

# A Fast and Effective Approach for the Analysis of Urinary Cortisol, Cortisone, Prednisolone and Prednisone Using SPE and LC/MS/MS

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## **Overview**

The existing methods for the quantification of cortisol, cortisone, prednisolone, and prednisone are very diverse. While liquid-liquid extraction, protein precipitation, and "dilute-and-shoot" procedures offer quick and dirty methodologies, these same methodologies risk increases in instrument downtime and analytical column costs. We evaluated a variety of silica-based and polymer-based SPE sorbents, each of which provides a different retention mechanism, including; hydrophobic, anion-exchange,  $\pi$ - $\pi$  interactions, and any combination thereof. The evaluation showed that the Strata<sup>T-</sup>X sorbent, with a unique elution solvent has been found to be a robust, reproducible, and cost effective solution for the laboratory, while providing a LLOQ of 10.0 ng/mL in human urine for all four corticosteroids.

#### **STRUCTURES**





OH

### Introduction

The measurement of the urinary free cortisol-cortisone ratio is useful in diagnosing patients with low-renin hypertension caused by apparent mineralocorticoid excess. In samples from patients who have been treated with prednisolone, methylprednisolone, or prednisone, falsely elevated concentrations of cortisol may be reported. These false elevations may be due to crossreactivity. Also prednisolone is an isomer of cortisone, which causes interference with cortisone analysis in samples.

The SPE media was divided into two main groups, silica particles and polymeric resin beads. The silica-based SPE consists of end-capped C18 ligand which presents a strong hydrophobic retention mechanism. The polymeric resin comprised of a polystyrene backbone with a quaternary amine moiety (strong anion-exchange), a sulfite moiety (strong cation-exchange), or a pyrolidone moiety for neutral compounds (see below). The styrene backbone provides both hydrophobic and  $\pi$ - $\pi$  interaction and/or retention mechanism.

In this poster, we evaluate selected SPE sorbents, and optimize the best performing Strata-X SPE extraction methods to reach acceptable recoveries of four corticosteroids. The separation of all analytes, especially two isomers of cortisone and prednisolone, were successfully resolved by using the Kinetex<sup>®</sup> Core-Shell Biphenyl HPLC column.



End-capped C18 Strata<sup>®</sup> C18-E



Strong Cation-Exchanger Strata-X-C

O N **Neutral Polymer** 

Strata-X

CH<sub>3</sub> CH<sub>3</sub>

Strong Anion-Exchanger Strata-X-A

#### **SPE Method**

Phenomenex Strata-X (60 mg), 96-well plate		Elute:	2x 500 µL o	of 2 % Formic Acid in Ethyl
Part No.: Condition: Load sample:	8E-S100-UGB 1) 1 mL Methanol; 2) 1 mL Water 300 µL human urine + 300 µL Water /w 1 µg/mL IS (Cortisol-D4)	Dry down : Rconstitute :	acetate/Isopropanol (85:15) Under $N_2$ at 50 °C with 100 µL of Ammonium acetate/Ammonium acetate in Methanol (50:50)	
Wash:	1) 1 mL Water; 2) 1 mL 10 % Methanol			
<b>LC/MS</b> Condition	าร			
LC Conditions:				
LC system:	Agilent <sup>®</sup> 1200SL with binary pumps	F	low Rate:	0.4 mL/min 40 °C 10 μL
Column:	Kinetex Core-shell Biphenyl, 2.6 µm, 50 x 3.0	mm Colu	mn Temp:	
Part No.:	00B-4622-Y0	Injectio	n Volume:	
Mobile Phase:	A: 10 mM Ammonium acetate in Water			

B: 10 mM Ammonium Acetate in Methanol

40

40

Time(min) B (%) 0.01

0.5

2.0

3.0	
3.01	
5.0	
	3.0 3.01 5.0

#### **MS Conditions**

• AB SCIEX API 4000<sup>™</sup> LC/MS/MS

Gradient:

• Positive, ESI, MRM

ID	Q1 Mass (Da)	Q3 Mass (Da)	
Cortisone 1	361	163	
Cortisone 2	361	121	
Cortisol 1	363	121	
Cortisol 2	363	309	
Prednisolone 1	361	147	
Prednisolone 2	361	173	
Prednisone 1	359	147	
Prednisone 2	359	237	
Cortisol – D4 1	367	121	
Cortisol – D4 2	367	331	

#### FIGURE 1. SPE Media Selection



#### FIGURE 2. Wash Solvent Optimization



#### FIGURE 3. Recovery using Strata-X



#### FIGURE 4. Representative of Curve



# TABLE 1.Accuracy and Precision

	LL0Q	QCL	QCM	QCH	
Nominal Conc. (ng/mL)	10	30	500	1600	
	Cortisone				
Mean Conc. Fund (ng/mL)	10.1	30.4	527	1678	
STDV	0.649	2.32	8.18	50.8	
CV%	6.40	7.64	1.55	3.02	
Accuracy (%)	101	101	106	105	
	Cortisol				
Mean Conc. Fund (ng/mL)	10.5	31.3	518	1660	
STDV	0.399	2.41	6.65	17.9	
CV%	3.82	7.71	1.28	1.08	
Accuracy (%)	105	104	104	104	
	Prednisolone				
Mean Conc. Fund (ng/mL)	10.8	33.1	537	1587	
STDV	0.973	3.49	14.9	66.5	
CV%	8.99	10.5	2.78	4.19	
Accuracy (%)	108	110	107	99.3	
	Prednisone				
Mean Conc. Fund (ng/mL)	10.2	31.1	540	1623	
STDV	0.816	1.97	14.1	45.0	
CV%	7.97	6.31	2.63	2.77	
Accuracy (%)	102	104	108	101	

FIGURE 5. Chromatograms\_LLOQ (10 ng/mL)





### Results

The selected SPE media was compared based on manufacturer recommended procedures for each sorbent, Strata-X provided the best recovery. (**Figure 1**)

Low recoveries of analytes were observed using manufacturer recommended procedures. Optimization of the 2nd strong wash solvent was performed to maximize recovery using Strata-X. 10% Methanol in water was found to have better recovery across all the concentrations and analytes. (**Figure 2**)

Recoveries of four corticosteroids were acceptable. (Figure 3)

Assay linearity of all analytes were 10.0 - 2,000 ng/mL. (Figure 4) showed representative of the curve of cortisol.

Accuracy and precision of 4 level QCs against the curves (**Table 1**). Accuracy of four level QCs were 99.3-110 % with CV% at 1.08-10.55 %, respectively.

Kinetex Core-shell Biphenyl analytical column offers better separation of two pairs of cortisone/cortisol and prednisone/prednisolone, especially the separation in between the isomers of cortisone and prednisolone. (**Figure 5, 6**)

# Conclusion

- A fast, robust LC/MS method was developed for quantitation of cortisone, cortisol, prednisone, and prednisolone using Phenomenex Strata-X 96-well plates
- Kinetex Core-shell Biphenyl analytical column offers better separation and sensitivity
- The LLOQ of four corticosteroids in human urine was 10.0 ng/mL
- The unique elution solvent provided cleaner sample extraction and maximized the analyte recoveries
- Strata-X has both individual columns and 96-well formats; it's automation friendly

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