A Rapid Sample Preparation for Quantitative Analysis of Cortisol in Saliva and Urine by LC-MS/MS

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HIGHLIGHTS: Reduce manual sample prep. time

WAX-S- XTR

ABSTRACT

For determining saliva and urine cortisol levels, LC-MS/MS is the measurement method of choice, with high sensitivity and specificity. However, the conventional LC-MS/MS approach requires lengthy manual sample preparation by liquid-liquid extraction [LLE] or solid phase extraction [SPE], and patient samples are processed in a batch mode. To shorten sample preparation time, Stanford investigated Dispersive Pipette XTRaction tips that contained weak anion exchange (WAX) sorbent and salt (S) necessary for SALLE (Salting-out Assisted Liquid-Liquid Extraction) as a quick alternative. Stanford validated this improved LC-MS/MS method in a clinical chemistry laboratory. Assay characteristics such as sensitivity, specificity, linearity, and precision are presented here. We conclude that the improved LC-MS/MS method is rapid and as sensitive as the conventional LC-MS/MS approach much more suitable for clinical diagnostic use.

INTRODUCTION

Late night saliva and 24-hour urine cortisol are the first line screening tests for cortisol excess [i.e. Cushing's syndrome or disease]. Early morning saliva cortisol may be used to assess and rule out adrenal insufficiency [i.e. Addison's syndrome]. LC-MS/MS is the measurement method of choice, due to improved sensitivity and specificity over traditional immunoassay. To utilize the LC-MS/MS method with superior sensitivity and specificity, WAX-S-XTR tips were validated in order to shorten the manual sample preparation time and to make it amendable to be processed in a random access mode.

MATERIALS AND METHODS

Stable deuterium labeled cortisol (cortisol-d4; CDN Isotopes, Inc., Pointe-Claire, Quebec) was used as internal standard (IS). BSA [0.2%] based calibration standards and quality control standards were made by spiking pure cortisol compound. A Shimadzu Liquid Chromatography system (model LC-20AD) with API 5000 tandem mass spectrometer (Turbo V Source; AB Sciex, Redwood City, CA) was used for all the analysis. Universal style 1mL tips with 55-65 µ – 10 mg + 40 mg salt (WAX-S) from DPX Technologies, Columbia, SC. LC column was from Phenomenex (Torrance, CA). All patient saliva samples were collected in Salivette [Sarstedt, Numbrecht, Germany].

Table 1. MS/MS parameters

Mass Spectrometry Settings and Multiple Reaction Monitoring						
Collision Gas=9; Curtain Gas =30; Ion Source Gas=50						
Neubilizer Current=5; Source Temp=500°C; Interface heater=on						
Analyte	**Parent Ion**	**Product Mass**	**CE**	**DP**	**EP**	**CXP**
Cortisol	363.21	121.1	30	80	10	20
	363.21	97.0	35	80	10	15
Cortisol-d4	367.3	121.0	30	66	10	20

Table 2. Sample Preparation

Pretreatment	Add 50 µL Internal standard and 500 µL acetonitrile to 250 µL of patient saliva and patient urine samples. Vortex and incubate at room temperature for ten minutes.
Extraction	Aspirate/Dispense 3x with WAX-S-XTR tips
Elution	Transfer 300 µL organic upper layer
Prep for injection	Solvent evaporation and reconstitute in 120 µL of H_2O/MeOH

RESULTS

The improved LC-MS/MS method with rapid sample preparation are is shown to have comparable characteristics to a conventional LC-MS/MS method. The new sample preparation method was much more rapid [up to 1 hour] compared with the conventional approach [~6 hours].

CONCLUSIONS

Utilizing XTR tips may reduce sample preparation by 80%. The improved LC-MS/MS method with rapid sample preparation retained satisfactory assay characteristics and is much more suitable for a clinical diagnostic lab.

Table 3.

