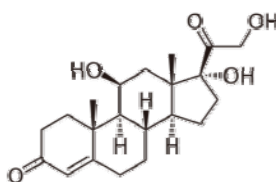


Cortisol

– the most important corticosteroid

Cortisol is the main glucocorticoid in humans. It is synthesized in the adrenal cortex and follows ACTH's diurnal variation causing the cortisol concentration in plasma to vary during the day, with the highest concentration in the morning and lowest concentration around midnight.

Cortisol is released in response to stress and a low level of blood glucocorticoids. Its primary functions are to increase blood sugar; suppress the immune system; and aid in fat, protein and carbohydrate metabolism. It also decreases bone formation. Cortisol is also used for diagnosing Cushing's syndrome. In plasma cortisol is transported bound. The free fraction represents about 10% of the total content of cortisol. The main indication for the determination of serum cortisol is suspicion of adrenocortical hyperfunction. One of the earliest signs of overproduction of cortisol is an absence of diurnal variation in serum cortisol levels. Salivary cortisol is a measure of the levels of free cortisol, but urine samples are also commonly taken from patients for clinical analysis.



Cortisol

On the following pages, we show a new method for analysis of cortisol using reversed phase LC-MS/MS were patient samples were analysed along with standards and control samples.

Cortisol in Saliva and Urine

Purospher® STAR RP-18 endcapped

Recommended column:
Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100-2.1 mm (1.50648.0001)

Recommended solvents and reagents:

Methanol: hypergrade for LC-MS LiChrosolv® (1.06035)

Water: Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q® water purification system

Formic acid 98-100% for analysis EMSURE® ACS, Reag. Ph Eur (1.00264)

Mobile phase: A: 0.1% formic acid in Milli-Q water
B: 0.1% formic acid in methanol

Time (min)	A (%)	B (%)	Flow Rate (mL/min)
0.00	70	30	0.40
0.50	50	50	0.40
4.80	50	50	0.40
4.81	0	100	0.60
5.80	0	100	0.60
5.81	70	30	0.40
7.00	70	30	0.40

HPLC Sample Preparation

Saliva and Urine samples:

Centrifuged saliva or urine samples. Add internal standard (Cortisol-D4).

(IS solution preparation (50 nM): 1.0 mL IS-standard (Cortisol-D4 1µM) in 20 mL milli-Q water)

The samples are thereafter prepared using supported liquid extraction (SLE) where methyl tert-butyl ether is used as eluent. Eluted sample is taken to dryness with nitrogen and reconstituted in initial mobile phase composition prior to injection on LC-MS/MS system.

Quantitation of Cortisol in saliva and urine

Linear range: 0.25 – 300 nM (nmol/L).

Lower limit of Quantitation = 0.25 nM

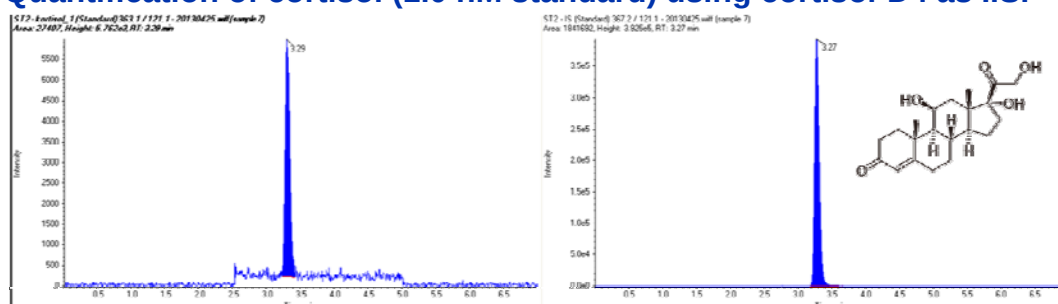
Cortisol in Saliva and Urine

Purospher® STAR RP-18endcapped

Chromatographic Conditions

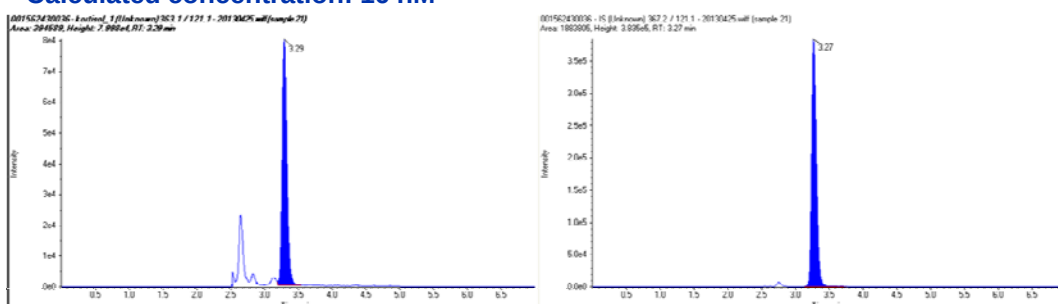
Column: Purospher® STAR RP-18 endcapped (2 µm) Hibar® HR 100-2.1 mm (1.50648.0001)
Injection: 10 µL
Detection: LC-ESI MS/MS; MRM transitions: m/z 363.1/121.1 (cortisol) and m/z 367.1/121.1 (Cortisol-D4)
Flow Rate: 0.4 mL/min
Gradient: See table
Mobile Phase: A: 0.1% formic acid in Milli-Q water
B: 0.1% formic acid in methanol
Temperature: 50 °C
Sample: Urine samples treated according to sample preparation protocol.
Backpressure: 360 bar (5184 psi) at start of gradient

Quantification of cortisol (1.0 nM standard) using cortisol-D4 as I.S.



Analysis of cortisol in patient samples (urine)

- Calculated concentration: 16 nM



Chromatographic Data

No.	Compound	Retention Time (min)	Precursor ion (m/z)	Product ions (m/z)
1	Void volume	1.3	-	
2	Cortisol-D4	3.27	363.1	121.1
3	Cortisol	3.29	367.1	121.1