CORTISOL and CORTISONE IN URINE by LC/MS – Code LC91015

INTRODUCTION

Hormones and hormonal mechanisms of action

The term "hormone" means a substance that is produced by an endocrine cell, that is, of internal secretion and is released into the bloodstream, causing functional responses in cells located at various distances from its point of production. Fulfilment of hormone action requires, in addition to the synthesis and secretion, transport into the bloodstream and the targeting of tissues where receptors are present. These are specialized structures that recognize the specific stimulus and translate the message.

The receptors may be on the cell membrane or within it. Hormones that cannot cross the membrane (eg. A peptide) bind to receptors located on the plasma membrane, while those that diffuse through the plasma membrane to the interior of the cell (steroids, iodothyronines) bind to intracellular receptors (typically located in the nucleus).

Hormone receptors have common characteristics regardless of their structure and type. They all feature a region able to recognize and bind the hormone and other MEP to generate an intracellular signal that translates the hormonal message into functional responses in the target cell. Additionally the properties that regulate the hormone binding (affinity, specificity, saturability, transduction capacity, ie to evoke specific effects) are common for all the receptors.

The communication entrusted to hormones takes place for the most part through the bloodstream (endocrine action) and to a lesser extent, by means of other methods. Some hormones act on cells immediately surrounding the cell that produces them (paracrine action), whilst others interact with the same cell through secretion (autocrine action). Finally, other hormones, are produced by the neurons of the nervous system (neurocrine action, which in reality is a specialized form of paracrine action).

Steroid Hormones

They are fat-soluble, freely diffuse within the cell and exert their effects after binding to receptors located in the nucleus. Their chemical structure, that is polycyclic, derives from cholesterol. They are divided, according to the site of production, in gonadal and adrenal steroid; also included in this category is Vitamin D and its analogues. The steroid hormones produced by the adrenal gland and gonads are divided into subgroups according to the number of carbon atoms of the steroidal nucleus.

Unlike peptide hormones, the secretion of steroids in the circulation does not proceed through storage within the cells but immediately follows the synthesis. They travel in the plasma bound to specific transport proteins, with high affinity, such as cortisol-binding globulin (CBG), the sex steroid binding globulin (SHBG) and vitamin D-binding protein (DBP). CBG is a glycoprotein able to bind with equal affinity cortisol and progesterone. SHBG are globulins which bind testosterone with high affinity, while estradiol is bound predominantly to albumin. 98% of gonadal steroids, 95% of cortisol and 50% of aldosterone bound to their transport proteins. Since only free hormones are able to interact with the receptors, and thus to express the biological activity, plasma protein binding is an important reserve phase in the metabolism of these hormones.

Cortisol-Cortisone

Cortisol is a steroid hormone synthesized in the adrenal glands from cholesterol, through an enzymatic cascade. It's the main glucocorticoid in humans and acts as a gene transcription factor influencing a variety of cellular responses in many tissues.

Cortisol plays a key role in the metabolism of glucose, in the maintenance of vascular tone as well as regulates the immune response and the body's response to stress. Its production is under the control of the pituitary gland hypothalamus.

Only a small percentage of circulating cortisol is biologically active, most cortisol is inactive (protein bound). When the plasma cortisol value rises, the free cortisol (cortisol unmarried or hydrocortisone) also increases, and is filtered by the glomerulus. The urinary free cortisol is well correlated with the concentration of serum free cortisol. The urinary free cortisol is biologically active, and is responsible for the signs and symptoms of hypercortisolism (Cushing's Syndrome).

The measurement of free cortisol excretion over 24 hours associated with liquid mass spectrometry (LCMS/MS) is the preferred screening test for Cushing's syndrome. It eliminates interference from such factors as the taking of carbamazepine and synthetic corticosteroids, which can alter the results of cortisol.

Cortisone, cortisol metabolite, is an additional parameter to help in the diagnosis of various adrenal disorders, including the abnormality in the operation of 11-beta-hydroxy-dehydrogenase (11-beta HSD), the enzyme that converts cortisol to cortisone. The lack of 11-beta HSD occurs in a state in which it has a mineralocorticoid excess, since cortisol (but not cortisone), acts as an agonist to the mineralocorticoid receptor.

