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

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Background: Late night saliva and 24-hour urine cortisol are among the first line screening tests for cortisol excess [i.e., Cushing's syndrome or disease][1]. Early morning saliva cortisol may be used to assess and rule out adrenal insufficiency [i.e., Addison's syndrome][2]. Saliva and urine cortisol measurement by LC-MS/MS is the method of choice. However, conventional LC-MS/MS approach requires lengthy manual sample preparation by liquid-liquid extraction (LLE) or solid phase extraction (SPE), and is often processed in a batch mode with much delayed turnaround time [TAT][3]. We investigated commercially available dispersive pipette extraction tips [DPX tips] to shorten the manual sample preparation time and to make it amendable to be processed in a random access mode, much more suitable for a clinical diagnostic lab.

Methods:

Calibrator/QC/urine/saliva sample [250 µL] were mixed with 50 µL of IS [cortisol-d4] and 500 µL of acetonitrile and incubated at RT for 10 minutes. WAX-S tips [DPX Labs, LLC, Columbia, SC] were pre-wetted with acetonitrile and were used to aspirate and expel each of the above sample mix 3-4 times. The organic upper layer [300 µL] was transferred to a new set of tubes and was evaporated under N_2 gas at 37°C on a heating block. The dried samples were reconstituted with 120 µL of water and methanol (80:20) and injected [20 µL] to a LC-MS/MS system. Quantification was performed in the multiple reaction monitoring mode [positive] after electrospray ionization [ESI]. The new sample preparation method was validated and results were compared directly with a conventional LC-MS/MS method in clinical use for assay characteristics such as sensitivity [LLOD and LLOQ], linearity, and precision.

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

Table 1.

	Saliva (ng/dL)	**Urine (ng/dL)**
AMR	30 – 5,000	30 – 5,000
CRR	30 – 5,000	30 – 50,000
LLOD	5	5
LLOQ	30	30

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%
Recovery	85% (at 30), 90% (at 500)	80% (at 500), 75% (at 4000)
Ion Suppression	90%	N/A

Conclusion: Utilizing DPX WAX-S tips may reduce sample preparation time by up to 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.

References:

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