STEROID HORMONES IN SERUM/PLASMA by LC/MS – Code LC72415

(17-OH-Progesterone, Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone sulfate (DHEAS), Androstenedione, Cortisol, 11-Deoxycortisol, Corticosterone, Aldosterone, Testosterone, Dihydrotestosterone, Androsterone, Estrone, Estradiol, Pregnenolone, 17-OH-Pregnenolone, Progesterone, 11-Deoxycorticosterone, 21-Deoxycortisol, Cortisone)

INTRODUCTION

Hormones and hormone action mechanisms

The term "hormone" indicates a substance that is produced by an endocrine cell by internal secretion. Hormones are 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. Hormone receptors have common characteristics regardless of their structure and type.

The receptors may be on the cell membrane or inside the cell. 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). In both cases, the hormone-receptor complex will activate a cascade of molecular event 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 mostly occurs through the bloodstream (endocrine action). Some hormones act on cells immediately surrounding the cell that produces them (paracrine action), conversely 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).

More than fifty hormones have been identified and their functional characteristics are determined by their different molecular structure. Based on this, they are divided into four broad categories: proteins and peptides, steroids, those derived from amino acids and those derived from polyunsaturated fatty acids. Hormones, interacting with receptors located at the level of target tissues, evoke specific responses that regulate the enzymatic activities, gene expression and protein synthesis.

Classification of steroid hormones

They are fat-soluble, diffuse freely within the cell and exert their action after binding to receptors located in the nucleus. Their chemical structure, which is polycyclic, derives from cholesterol. They are termed adrenal and gonadal steroids according to the site of production.
Also Vitamin D and its analogues belong to this class. The steroid hormones produced by the adrenal gland and gonads are divided into subgroups according to the number of carbon atoms in the steroid nucleus (21C-19C-18C). In particular, progesterone,glucocorticoids and mineralocorticoids derive for subsequent synthesis by pregnane, a simple substance that contains 21 carbon atoms, while estrogen comes from the

core of estrane which has 18 carbon atoms, and androgens are derived from a 19-carbon tetracyclic hydrocarbon nucleus known as Androstane.

The synthesis of steroid hormones following identical biosynthetic stages in both the adrenal and ovary or testis and differentiation in the three endocrine glands, depends on a different distribution of tissue-specific enzyme synthesis. Steroidogenesis is a multistep enzymatic process, mostly catalyzed by cytochrome P450 enzymes (cP450) localized within cells, and starts with the conversion of cholesterol to pregnenolone. This enzymatic stage is the most important as it controls the synthesis of all steroid hormones, even in the presence of significant amounts of cholesterol, the possibility of the continuation of the biosynthetic pathway appears to be limited, since it depends on the enzymatic activity of cP450.
The produced pregnenolone comes out of the mitochondria and is transferred to the endoplasmic reticulum, where it undergoes subsequent enzymatic modifications by cP450. Pregnenolone is then the common precursor of the major steroid hormones.

Androgens are synthesized by the Leydig cells of the testis, from the reticular zone of the adrenal glands and from theca cells of the follicle and interstitium of the ovary. The biosynthetic pathway that proceeds from pregnenolone follows two possible ways: the first consists of the transformation of pregnenolone to 17-hydroxypregnenolone, to dehydroepiandrosterone, to androstenediol, and finally into testosterone, which is the main male hormone. The second way consists of the transformation of pregnenolone to progesterone, then to 17-hydroxyprogesterone, then the formation of androstenedione, which subsequently transforms into testosterone. In some tissues testosterone requires further transformation reaction in 5α-dihydrotestosterone to exert its effect. In the adrenal gland where, in relation to the testicle, there is a different distribution of enzymes that catalyze these biosynthetic pathways, most of adrenal androgen production is directed towards the development of precursors of testosterone, particularly androstenedione and dehydroepiandrosterone, a hormone of more modest activity. About 50% of pregnenolone metabolized in the adrenal cortex is converted into dehydroepiandrosterone.

Estrogen and progesterone steroids, the main female hormones, are produced in greater amounts by the ovaries and are involved in regulating the menstrual cycle and pregnancy. The formation of these steroids derive from enzymatic steps in different cellular compartments of the ovaries: follicles and interstitial cells. The granulosa cells that surround the egg in the context of the follicle, undergo transformation into luteal cells after ovulation and the product of the transformation of pregnenolone is represented by progesterone. Theca cells of the follicle and those interstitial produce mainly androstenedione through biosynthetic pathways that have already been described for androgens. The enzymatic steps involved in the following metabolic fate androstenedione also have a characteristic cellular localization: in fact testosterone is synthesized only in the cells of the hilum, while inside granulosa cell androstenedione is transformed into testosterone then in estrone and estradiol. The interstitial cells also have the ability to turn testosterone into dihydrotestosterone.