This method allows to determine simultaneously both the cortisol and Cortisone in 24-hour urine sample by LCMS/ MS.

Bibliografy

Enciclopedia della Scienza e della Tecnica (2007)

Andreani, Cassano 1977: Andreani, Domenico - Cassano, Cataldo, *Trattato italiano di endocrinologia*, Roma, SEU, 1977.

Becker 1995: Becker, Kenneth L., *Principles and practice of endocrinology and metabolism*, 2. ed., Philadelphia, Lippincott, 1995 (1. ed.: 1990).

Faglia 1998: Faglia, Giovanni, *Malattie del sistema endocrino e del metabolismo*, 2. ed., Milano, McGraw-Hill, 1998 (1. ed.: 1993).

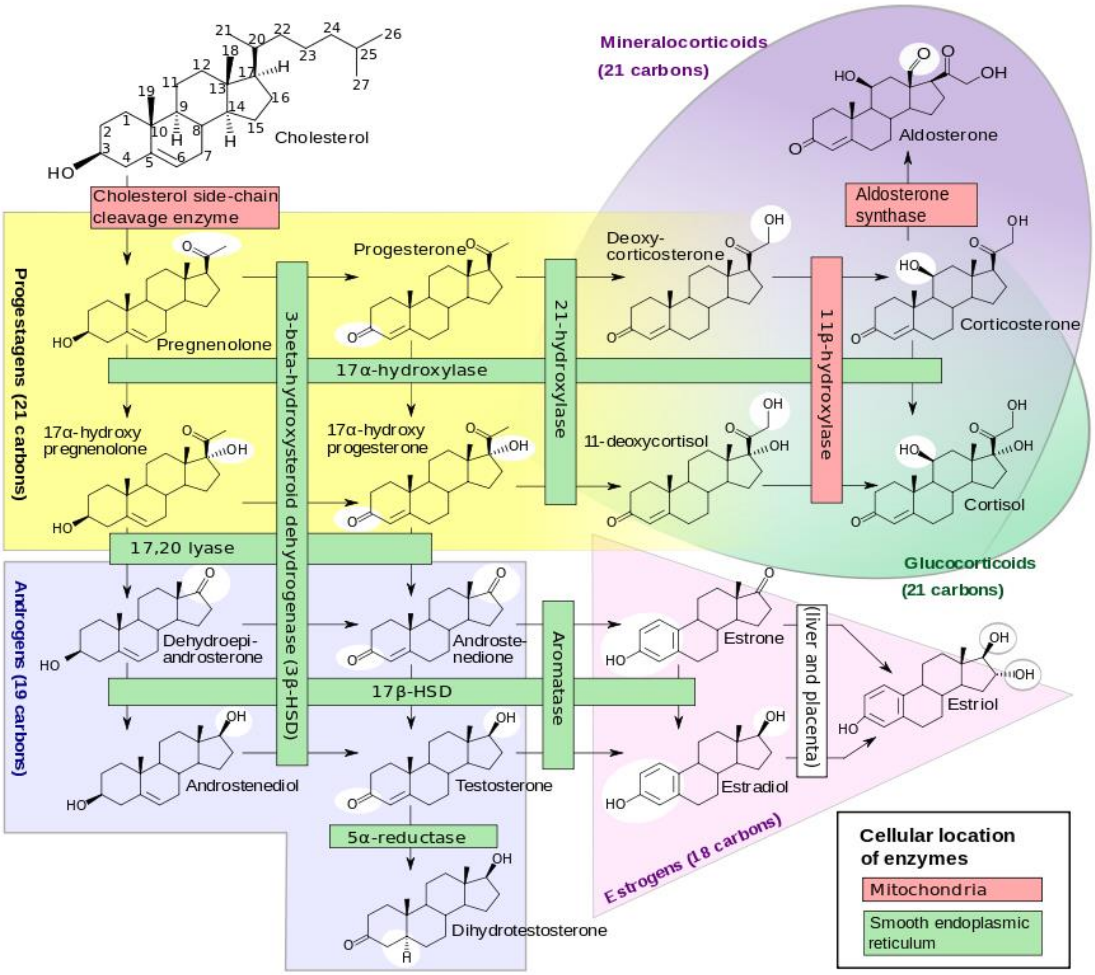
Felig 1995: Felig, Philip - Baxter, John D. - Frohman, Lawrence A., *Endocrinology and metabolism*, 3. ed., New York, McGraw-Hill, 1995 (1. ed.: 1981).

