

Analysis of Cortisol in Hair:

Quantification of Cortisol in Hair Using Luxon Ion Source®

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Introduction

Cortisol analysis is used as biomarker of hypothalamic-pituitary-adrenal (HPA) activity associated to physiological stress^{1,2}. Cortisol is slowly and continuously incorporated into growing hair. This approach provides a long-term index of stress assessment. Gonzalez et al³ suggest a hair cortisol concentration median of 55 pg/mg hair (range 40-128 pg/mg hair) in healthy individuals with low levels of stress and 250 pg/mg hair (range 182-520 pg/mg hair) in stressed individuals.

Our goal for this application note is to develop a diagnostic tool to rapidly quantify cortisol in hair as a biomarker for long-term 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 unmatched 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 suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 8 seconds sample-to-sample and allows working with very small volumes.



Figure 1 - Luxon Ion Source®



Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Sample Collection

Thirty milligrams of hair samples (approximately 1 cm length) were transferred into the Precellys MK28R 2mL lysing kit (2 mL reinforced empty vials with 2.8 mm stainless steel beads from Bertin Technologies, Montigny-le-Bretonneux, France, p/n : P000917-LYSKO-A.O).

Decontamination and Pulverization

To remove undesired Cortisol from the hair surface, 1 mL of isopropanol (IPA) was added to the samples, and they were soaked for 3 minutes at room temperature. The washing solution was then discarded. This washing step was repeated twice. Afterwards, samples were dried with air flow (5 psi) for 60 minutes. Next, the cleaned hairs were pulverized into a fine powder (3 X 60 sec at 6500 rpm, 15 seconds pause time) using a Precellys 24 Touch system (Figure 3).



Figure 3 – Hair pulverization system (Precellys 24 Touch) from Bertin Technologies, Montigny-le-Bretonneux, France.

Sample Extraction

Samples were extracted as follows:

- 1500 µL of methanol into vial.
 - Cap vial, vortex and place in sonicator bath for 1 hour.
 - After incubation step, centrifuge vial at 5000 rpm for 5 minutes.
- Transfer 200 µL of extracted sample into borosilicate tubes (10X75mm)
- Add 40 µL of internal standard.
- Add 40 µL of methanol or working solution.
- Evaporate to dryness.
- Add 120 µL of Methanol:Water (1:1) into dry sample tube.
 - Vortex
- Spot 6 µL of mixture on a LazWell™96-CORT plate
 - Dry 8 minutes at 40°C

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix

Carrier gas: 9 L/min (air)

Laser pattern:

- 6-second ramp to 65% power

MS/MS

MS model: Q-Trap System® 5500, Sciex

Scan Time: 50 msec

Total run time: 8 seconds per sample

Ionization: APCI

Analysis Method: Negative MRM mode

Table 1 – Negative MRM transitions for LDTD-MS/MS

	Transition	CE
Cortisol	394.1 → 331.0	-25
Cortisol-d ₆	400.1 → 335.0	-25

Results and Discussion

O₂ Adduct Formation

Luxon Ion Source allows the formation of O₂ adduct in negative ionization mode for chemical structure having conjugated keto in the structure⁴ (Figure 4).

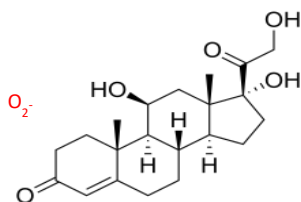


Figure 4 – O₂ adduct of Cortisol

This adduct formation increases the cortisol signal and the specificity of the method.

Method of Standard Addition (MSA)

The method of standard addition (MSA)⁵ involves adding different known amounts of a target analyte to fixed amounts of a sample to compensate for a sample matrix effect that enhances or depresses the analyte signal and used to evaluate the endogenous concentration of the compound in this matrix.

Zero and three additional fortifications of Cortisol were used to generate MSA curve. Cortisol addition concentration was plotted against the peak area ratio Cortisol/Internal standard (ISTD). A linear regression curve was performed, and the X-intercept was evaluated to get the endogenous concentration value of Cortisol in this matrix (Figure 5).

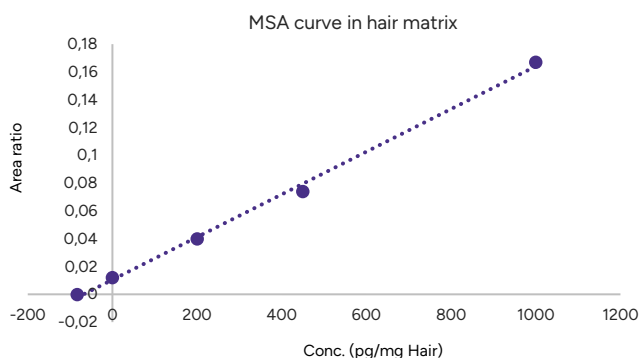


Figure 5 – MSA curve of Cortisol in hair matrix

Method Precision Evaluation

A pool of digested matrix was fortified with a Cortisol addition and the endogenous concentration was evaluated using a MSA curve. Table 2 shows the linearity results ($Y = mX + b$) and the calculated concentration. A precision of 13.1% was obtained.

Table 2 - Intra-Run Precision

Cortisol	m	b	Conc. (pg/mg)
Curve 1	0.00015587	0.01059	67.9
Curve 2	0.00016095	0.01301	80.8
Curve 3	0.00015999	0.00986	61.6
Curve 4	0.00014554	0.0131	90.0
Curve 5	0.00015596	0.01184	75.9
Curve 6	0.00016204	0.01081	66.7
Curve 7	0.00014794	0.01234	83.4
Curve 8	0.00016047	0.01351	84.2
Mean			76.3
%CV			13.1

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 4 days, sample extracts are spotted on a LazWell™ plate, dried and analyzed. MSA curve was generated. Precision of endogenous concentration and % difference against (T=0) values are reported in Table 3. All the results are within the acceptable criteria range (%CV ≤ 20% and %Difference ±20%) for 4 days 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 3 hours before analysis. MSA curve was generated. Precision of endogenous concentration and % difference against (T=0) values are reported in Table 3. All the results are within the acceptable criteria range (%CV ≤ 20% and %Difference ±20%) for 3 hours at room temperature.

Table 3 - Wet and Dry stability of Cortisol

Parameters	Dry stability		Wet stability	
	T0	3h/RT	T0	4 days / 4°C
Stability time				
Conc.(pg/mg)	76.3	87.1	76.3	86.1
%CV		13.3		2.2
%Diff		13.2		12.1

Multi-matrix evaluation

Hair samples were collected from 10 volunteers. Samples were analyzed using MSA approach. Table 4 shows calculated concentration of samples. Based on Gonzalez range:

- Range 40-128 pg/mg hair: Low levels of stress
- Range 182-520 pg/mg hair: High levels of stress
- A concentration between these ranges were reported as: Medium levels of stress.

Three samples were detected with Cortisol concentrations associated to a low level of stress. Seven samples show high levels of stress.

Table 4 - Sample evaluation

Sample ID	Cortisol (pg/mg)	Level of stress
Sample 1	314.0	High
Sample 2	214.8	High
Sample 3	92.1	Low
Sample 4	335.3	High
Sample 5	60.0	Low
Sample 6	278.2	High
Sample 7	249.8	High
Sample 8	371.8	High
Sample 9	195.2	High
Sample 10	107.0	Low

Conclusion

The Precellys®24 Touch can be successfully used to pulverize hair samples for mass spectrometry analysis of Cortisol. The Precellys 24 Touch combined with the Luxon Ion Source and Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (8 seconds per sample) screening of Cortisol in hair using a simple sample preparation method.

References

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