Phytronix Breakaway Technologies

Sciex

Clinical

Application Note : 2208

Analysis of Cortisol in Saliva:

Quantification of Cortisol in Saliva Using Luxon Ion Source®

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Introduction

The rapid quantification of cortisol in saliva can be useful for the screening of adrenal pathologies like Cushing's syndrome[1,2]. Saliva analysis of cortisol can be use as biomarker for stress assessment. It was shown that salivary cortisol can provide important information about hypothalamic-pituitary-adrenal (HPA) axis activity under normal and stress conditions[3].

Our goal for this application note is to develop a diagnostic tool to rapidly quantify cortisol in saliva, which will allow the rapid diagnosis of certain adrenal pathologies or as biomarker for stress assessment.

LDTD-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled quantification method. To develop this application, we focused on performing an efficient sample preparation, which will increase the speed of the analysis process.

Luxon Ionization Source

The Luxon Ion Source® (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing more precision, accuracy, and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic saturation characterize this type of ionization and is the result of the absence of solvent and mobile phase.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Stock solutions of Cortisol were prepared in methanol at 1 mg/mL. Then, artificial saliva samples were spiked to generate a calibration curve and QCs.

Automated Sample Extraction

An automated system (**Figure 3**) is used to extract the samples using the following conditions:

- 8 µL of Internal standard in methanol
- 400 µL of Saliva sample.
  - Vortex.
- 320 µL of Buffer (KH_2PO_4, 1M).
  - Vortex.
- 800 µL of Methyl-*tert*-butyl ether
  - Vortex and Centrifuge (3000 rpm/2 min.).
- Transfer 400 µL of the upper layer in a borosilicate test tube
  - Evaporate to dryness.
- Add 120 of Methanol/Water (1:1) mixture.
  - Vortex.
- Spot 6 µL of mixture on a LazWell™ CORT plate (96 wells).
  - Dry 5 minutes at 40°C

Figure 3 - Automated extraction system

LDTD®-MS/MS Parameters

LDTD
Model: Luxon S-960, Phytronix
Carrier gas: 6.0 L/min (air)
Laser pattern:

- 6-seconds ramp to 75% power
- 2-seconds hold

MS/MS
MS model: QTrap® System 5500, Sciex.
IonSpray Voltage: -4200
Temperature, GS1 and GS2 set to zero.
Scan Time: 50 msec
Analysis Method: Negative MRM mode

Table 1 - MRM transitions for Luxon-MS/MS

	Transition	CE (V)
Cortisol	361.2 → 331.1	-15
Cortisol-d_6	367.1 → 335.0	-15

Results and Discussion

Validation Test

Calibration curves ranging from 1 to 20 ng/mL were prepared in synthetic saliva. Sets of QCs were prepared (LLOQ, QCL, QCM, QCH, and ULOQ) in synthetic saliva. Replicate extractions were deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio was used to normalize the signal.

Linearity

The calibration curves were plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criteria was used:

- Linear regression (r) must be ≥ 0.995

Table 2 shows the inter-day correlation coefficients for Cortisol. Values greater than 0.995 are obtained. **Figure 4** shows a typical calibration curve result for Cortisol.

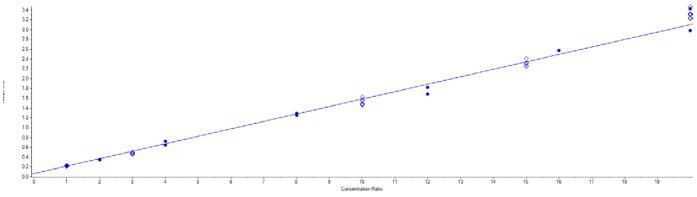

Figure 4 – Cortisol calibration curve

Table 2 – Inter-day calibration curve correlation coefficients

	Cortisol	R
Curve 1	Y = 0.15169 X + 0.06757	0.99623
Curve 2	Y = 0.15002 X + 0.066340	0.99700
Curve 3	Y = 0.13005 X + 0.06599	0.99741
Curve 4	Y = 0.08740 X + 0.06370	0.99789
Curve 5	Y = 0.11970 X + 0.05502	0.99517

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria were used:

- Each concentration must not exceed 15%CV.
- Each concentration must be within 100 ± 15% of the nominal concentration.

For the intra and inter-run precision and accuracy experiment, each QC was analyzed in sextuplicate, on four different runs. **Table 3 and 4** show the intra and inter-run precision and accuracy results for Cortisol. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value.

Table 3 - Intra-Run Precision and Accuracy of Cortisol

Cortisol	LLOQ	QCL	QCM	QCH	ULOQ
Conc (ng/mL)	1	3	10	15	20
N	6	6	6	6	6
Mean (ng/mL)	1.0	2.7	9.6	14.9	21.4
%CV	5.0	3.5	4.3	3.0	2.6
% Nom	102.0	89.9	96.3	99.5	107.0

Table 4 - Inter-Run Precision and Accuracy of Cortisol

Cortisol	LLOQ	QCL	QCM	QCH	ULOQ
Conc (ng/mL)	1	3	10	15	20
N	12	30	30	30	12
Mean (ng/mL)	1.0	2.8	9.4	14.9	20.8
%CV	7.6	11.0	7.4	9.6	10.9
% Nom	104.2	93.3	94.2	99.3	104.0

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers protected from light. After five days, sample extracts are spotted on a LazWell™ plate, dried and analyzed. The precision and accuracy results of QCs in synthetic saliva samples are reported in **Figure 5**. All the results are within the acceptable criteria range for one week at 4°C.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 2 hours before analysis. The precision and accuracy results of QCs in synthetic saliva samples are reported in **Figure 5**. the results are within the acceptable criteria range for 2 hours at room temperature.

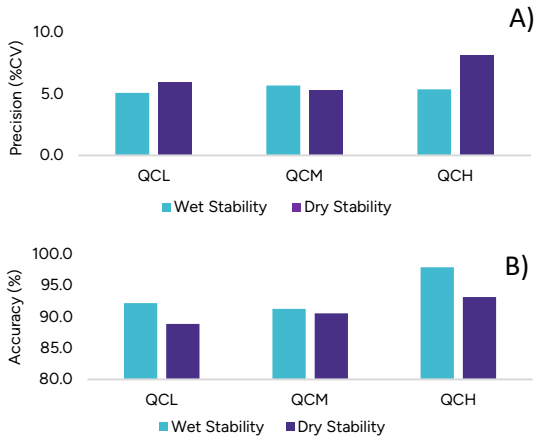

Figure 5 - Wet and Dry Stability of Cortisol in Synthetic Saliva Sample. A) Precision data; B) Accuracy data

Cortisol study

The study of cortisol in saliva over a period of 24 hours was carried out. The results obtained agree with those obtained in Chan *et al.* (2020). The level of cortisol rises rapidly when an individual wakes up and decreases throughout the day until bedtime.

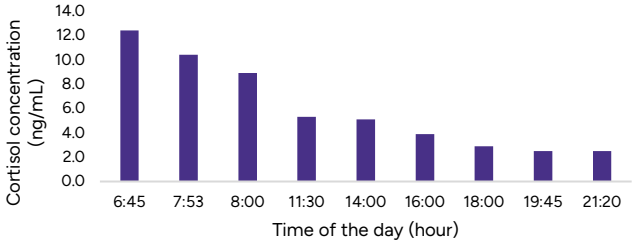

Figure 6 – Cortisol study (one day)

Conclusion

The Luxon Ion Source® combined with the Sciex QTrap® 5500 mass spectrometer system enables the rapid analysis of cortisol in saliva in less than 10 seconds.

References

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