Giusti, Serio 1988: Giusti, Giorgio - Serio, Mario, *Endocrinologia, fisiopatologia e clinica*, Firenze, USES, 1988.

Pinchera 1991: Pinchera, Aldo e altri, *Endocrinologia e metabolismo. Fisiopatologia e clinica*, Milano, Ambrosiana, 1991.

Williams 1998: Williams, Robert H., *Williams textbook of endocrinology*, 9. ed., edited by Jean D. Wilson e altri, Philadelphia-London, Saunders, 1998 (1. ed.: 1950).

Biosynthetic pathway diagram



EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com

This product fulfills all the requirements of Directive 98/79/EC and Dl.ivo 08/09/2000 n.332 on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

TECHNICAL FEATURES

Principle of the Method:

Urine is diluted with appropriate reagent containing the internal standard and injected into the LC-MS/MS system.

Recovery :	100%	
Sensitivity (LLOD) :	Cortisol	0,1 ng/ml
	Cortisone	0,1 ng/ml
Minimum Concentration analyzable (LLOQ) :	Cortisol	0,25 ng/ml
	Cortisone	0,25 ng/ml
Linearity :	Cortisol	0,25 – 300 ng/ml
	Cortisone	0,25 – 600 ng/ml

Normal Range:

CORTISOL

0-2 years: n.d.

3-8 years: 1.4-20 ug/24 hours

9-12 years: 2.6-37 ug/24 hours

13-17 years: 4.0-56 ug/24 hours

> o =18 years: 3.5-45 ug/24 hours

CORTISONE

0-2 years: n.d.

3-8 years: 5.5-41 ug/24 hours

9-12 years: 9.9-73 ug/24 hours

13-17 years: 15-108 ug/24 hours

> o =18 years: 17-129 ug/24 hours

Use the conversion factors listed below for each analyte from 24 hours nmol/ug/:

Conversion factors:

Cortisol: ug/24 hours x 2.76=nmol/24 hours (molecular weight=362.5)

Cortisone: ug/24 hours x 2.78=nmol/24 hours (molecular weight=360)

Accuracy intra serie (relative error %) Cortisol:

C1	C2
2,5 ng/ml	75 ng/ml
11,1%	4,0%

Accuracy inter serie (relative error %) Cortisol:

C1	C2
2,5 ng/ml	75 ng/ml
14,6%	4,9%

Reproducibility intra serie (relative error %) Cortisol :

CLLOQ	Cm	Cs
0,25 ng/ml	30 ng/ml	160 ng/ml
0,4%	2,4%	1,4%

Reproducibility inter serie (coefficient of variation %) Cortisol :

CLLOQ	Cm	Cs
0,25 ng/ml	30 ng/ml	160 ng/ml
12,7%	6,2%	3,1%

Accuracy intra serie (relative error %) Cortisone:

C1	C2
2,5 ng/ml	150 ng/ml
6,7%	3,2%

Accuracy inter serie (relative error %) Cortisone:

C1	C2
2,5 ng/ml	150 ng/ml
10,2%	3,3%

Reproducibility intra serie (relative error %) Cortisone:

CLLOQ	Cm	Cs
0,25 ng/ml	25 ng/ml	300 ng/ml
7,8%	2,3%	1,2%

Reproducibility inter serie (coefficient of variation %) Cortisone:

