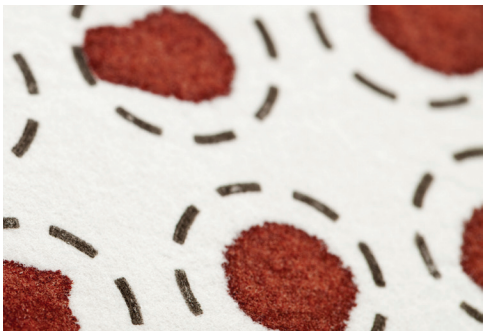
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Rapid UPLC-MS/MS Dried Blood Spot Analysis of Steroid Hormones for Clinical Research

Dominic Foley, Heather Brown, and Lisa Calton
Waters Corporation, Wilmslow, UK



GOAL

To demonstrate a rapid UPLC-MS/MS method for analysis of steroid hormones in dried blood spots using the Xevo™ TQ-S micro Mass Spectrometer.

BACKGROUND

Dried blood spots (DBS) are an established micro sampling technique providing a low-cost approach of collecting, shipping, and analyzing samples for clinical research. Ligand-binding assays (LBAs) are the established frontline testing methodologies for DBS samples in steroid hormone analysis. Although rapid, the relatively low analytical specificity of the LBAs may necessitate follow-up, using liquid chromatography – tandem mass spectrometry (LC-MS/MS). The multiplexed LC-MS/MS analysis of steroid hormones panels provides greater detail about the underlying enzyme activity, compared with single-analyte LBAs, which is important for the assessment of biomarkers in clinical research. The challenge is to create a LC-MS/MS methodology which separates key analytes from matrix and isobaric interferences, while maximizing throughput.

Rapid separation and analysis of steroid hormones in dried blood spots was achieved using the ACQUITY UPLC I-Class System/Xevo TQ-S micro Mass Spectrometer and CORTECS Column.

