ABSTRACT – Salivary Cortisol

Fast and Reliable Method of Salivary Cortisol Quantification Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Background: Cortisol is one of the most important glucocorticoids in regulating human metabolism. Midnight salivary cortisol has been shown to correlate well with free cortisol (F-cortisol) concentration in serum and is one of the first tests recommended for the diagnosis of Cushing's syndrome. The purpose of this study was to validate the Cortisol salivary quantification using LC-MS/MS Waters® Xevo-TQS. **Materials and Methods** The procedure described here involves centrifugation of the saliva samples to remove solids and mucus strands before they are diluted with methanol containing carbonate internal standard cortisol-$^{13}C_3$ (IS). The samples are then subjected to separation on an Acquity BEH C18® analytical column (50 x 2.1mm, 1.7μm). Quantification is achieved by comparing the responses of a given sample to the responses of the calibrators of known concentrations. Analytical specificity is ensured by using multiple reaction monitoring with fragment ions that are unique to cortisol and IS, 363.5 >121 and 366.6 >124.3 respectively. The mobile phase was H_2O:Methanol with formic acid 0.1% (88:12-v/v) with a flow rate of 0.3 mL/min. The linearity was observed in the expected concentration range. F-cortisol saliva was used as a biological matrix for the study. **Results**: The linearity was studied using enriched samples of cortisol from 1.0 to 30.0 ng/mL and the saliva samples were evaluated six times, each. The intra-assay and inter-assay precision and accuracy are demonstrated in table 1. The retention time was 0.76 min and the total analysis time was 2.5 min. Linearity was studied in the concentration range from 1.0 to 30.0 ng/mL with a coefficient of determination (R^2) of 0.993639. **Conclusion:** The method was quick and efficient in determining salivary cortisol. The efficiency and selectivity combined with the technical robustness can be employed to cortisol dosage in the control of diagnosis of Cushing's syndrome.

Concentration ng/mL	Precision		Accuracy	
	Intra-Assay (RSD%) n=6	Inter-assay (RSD%) n=18	Intra-Assay % n=6	Inter-assay % n=18
0.1	7.70	N.D.	100.0	N.D.
0.2	6.85	N.D	100.0	N.D
0.5	5.74	9.6	94.0	94.0
1.0	6.38	N.D	95.0	N.D
2.0	4.33	N.D	95.0	N.D
5.0	1.23	N.D.	95.2	N.D.
7.5	0.75	N.D	108.5	N.D
10.0	1.85	4.6	98.9	100.2
20.0	0.54	5.6	108.9	103.3
30.0	2.33	N.D	102.3	N.D

N.D. Not determined

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