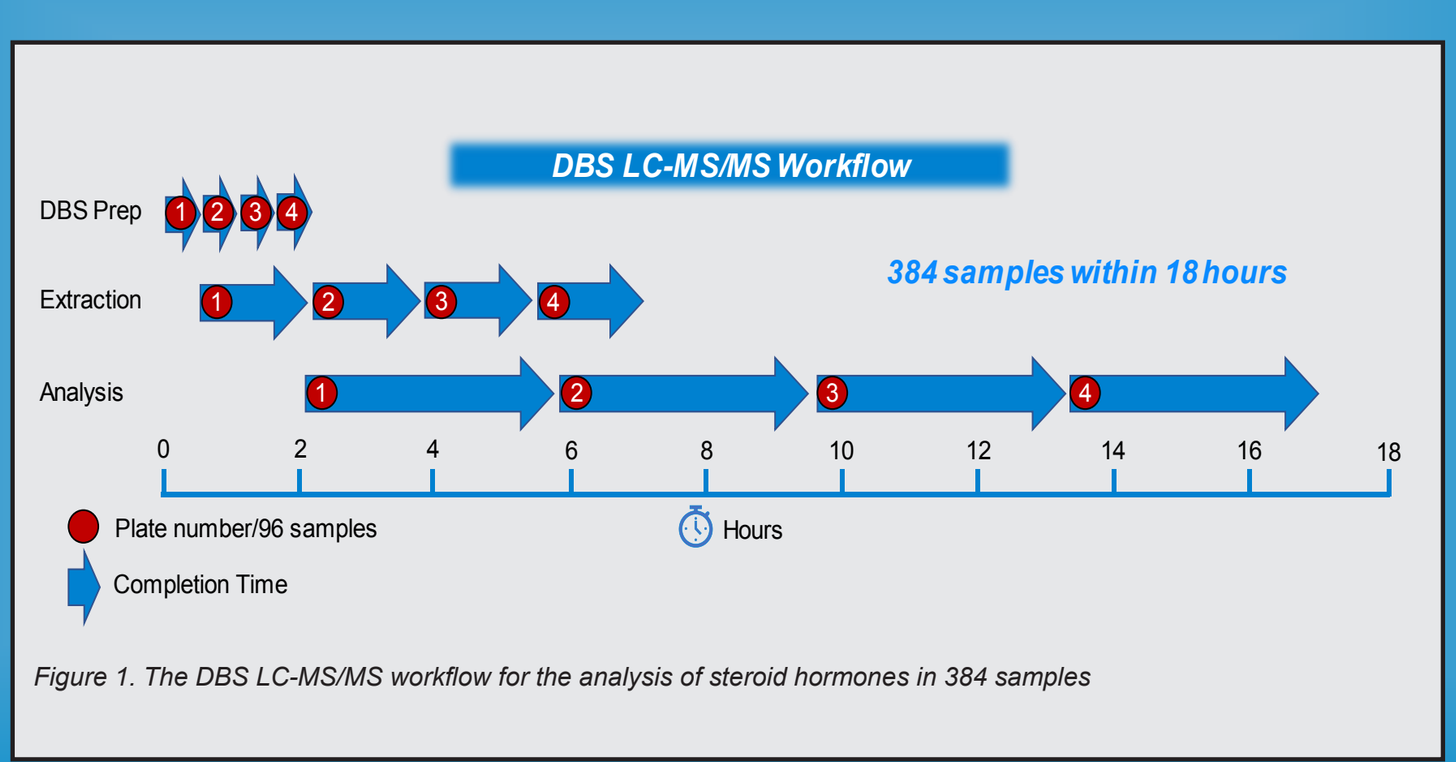

Figure 1. The DBS LC-MS/MS workflow for the analysis of steroid hormones in 384 samples

METHODS

Materials

- Certified androstenedione, 17-hydroxyprogesterone (17-OHP), cortisol, 11-deoxycortisol and 21-deoxycortisol reference material purchased from Merck (Poole, UK) was used to prepare calibrators and QC material in stripped serum from Golden West Biologicals (CA, USA) and then mixed 50/50 (v/v) with red blood cells from BioIVT (West Sussex, UK).
- Dried Blood Spots (DBS) were prepared by aliquoting 75µL of sample onto Whatman 903 Protein Saver cards from Merck (Poole, UK).

  Total precision was determined by extracting and quantifying five replicates of tri-level QC material on one occasion per day over five consecutive days (n=25). Repeatability was determined by analyzing five replicates at each QC level.

Methods

- 2 x 3mm DBS samples were added to a 96 well plate, followed by addition of internal standard (90% Methanol) and shaken for 5 minutes. Water was added and the plate was shaken for 1 minute prior to SPE.
- Sample supernatant was loaded onto a Waters Oasis™ MAX µElution plate and washed with 1% ammonia in 10% acetonitrile. Analytes were eluted with 70%$_{(aq)}$ 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 50mm Waters CORTECS™ C_{18} 2.7µm column with a pre-column CORTECS C_{18} VanGuard™, using a methanol and 0.05mM ammonium fluoride gradient and analyzed with a Waters Xevo™ TQ-S micro detector (Figure 1) in positive ESI, using Multiple Reaction Monitoring (Table 1).
- The analysis time per sample was approximately 2.3 minutes injection to injection.
- The workflow for this analysis is illustrated in Figure 1.