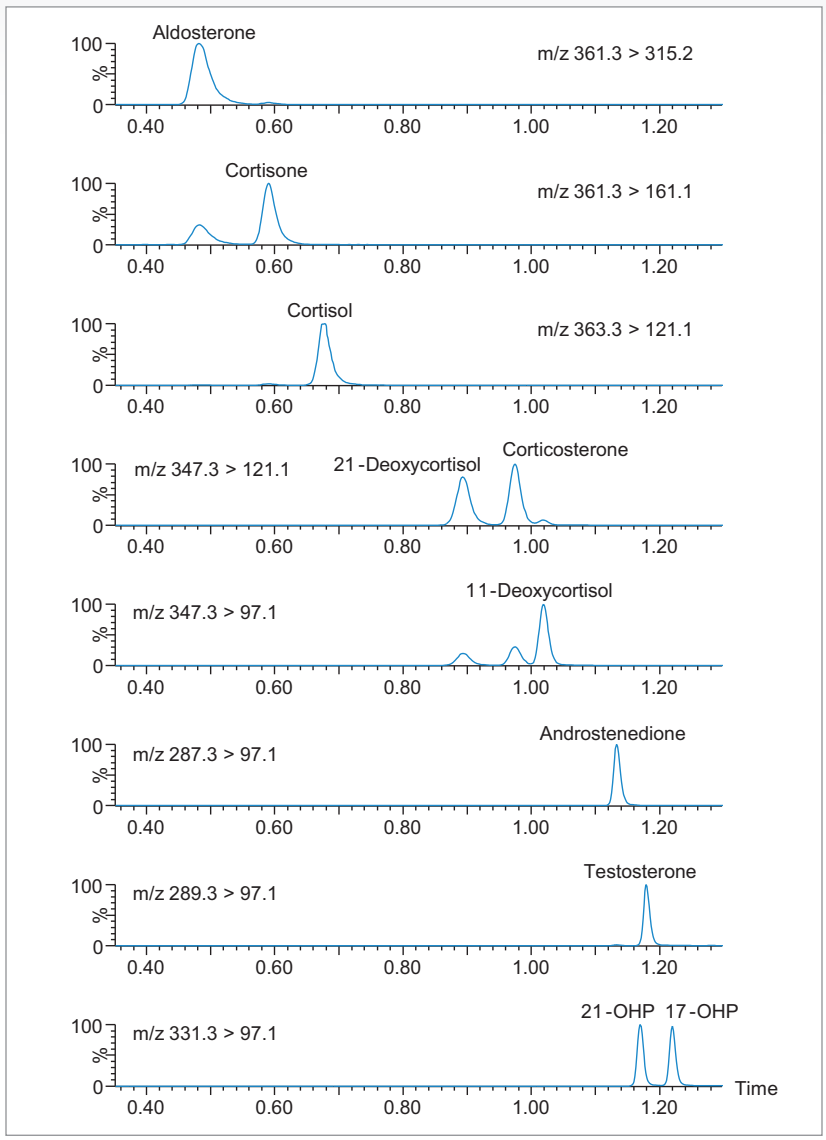


Figure 1. Chromatographic selectivity of the CORTECS™ C_{18} 2.7 µm Column for the separation of cortisol, 11-deoxycortisol, 21-deoxycortisol, androstenedione, and 17-OHP from other endogenous steroid hormones.

UltraPerformance Liquid Chromatography (UPLC™) on the ACQUITY™ UPLC I-Class System enables high pressure chromatographic separations in short analysis times. Combining the ACQUITY UPLC with a CORTECS 2.7 µm particle Column provides UPLC separations at high linear velocities without a loss in column performance, particularly in regard to critical pair separations.

THE SOLUTION

Through utilization of the ACQUITY UPLC I-Class System and the CORTECS Column technology, separation of cortisol, 21-deoxycortisol, 11-deoxycortisol, androstenedione, and 17-hydroxyprogesterone (17-OHP) from other endogenous steroid hormones was achieved within a 2.3 minute injection cycle time (Figure 1). Analytically sensitive detection between 0.5–1.0 ng/mL in DBS samples was achieved for the steroid hormones using the Xevo TQ-S micro, following extraction employing Oasis™ MAX µElution SPE on the Tecan® Freedom Evo 100 Liquid Handling System (LHS).

Sample preparation and UPLC-MS/MS analysis

Using the LHS, 150 µL internal standard was added to two 3 mm DBS samples, shaken for 5 minutes and diluted with water. The Oasis MAX SPE µElution Plate was conditioned with methanol prior to 700 µL supernatant being applied. This was followed by a wash with 1% (v/v) ammonia in 10% (v/v) acetonitrile (aq). Samples were eluted with 70% acetonitrile and diluted with water.

25 µL of extracted sample was injected on the ACQUITY UPLC I-Class System utilizing a 0.05 mM Ammonium fluoride/methanol gradient and a 2.7 µm, 2.1 mm x 50 mm CORTECS C_{18} Column with VanGuard™ Pre-Column. Detection was performed using Multiple Reaction Monitoring (MRM) on the Xevo TQ-S micro. The complete analytical workflow is shown in Figure 2.

Results

DBS calibration lines were linear from 0.5–500 ng/mL for androstenedione and 11-deoxycortisol; and 1.0–500 ng/mL for cortisol, 17-OHP, and 21-deoxycortisol with correlation coefficients (r2) >0.99 over five occasions.

Signal/Noise (S/N) at the LLOQ calibrators are shown in Figure 3, demonstrating the analytical sensitivity of the method from two 3 mm dried blood spots.

Total precision and repeatability of the method for analyzing the steroid hormones over five occasions was ≤9.3% CV (Table 1).

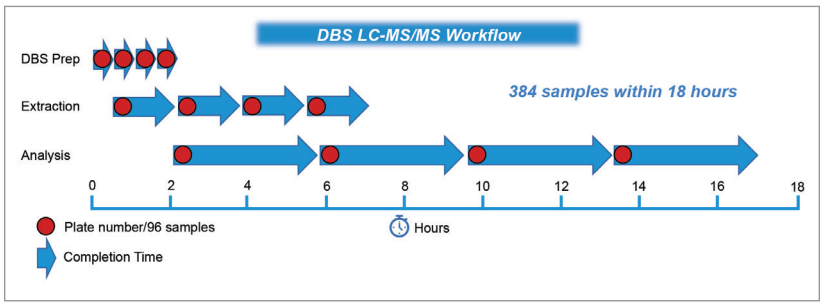


Figure 2. Analytical workflow for the analysis of DBS samples using Oasis MAX µElution SPE and UPLC-MS/MS analysis.

