ANALYSIS OF PLASMA 17-HYDROXYPROGESTERONE, ANDROSTENEDIONE, AND CORTISOL USING A NOVEL SOLID-PHASE EXTRACTION SORBENT FOR UPLC-MS/MS ANALYSIS

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INTRODUCTION

Bioanalysis of drugs and endogenous molecules from plasma samples often involves using solid phase extraction (SPE) to clean up the matrix and concentrate the analytes of interest. Despite the advances in SPE sorbents and formats, residual phospholipids often remain in extracts and can interfere with analyses by causing ion suppression and by prematurely fouling analytical columns and MS sources. Using a novel SPE sorbent that is designed to remove phospholipids from samples, a panel of corticosteroids has been extracted from plasma. This sorbent is also water wettable, enabling extraction without the usual requisite preconditioning and equilibration steps. This has resulted in a method with excellent sensitivity that demonstrates consistent recovery, minimal matrix effects, and the elimination of >95% of phospholipids compared to simple protein precipitation.

METHODS

Calibration standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All deuterated internal standards were purchased from Cerilliant (Round Rock, TX). Double charcoal-stripped plasma was used for calibrators and QC samples.

Sample Preparation: 150 µL plasma samples were precipitated with 300 µL of a solution of 4:1 MeOH and 89 g/L $ZnSO_4$ (V:V). After centrifugation, 300 µL of supernatant was diluted with 900 µL 4% H_3PO_4 and directly loaded onto Waters' Oasis PRiME µElution SPE plates. All wells were then washed with 25% MeOH and eluted with 2 x 25 µL of 90:10 ACN:MeOH. The sample eluates were diluted with 25 µL water and analyzed by UPLC/MS/MS.

Calibration curves were spiked from 1-500 ng/mL for cortisol and from 0.05-25 ng/mL for 17α-hydroxyprogesterone (17-OHP) and androstenedione (Adione) .

Chromatographic Conditions

UPLC System:	ACQUITY UPLC® I-Class-FL
MS:	XEVO TQ-S
Column:	ACQUITY UPLC® HSS T3, 1.8 µm 2.1 x 50 mm
Mobile Phase A (MPA)	0.1% Formic Acid in Water
Mobile Phase B (MPB)	0.1% Formic Acid in ACN
Column Temp:	40 °C
Sample Temp:	10 °C
Strong Needle Wash	25:25:50 ACN:MeOH:Water
Weak Needle Wash	10% ACN

Table 1—UPLC Gradient

Time	Flow	%A	%B
0.0	0.6	70	30
1.0	0.6	50	50
2.0	0.6	45	55
2.5	0.6	5.0	95
3.5	0.6	5.0	95
3.6	0.6	70	30
4.5	0.6	70	30

Waters Xevo® TQ-S Conditions, ESI+

Capillary Voltage:	1.0 kV
Desolvation Temp:	500 °C
Cone Gas Flow:	150 L/Hr
Desolvation Gas Flow:	1000 L/Hr
Source Temp:	150 °C
MRM transitions, ESI+:	See Table 2

Table 2

Compound	RT	MRM Transitions	Cone (V)	Coll. Energy
Cortisol	0.72	337.2>121.1 337.2>91.0	42 42	22 52
Androstenedione	1.50	287.2>97.1 287.2>109.0	58 58	20 26
17α-OH progesterone	1.55	331.2>97.1 331.2>295.2	58 58	26 16

RESULTS

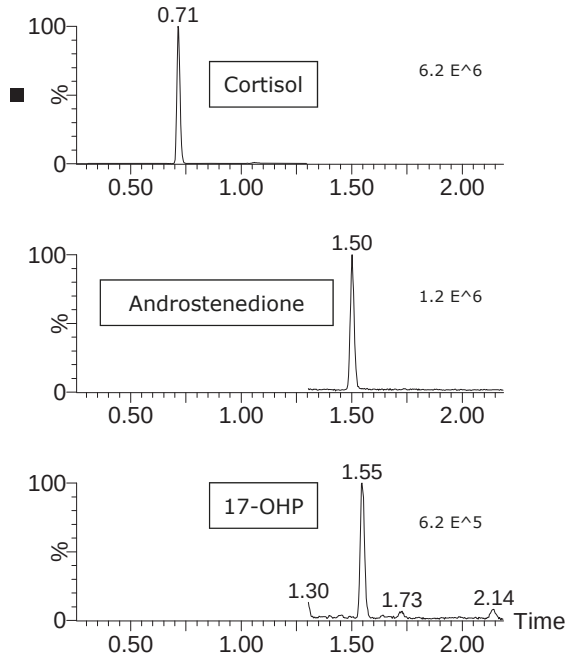

Figure 1. *Chromatography of a calibration standard of plasma steroids. Cortisol—20 ng/mL. Other steroids—1 ng/mL.*