On the contrary, in the case of peptide hormones, the secretion of steroids in circulation does not proceed through their storage within the cells but the synthesis immediately follows. They travel in the plasma bound to specific transport proteins, with high affinity, such as cortisol-binding globulin (CBG, Cortisol binding protein), the sex steroid binding globulin (SHBG, Sex hormone binding protein) and vitamin D (DBP, vitamin D binding protein). CBG is a glycoprotein able to bind cortisol and progesterone with equal affinity. SHBG are globulins that bind with high affinity to testosterone, while estradiol is mainly bound to albumin.
98% of gonadal steroids, 95% of cortisol and 50% of aldosterone circulate bound to their transport proteins. Since only free hormones are able to interact with the receptors, and therefore to express the biological activity, protein binding in plasma is an important reserve phase in the metabolism of these hormones. Synthetic steroids used in therapy usually do not bind transport proteins and are therefore able to exert biological effects immediately. Glucocorticoids and mineralocorticoids are metabolized through enzymatic reactions that result in the loss of their hormonal activity or through conjugation with chemical groups that make them water-soluble leading to their elimination in the urine. The only metabolic reaction that does not result in a loss of biological activity is represented by the conversion of testosterone into dihydrotestosterone in target cells.*

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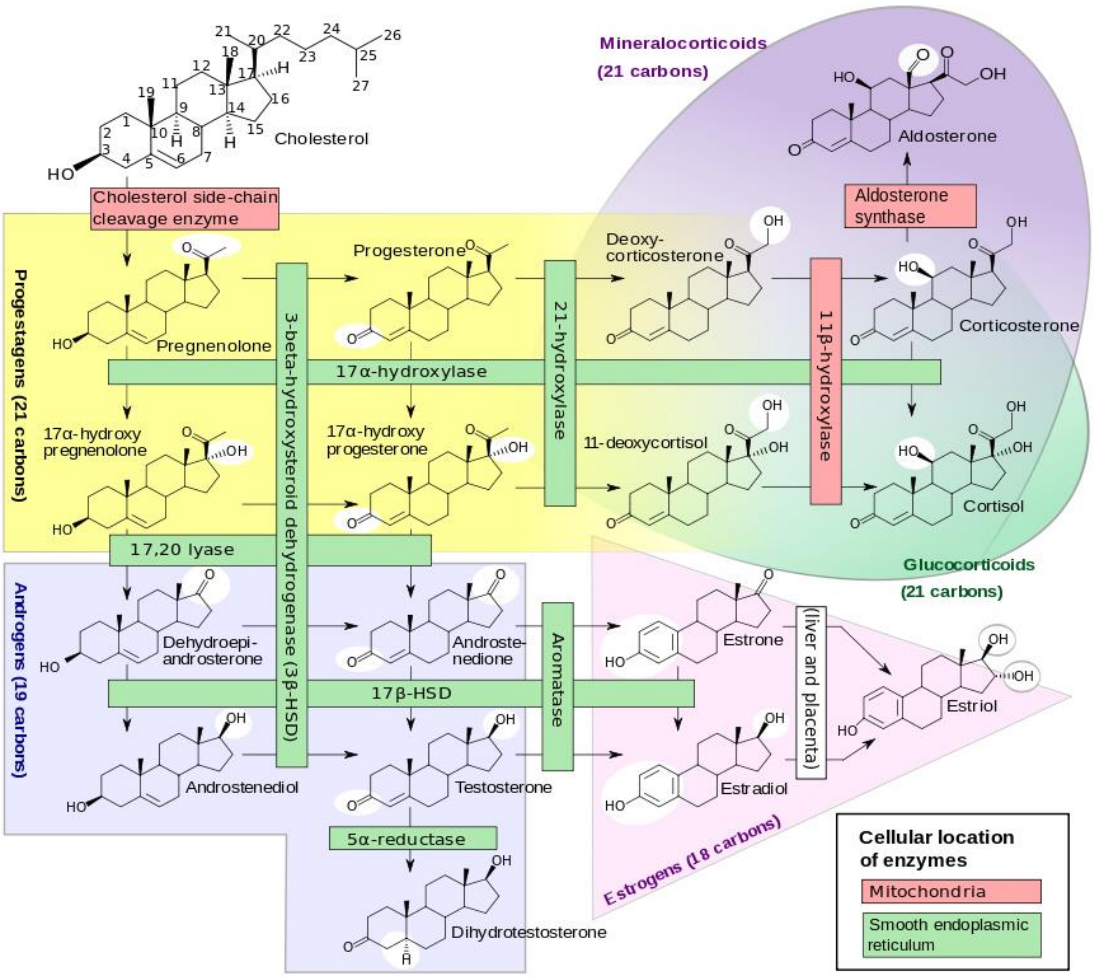
Biosynthetic pathway diagram



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TECHNICAL FEATURES

Principle of Method:

Steroid Hormones are deproteinized with an appropriate reagent containing the internal standard, centrifuged, diluted and injected in LC/MS.