CLLOQ	Cm	Cs
0,25 ng/ml	25 ng/ml	300 ng/ml
10,3%	2,7%	3,8%

Coefficient of Correlation R2 + Dev Std

0,9984 ± 0,0004 Cortisol
0,9989 ± 0,0002 Cortisone

Components of the kit (500 tests) :	All the Reagents are ready to use and stable 3 years at 2–8 °C.
Reagent A – Extraction Solution, **3 x 75 ml**	
Reagent B – Deuterated Internal Standard, **1 x 500 ul**	**Store at -20 °C**
Calibrator in urine – Level 0, 3 x 1 ml	**Code LC91016 (packed separately – see data sheets)**
Calibrator in urine – Level 1, 3 x 1 ml	**Code LC91016 (packed separately – see data sheets)**
Calibrator in urine – Level 2, 3 x 1 ml	**Code LC91016 (packed separately – see data sheets)**
Calibrator in urine – Level 3, 3 x 1 ml	**Code LC91016 (packed separately – see data sheets)**
Calibrator in urine – Level 4, 3 x 1 ml	**Code LC91016 (packed separately – see data sheets)**
Calibrator in urine – Level 5, 3 x 1 ml	**Code LC91016 (packed separately – see data sheets)**
Calibrator in urine – Level 6, 3 x 1 ml	**Code LC91016 (packed separately – see data sheets)**
Reagent M1 – Mobile Phase M1, **3 x 500 ml**	
Reagent M2 – Mobile Phase M2, **3 x 500 ml**	

Minimum Instrumental equipment required: LC-MS Instrument triple quadrupole ESI

Urine Collection Procedure: Collect 24 hours of urination in clean containers without preservatives and measure the volume (L/12:00 am). If necessary centrifuge precipitates before extraction. The specimen is stable 7 days at 2-8° C 3 months at-20° c. Freeze only once.

ANALITYCAL PROCEDURE

STEP 1 : Put 150 ul of **Reagent B** – Deuterated Internal Std in **Reagent A** – Extraction Sol. (stable 3 months at 2-8 °C)

STEP 2 : In vial dispense:

	Calibrator	**Sample**	**Control**
Calibrator	50 µl		
Sample		50 µl	
Controls			50 µl
Reagent A – Extraction Sol after **STEP 1**	450 µl	450 µl	450 µl

Vortex for 10 seconds

INJECTION :

- Inject 10 µl of solution in LC system

N.B: at this step, the sample is stable 2 days at 2-8 °C.

CORTISOL and CORTISONE - Warnings

REAGENT B – DEUTERATED INTERNAL STANDARD

Solution containing: **Cortisol-*d4.* Store at -20 °C.**	
Hormones:	Internal Standard to use for calibration:
CORTISOL	**Cortisol-*d4***
CORTISONE	**Cortisol-*d4***

COLUMN CONDITIONING

Install a new analytical RRHD Eclipse Plus C18 (50 x 2,1 mm, 1,8 µm), thermostated at 60 °C. Disconnect the detector and flux a solution of Acetonitrile : H_2O (95 : 5 v/v) set flow at 600 µl / min for 15 minutes. **Don't recycle the washing solutions**. Condition further on the column with a solution of Mobile Phase M1 : Mobile Phase M2 (85 : 15 v/v) set flow at 600 µl / minute for 15 minutes. Making two injections of HPLC grade water before proceeding with the analytical series.

It is NOT possible to make analysis at recycling phase.

If Room Tem. is > 20° C is better to preserve the Mobile Phase at 2 – 8° C among runs.

COLUMN CLEANING

Wash the column with a solution of Mobile Phase M2 : H_2O (95 : 5) at flow of 600 µl / minutes for 15 minutes. Store the column in a solution of Mobile Phase M2 : H_2O (95 : 5).

AUTOSAMPLER SYRINGE CLEANING

Wash the syringe, before each analytical session, with Methanol.