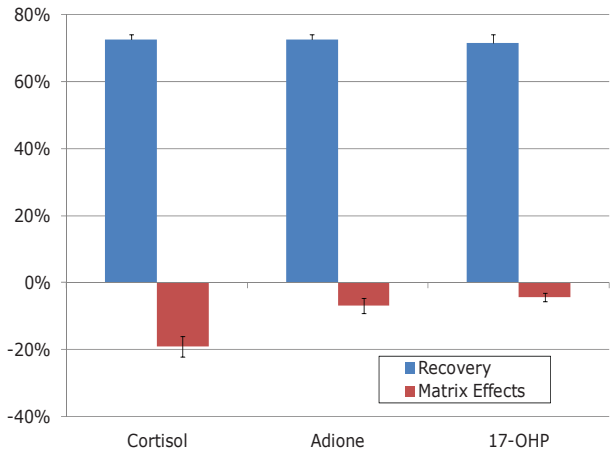

Figure 2. *Recovery and matrix effects for plasma steroids (N=4).*

PHOSPHOLIPID REMOVAL

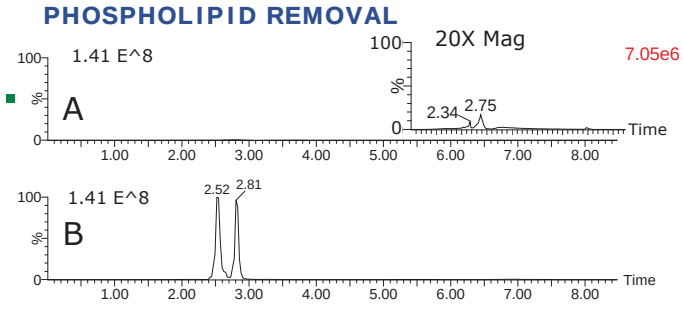

Figure 3. *Residual phospholipid traces for samples prepared with Oasis PRiME HLB (A) and by protein precipitation (B). > 97% of phospholipids were removed compared to PPT. A 20x magnification of the SPE extract is shown at upper right*

QUANTITATIVE RESULTS

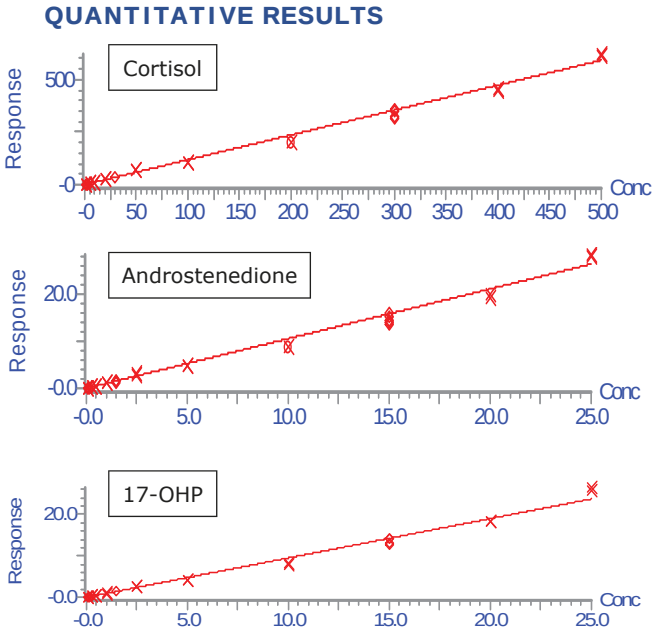

Figure 4. *Calibration curves for cortisol, androstenedione and 17-OHP*

	R^2	Mean % Dev.	Calibration Range (ng/mL)
Cortisol	0.996	8.4%	1.0-500
Androstenedione	0.989	8.0%	0.05-25.0
17α-OH progesterone	0.993	9.7%	0.05-25.0

Table 3. *Calibration summary for all analytes.*

	Cortisol	
QC Spike Conc.	Accuracy	%CV
3.0	92.3%	5.4%
30	94.8%	3.0%
300	94.9%	6.0%

	Androstenedione		17α-OH progesterone	
QC Spike Conc.	Acc	%CV	Acc	%CV
0.15	94.4%	5.7%	93.7%	6.5%
1.50	95.0%	3.6%	92.3%	5.6%
15	95.4%	5.5%	93.7%	6.5%

Table 4. *Quality Control (QC) results for plasma steroids extracted from plasma using Oasis PRiME HLB (N=6).*

CONCLUSIONS

- Direct loading and extraction of samples **without conditioning and equilibration** results in a faster extract with less solvent usage
- Near complete elimination of phospholipids compared to protein precipitation
- Consistent recovery with minimal matrix effects
- Excellent analytical sensitivity, linearity, accuracy and precision for all compounds

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