	Saliva (ng/dL)	Urine (ng/dL)
AMR	20-5,000	20- 5,000
CPR	20-5,000	20-50,000
LLOD	5	5
LLOQ	20	20
Correlation	y=0.97X+23 R^2= 0.95 [n=26]	y= 0.97X +88 R^2= 0.98 [n=50]
Precision	L= 18% H= 10%	L= 6% H= 4%

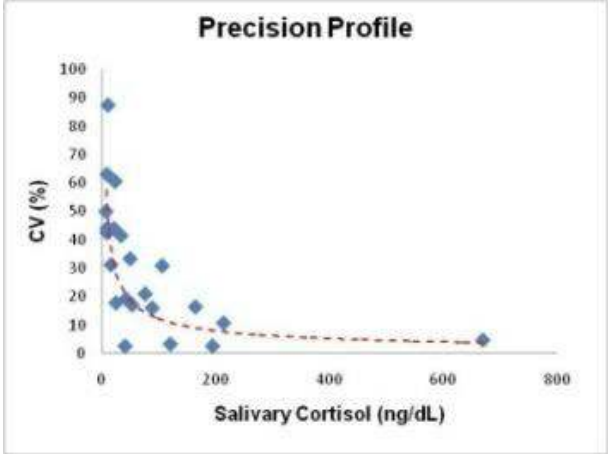

Figure 4. Saliva cortisol precision profile

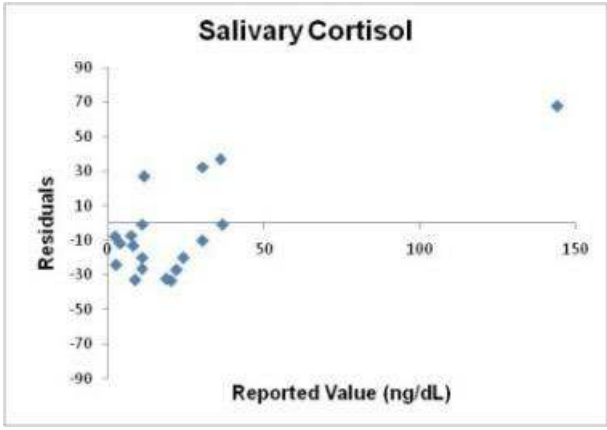

Figure 5. Saliva cortisol residual plot

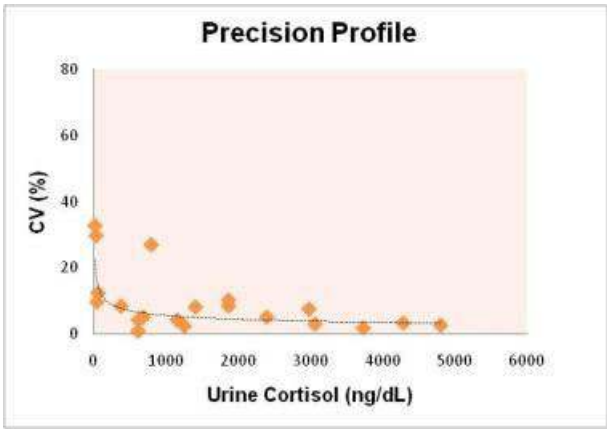

Figure 6. Urine cortisol precision profile

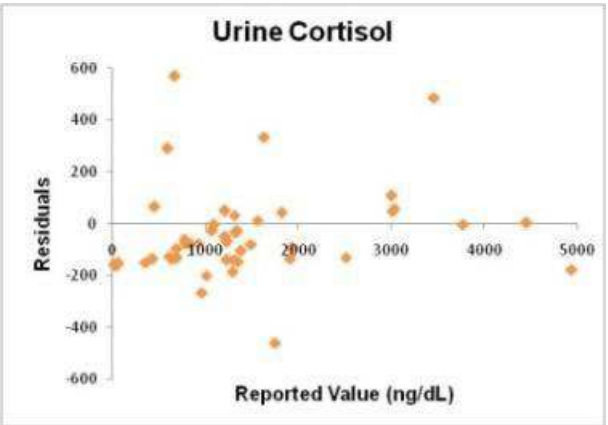

Figure 7. Urine cortisol precision profile