RESULTS

Chromatography

- The CORTECS C_{18} 2.7µm column provides baseline resolution between androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol (Figure 2).
- Figure 2 also demonstrates the baseline resolution of structurally similar steroid hormones.

Calibration and Analytical Sensitivity

- The calibration lines were linear across the calibration range of 0.5 – 500 ng/mL for androstenedione and 11-deoxycortisol, and 1.0 – 500 ng/mL for 17-OHP, cortisol and 21-deoxycortisol, with correlation coefficients (r^2) >0.995.
- The analytical sensitivity of the method for the method was calculated using S/N (PtP), where S/N was >10. The LLOQ for this method was 0.5 ng/mL for androstenedione and 11-deoxycortisol, and 1.0 ng/mL for 17-OHP, cortisol and 21-deoxycortisol.

Precision

- In-house QC concentrations were 2, 5, 50 and 400 ng/mL for androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol.
- Total precision and repeatability was ≤9.3% for the steroid hormones (Figure 5).
- Accuracy of the QCs to the nominal steroid hormone concentrations ranged from 94.2% - 110.1%.

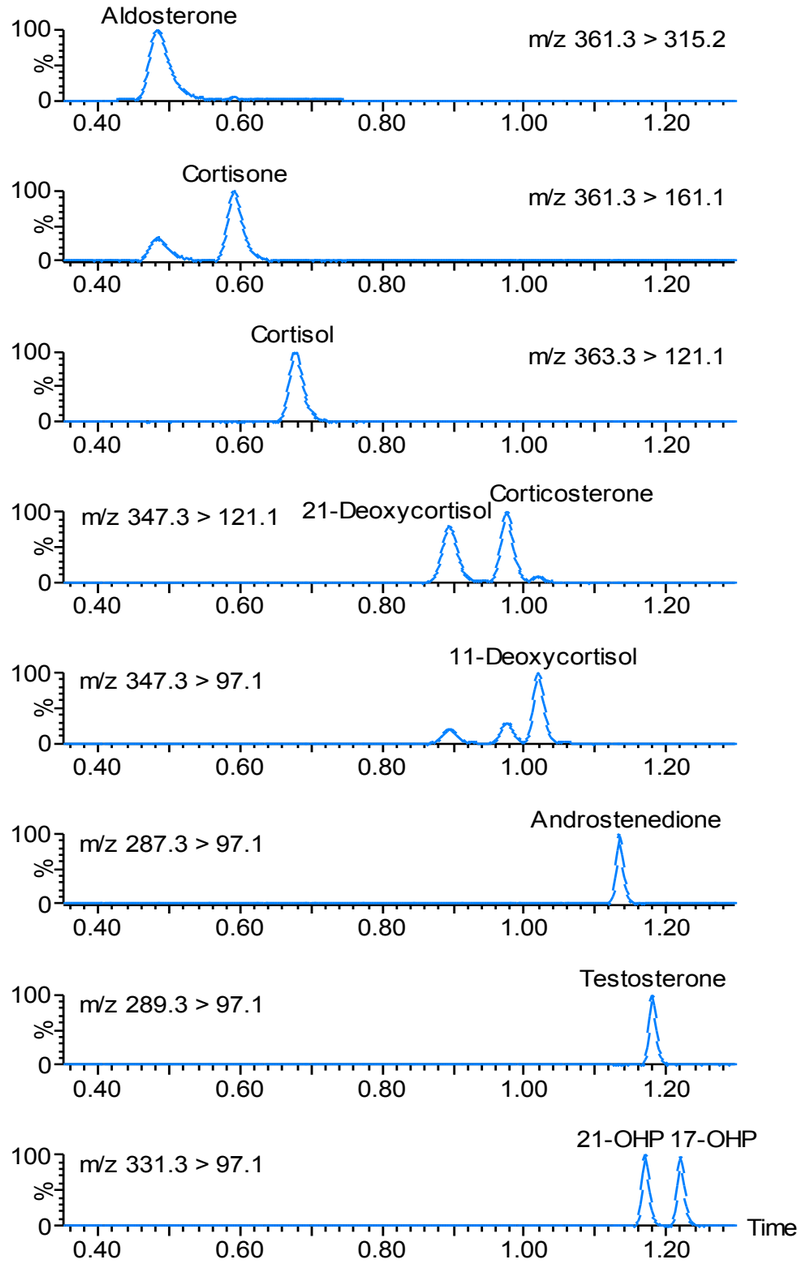

Figure 2. Separation using the CORTECS C_{18} 2.7µm column demonstrating baseline resolution of the steroid hormone isobars

CONCLUSION

- **A clinical research method to quantify androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol in DBS samples has been developed.**
- **The use of CORTECS 2.7µm columns coupled to offline automated Oasis MAX µElution sample preparation, provides a high throughput, analytically sensitive and selective method for analysing steroid hormones in DBS.**
- **The method demonstrates excellent linearity, precision and accuracy for all the steroid hormones.**

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