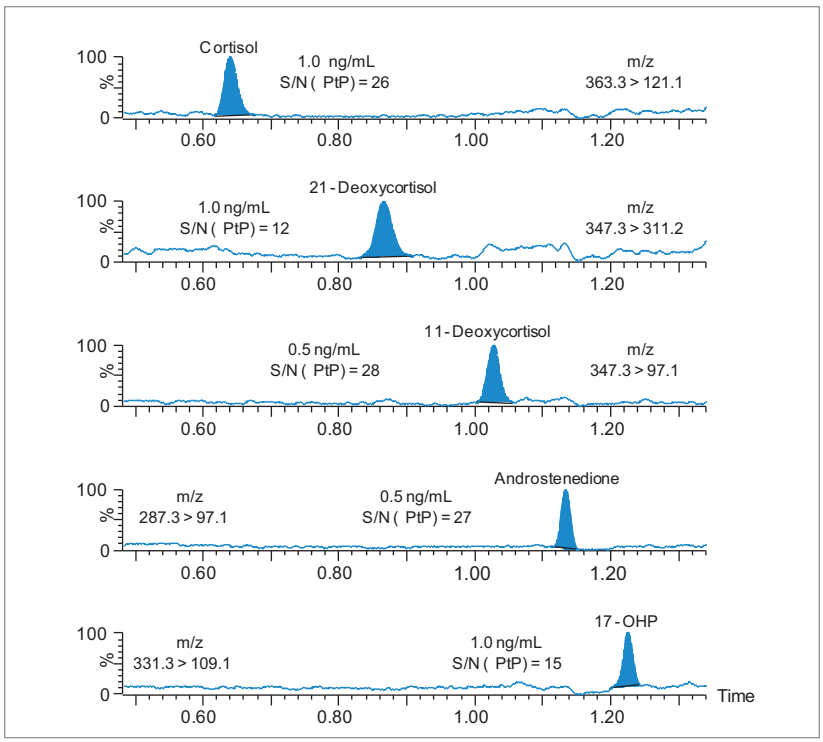


Figure 3. LLOQ chromatograms for cortisol, 21-deoxycortisol, 11-deoxycortisol, androstenedione, and 17-OHP in DBS samples.

	Total QC precision				QC repeatability			
Compound	2 ng/mL	5 ng/mL	50 ng/mL	400 ng/mL	2 ng/mL	5 ng/mL	50 ng/mL	400 ng/mL
17-OHP	7.6%	5.8%	6.1%	4.6%	7.1%	5.4%	4.6%	3.8%
Androstenedione	6.7%	4.7%	5.5%	4.4%	6.3%	4.4%	4.4%	4.1%
Cortisol	9.3%	6.9%	5.0%	3.8%	8.7%	6.8%	4.2%	3.8%
11-Deoxycortisol	7.0%	5.1%	5.3%	3.9%	5.8%	5.1%	4.2%	3.5%
21-Deoxycortisol	7.4%	6.6%	6.5%	6.0%	6.9%	6.6%	4.7%	6.0%

Table 1. Total precision and repeatability over five occasions for 17-OHP, androstenedione, cortisol, 11-deoxycortisol, and 21-deoxycortisol.

SUMMARY

A UPLC-MS/MS method for the separation and detection of steroid hormones in dried blood spots has been developed for clinical research purposes. The benefits of this method include:

- Rapid separation of steroid hormones within 1.4 minutes (2.3 minutes injection to injection) with baseline-resolution of steroid isobars, using the CORTECS 2.7 µm Column.
- Improved method analytical sensitivity, laboratory efficiency, reproducibility, and reduced sample-handling time by using an automated, offline, Oasis MAX µElution SPE protocol for the extraction of steroid hormones from the DBS samples.
- Extraction and analysis of 384 samples in less than 18 hours.
- Using two 3 mm blood spots, the method was analytically sensitive (0.5–1 ng/mL) and reproducible (<9.3% CV).

Acknowledgements

We would like to thank Susan Johnston and colleagues from NHS Glasgow and Clyde for the provision of 17-OHP DBS calibrators for use during initial investigations.

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

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com