Extraction Cortisol From Human Urine Using ISOLUTE® SLE+ Plates Prior to LC-MS/MS Analysis

This application note describes the supported liquid extraction clean-up of cortisol from urine prior to quantitative LC-MS/MS analysis.

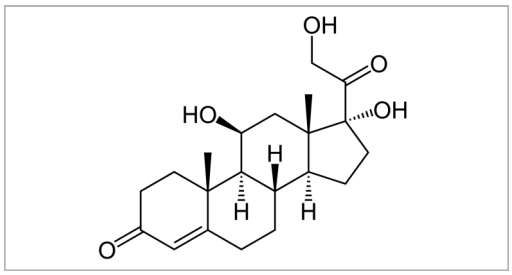

Figure 1. Structure of Cortisol

Introduction

Cortisol is a steroid hormone that in urine can be used to diagnose hyper or hypo cortisol diseases such as Cushing's Syndrome. This methodology has been designed to give an effective and efficient supported liquid extraction protocol for the clean-up and concentration of urinary cortisol levels.

Analyte recoveries achieved using this method ranged from 99-101% with RSDs below 5% for all analytes.

ISOLUTE SLE+ Supported Liquid Extraction plates offer an efficient alternative to traditional liquid liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

Analytes

Cortisol, Cortisol D4.

Sample Preparation Procedure

Plate configuration:	ISOLUTE SLE+ 200 µL Supported Liquid Extraction Plate, part number 820-0200-P01
Sample pre-treatment:	Dilute sample 1:1 (v/v) with water.
Sample loading:	Load the pre-treated sample (200 µL total volume) onto the plate and apply a pulse of vacuum (VacMaster 96 Sample Processing Manifold, 121-9600) or positive pressure (PRESSURE+ 96 Positive Pressure Manifold PPM-96) to initiate flow. Allow the sample to adsorb for 5 minutes.
Analyte extraction:	Apply MTBE (1 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent, collecting into collection plate.
Post extraction:	Evaporate the extract to dryness (40 °C) (SPE Dry 96 Sample Concentrator SD-9600-DHS-EU). Reconstitute in water:methanol (50:50, v/v) (100 µL).

HPLC Conditions

Instrument: Waters Aquity UPLC

Column Aquity UPLC BEH C18 1.7 µm x 2.1 x 50 mm

Mobile Phase:
A: 2 mM NH_4OAc 0.1% formic acid (aq)

B: 2 mM NH_4OAc 0.1% formic acid in methanol

Gradient:

Time (mins)	%A	%B	Flow	Curve
0.00	50	50	0.4	1
0.8	50	50	0.4	6
3.30	19	81	0.4	6
3.50	50	50	0.4	1

Injection: 10 µL (partial loop with needle overfill)

Sample temperature: 20 °C

Column temperature: 40 °C

Mass Spectrometry Conditions

Instrument: Waters Quattro Premier XE triple quadrupole mass spectrometer equipped with an electrospray interface

Source temperature: 150 °C

Desolvation temperature: 450 °C

Table 1. MRM transitions in positive mode

Compound	MRM	Dwell (s)	Cone voltage	Collision energy
Cortisol quant	363.30 > 121.00	0.08	25	25
Cortisol qual 1	363.30 > 105.00	0.08	25	45
Cortisol qual 2	363.30 > 91.00	0.08	25	45
Cortisol D4 (ISTD)	367.30 > 121.00	0.08	25	25

Results

This ISOLUTE SLE+ protocol demonstrates analyte recoveries ranges from 99-101% as shown in figure 3 (page 3) with RSDs below 5% for both Cortisol and Cortisol D4. Robustness testing was carried out across three days using three different sources of urine. Figure 2 shows the chromatogram at a concentration range of 25 ng/mL, with a calibration curve demonstrating linearity 25-2000 ng/mL.