PARAMETERS SET ON TRIPLE QUADRUPOLE SCIEX 4500

Parameters

Curtain Gas (CUR): 30
Collision Gas (CAD): 8
Ion Spry Voltage: (IS): 5500 V
Temperature (TEM): 550°C
Ion Source Gas 1 (GS1): 50
Ion Source Gas 2 (GS2): 60

FLOW SET

GRADIENT			
Time (min)	**% M1 (PUMP A)**	**% M2 (PUMP B)**	**FLOW (µl/min)**
0.00	60	40	400
1.00	60	40	400
2.50	5	95	400
3.50	5	95	400
3.51	60	40	400
5.00	60	40	400

Fragmentations (optimized on TRIPLE QUADRUPOLE AGILENT 6460)

Analyte	Q1	Q3	COLLISION ENERGY
Cortisol	363.1	121.0	8
Cortisone	361.2	163.0	8
Cortisol-D4	367.1	121	8

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC91016	Calibrator in urine for Cortisol/Cortisone	7 x 2 x 1 ml
LC91019	Control in urine for Cortisol/Cortisone – Levels 1, 2 and 3	3 x 5 x 1 ml
SK91010	Starter kit for Cortisol/Cortisone	1 Pc
LC91011	Mobile Phase M1	4 x 500 ml
LC91021	Mobile Phase M2	4 x 500 ml
LC91030	Deuterated internal Standard Solution in urine	1 x 100 µl
Z959757902	Analytical Column RRHD Eclipse Plus C 18 (50 x 2,1 mm – 1,8 µm)	1 Pc
Z51843550	Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
Z51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs

BIBLIOGRAPHY:

Quantification of corticosteroids in human plasma by liquid chromatography-thermospray mass spectrometry using stable isotope dilution
Hiromi Shibasaki, Takashi Furuta, Yasuji Kasuya

Steroid Hormone Analysis by Tandem Mass Spectrometry
Steven J. Soldin and Offie P.Soldin

Quantification of anabolic hormones and their metabolites in bovine serum and urine by liquid chromatography-tandem mass spectroscophy
R. Draisci, L. Palleschi, E. Ferretti, L.Lucentini, P. Cammarata

Identification of ten corticosteroids in human hair by liquid chromatography-ionspray mass spectrometry
V. Cirimele, P.Kintz, J.P. Goullè, B. Ludes

A Confirmatory HPLC-MS/MS Method for Ten Synthetic Corticosteroids in Bovine Urines, JOURNAL OF MASS SPECTROMETRY
S. Rhea Savu, L. Silvestro, A. Haag and F. Sorgel, VOL. 31, 1351-1363 (1996).

Automated solid-phase extraction for concentration and clean-up of female steroid hormones prior to liquid chromatography–electrospray ionization–tandem mass spectrometry.
B. Alvarez Sanchez, F. Priego Capote, J. Ruiz Jimenez, M.D. Luque de Castro. An approach to lipidomics, Journal of Chromatography A, 1207 (2008) 46–54.