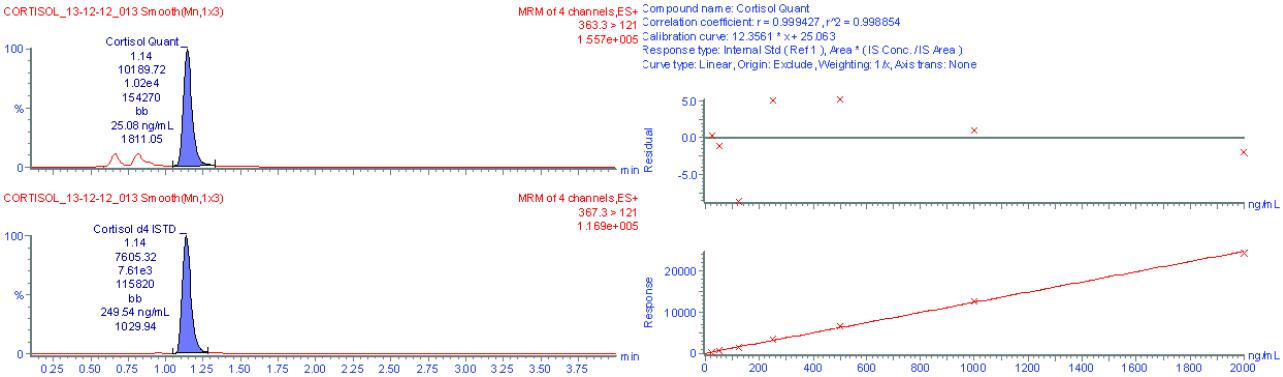

Figure 2. Typical Chromatogram showing extracted Cortisol at 25 ng/mL and calibration curve showing linearity from 25-2000 ng/mL

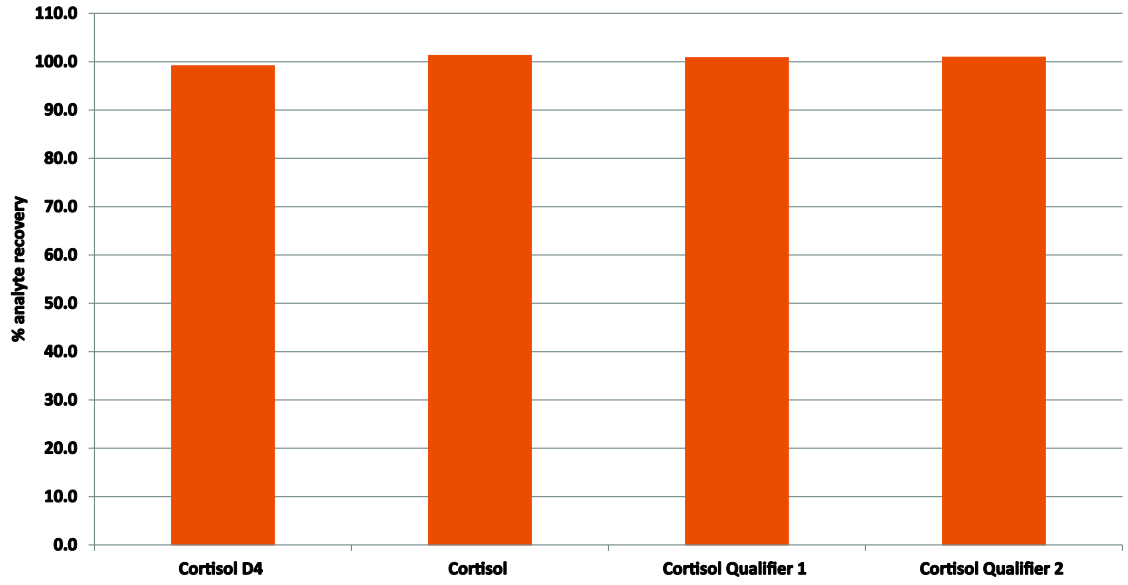

Figure 3. Typical analyte % recoveries at a level of 25 ng/mL for Cortisol and Cortisol D4 (n=7) using the ISOLUTE SLE+ protocol

In accordance with known clinical detection requirements this assay was demonstrated to a level of 25 ng/mL, however based upon the achieved linearity and signal to noise ratio, it is estimated that a lower limit of quantitation of 0.5 ng/mL could be reached.

Ordering information

Part Number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 µL Supported Liquid Extraction Plate	1
PPM-96	PRESSURE+96 Positive Pressure Manifold	1
121-9600	VacMaster 96 Sample Processing Manifold	1
SD-9600-DHS-EU	SPE Dry 96 Sample Concentrator	1

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