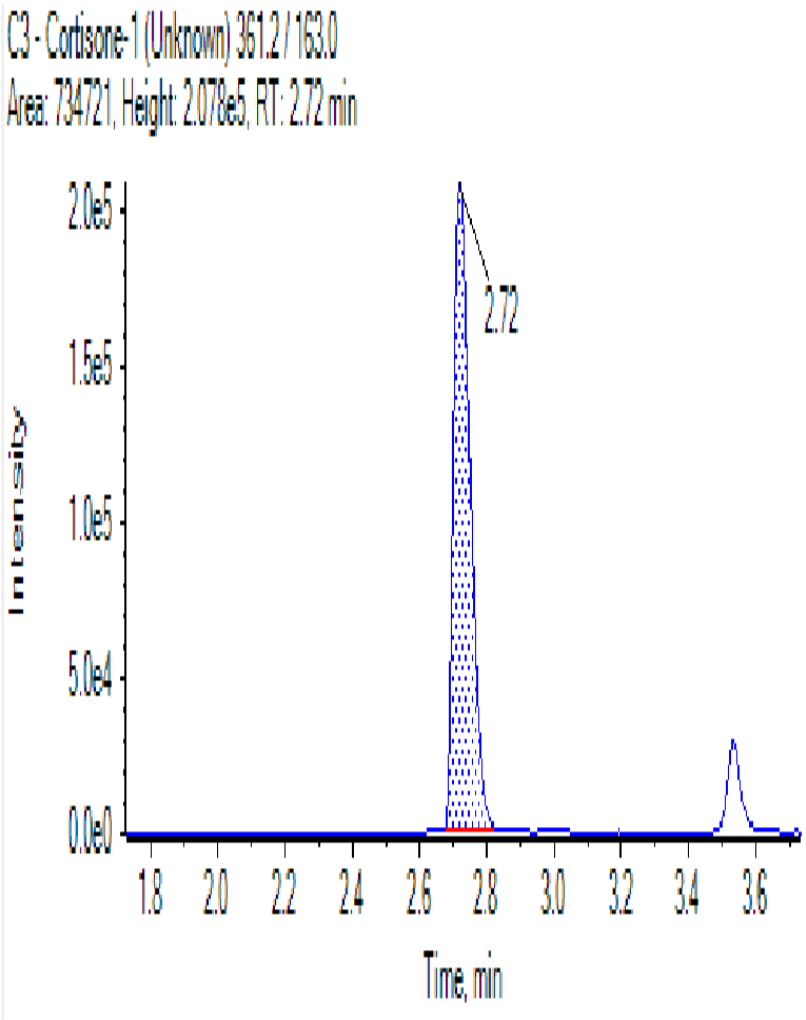
Detection, quantification and confirmation of anabolic steroids in equine plasma by liquid chromatography and tandem mass spectrometry
Fuyu Guan, Cornelius E. Uboh, Lawrence R. Soma, Yi Luo, Jeffery Rudy, Thomas Tobin., Journal of Chromatography B, 829 (2005) 56–68.

Liquid chromatography–electrospray ionization mass spectrometric analysis of corticosterone in rat plasma using selected ion monitoring,
Ashok Marwah, Padma Marwah, Henry Lardy. Journal of Chromatography B, 757 (2001) 333–342.

Quantification of corticosteroids in human plasma by liquid chromatography–thermospray mass spectrometry using stable isotope dilution,
Ashok Marwah, Padma Marwah, Henry Lardy, Journal of Chromatography B, 692 (1997) 7–14

CORTISOL AND CORTISONE IN URINE

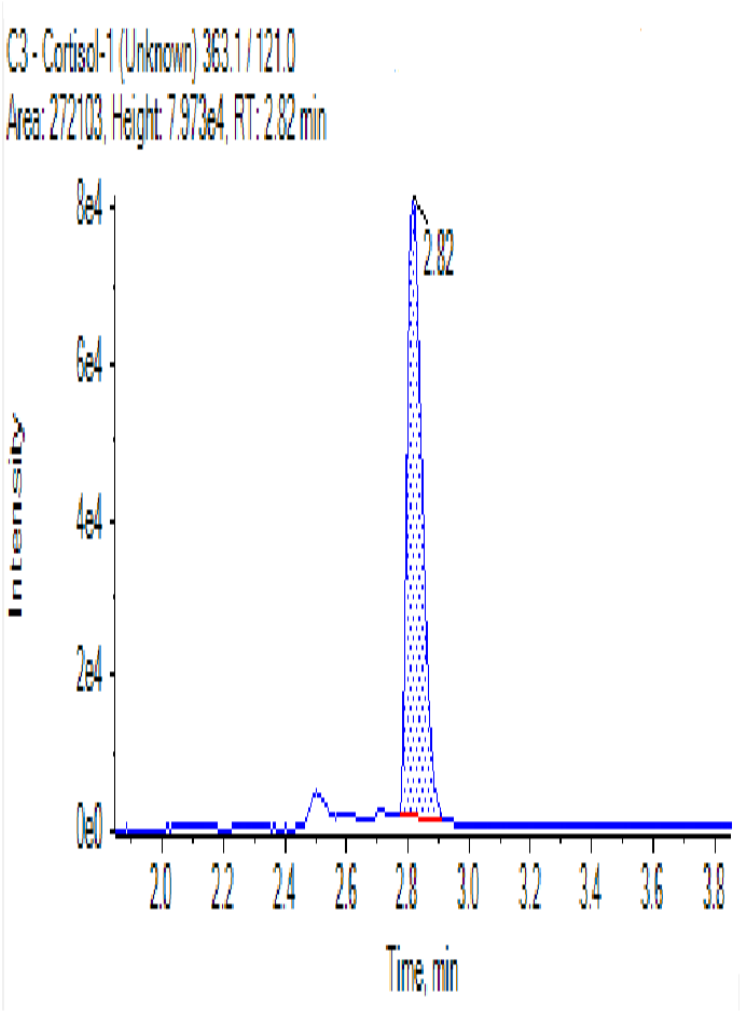
(Reference Chromatograms)



Hormones	Retention time
	Min
Cortisone 150 ng/ml	**2.72**

CORTISOL AND CORTISONE IN URINE

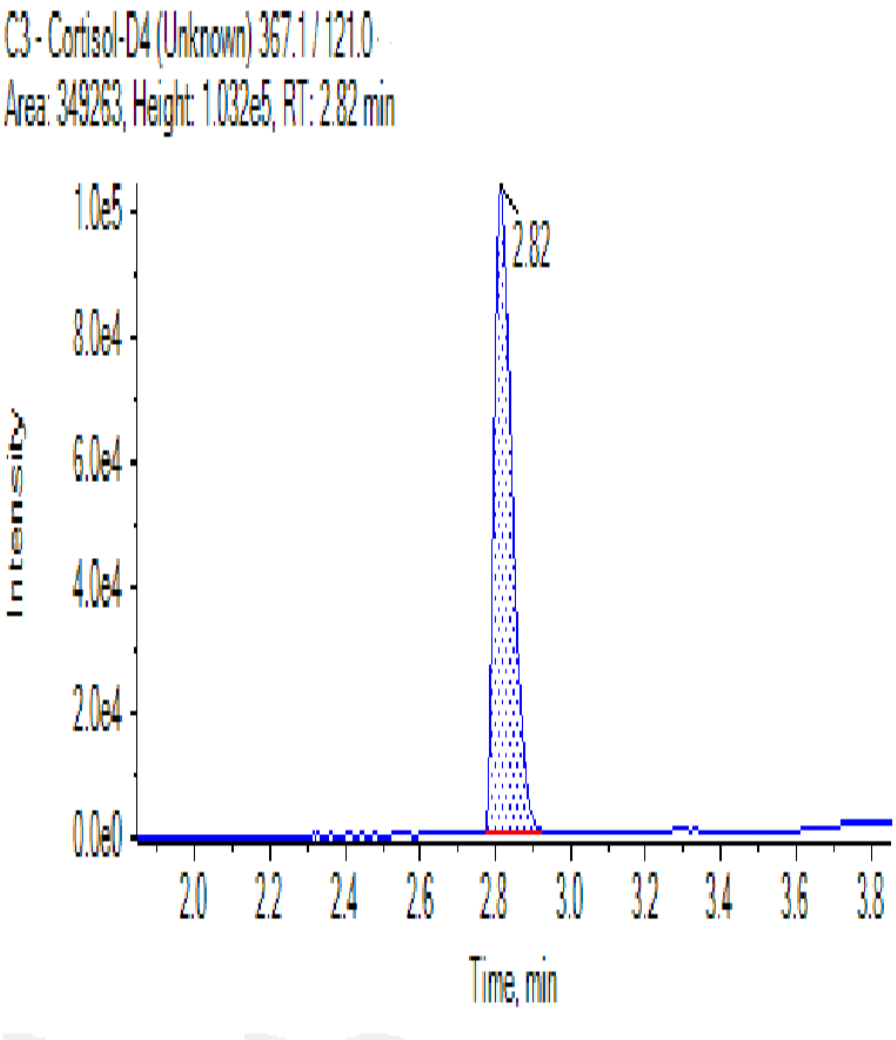
(Reference Chromatograms)



Hormones	Retention time
	Min
Cortisol 75 ng/ml	**2.82**

CORTISOL AND CORTISONE IN URINE

(Reference Chromatograms)



Internal Standard	Retention time
	Min
Cortisol-D4	**2.82**