Recovery %			
	C Loq	Cm	Cs
17-OH-Progesterone	113,33%	113,29%	137,20%
Androstenedione	109,26%	109,32%	133,01%
DHEAS	33,51%	36,23%	51,59%
DHEA	95,27%	102,64%	131,00%
Testosterone	115,66%	108,49%	132,23%
Cortisol	79,02%	83,31%	93,94%
Corticosterone	107,30%	103,81%	123,70%
Aldosterone	106,27%	64,57%	84,14%
11-Deoxycortisol	97,53%	107,30%	120,76%
Dihydrotestosterone	104,93%	104,83%	129,73%
Androsterone	114,53%	99,58%	127,62%
Estrone	86,91%	101,00%	110,40%
Beta-Estradiol	101,53%	106,49%	125,98%
Pregnenolone	99,76%	98,21%	125,96%
17-OH-Pregnenolone	95,38%	100,59%	130,62%
Progesterone	113,41%	108,95%	124,70%
11-Deoxycorticosterone	108,47%	108,79%	125,30%
Cortisone	71,91%	87,50%	97,35%
21-Deoxycortisol	116,58%	100,60%	123,21%

Sensitivity (LLOD) :

17-OH-Progesterone	0,002 ng/ml
Androstenedione	0,003 ng/ml
DHEAS	2,0 ng/ml
DHEA	0,015 ng/ml
Testosterone	0,002 ng/ml
Cortisol	0,15 ng/ml
Corticosterone	0,008 ng/ml
Aldosterone	0,005 ng/ml
11-Deoxycortisol	0,003 ng/ml
Dihydrotestosterone	0,002 ng/ml
Androsterone	0,04 ng/ml
Estrone	0,002 ng/ml
Beta-Estradiol	0,005 ng/ml
Pregnenolone	0,008 ng/ml
17-OH-Pregnenolone	0,02 ng/ml
Progesterone	0,007 ng/ml
11-Deoxycorticosterone	0,005 ng/ml
Cortisone	0,1 ng/ml
21-Deoxycortisol	0,005 ng/ml

Minimum concentration analizable (LLOQ) :

17-OH-Progesterone	0,006 ng/ml
Androstenedione	0,009 ng/ml
DHEAS	6,79 ng/ml
DHEA	0,044 ng/ml
Testosterone	0,007 ng/ml
Cortisol	0,444 ng/ml
Corticosterone	0,022 ng/ml
Aldosterone	0,014 ng/ml
11-Deoxycortisol	0,008 ng/ml
Dihydrotestosterone	0,007 ng/ml
Androsterone	0,111 ng/ml
Estrone	0,006 ng/ml
Beta-Estradiol	0,011 ng/ml
Pregnenolone	0,02 ng/ml
17-OH-Pregnenolone	0,061 ng/ml
Progesterone	0,02 ng/ml
11-Deoxycorticosterone	0,012 mg/ml
Cortisone	0,22 ng/ml
21-Deoxycortisol	0,01 ng/ml

Linearity :

17-OH-Progesterone	0,006 – 11 ng/ml
Androstenedione	0,009 - 16 ng/ml
DHEAS	6,79 – 9160 ng/ml
DHEA	0,044 - 80 ng/ml
Testosterone	0,007 - 24 ng/ml
Cortisol	0,444 – 800 ng/ml
Corticosterone	0,022 – 40 ng/ml
Aldosterone	0,014 – 5 ng/ml
11-Deoxycortisol	0,008 – 15 ng/ml
Dihydrotestosterone	0,007 – 12,5 ng/ml
Androsterone	0,111 – 40 ng/ml
Estrone	0,006 - 2 ng/ml
Beta-Estradiol	0,011 - 4 ng/ml
Pregnenolone	0,02 – 7,334 ng/ml
17-OH-Pregnenolone	0,061 - 22 ng/ml
Progesterone	0,02 – 36,667 ng/ml
11-Deoxycorticosterone	0,012 – 22 ng/ml
Cortisone	0,22 – 400 ng/ml
21-Deoxycortisol	0,01 – 4 ng/ml

ANALYTE	Reproducibility intra-serie			Reproducibility inter-serie		
	(CV%)			(CV %)		
	Concentration			Concentration		
	C Loq	Cm	C Loq	C Loq	Cm	Cs
17-OH-Progesterone	3,58%	1,76%	1,62%	6,49%	2,71%	2,04%
Androstenedione	6,13%	1,35%	1,58%	8,12%	2,39%	2,07%
DHEAS	6,32%	2,10%	2,55%	6,24%	3,08%	1,94%
DHEA	4,21%	2,73%	1,81%	5,21%	3,06%	1,69%
Testosterone	2,65%	1,91%	1,72%	2,95%	2,45%	1,87%
Cortisol	3,95%	0,77%	0,80%	5,81%	1,76%	0,97%
Corticosterone	2,91%	2,67%	1,11%	4,71%	3,53%	3,21%
Aldosterone	6,51%	4,04%	2,26%	13,2%	7,7%	3,2%
11-Deoxycortisol	6,06%	1,76%	2,17%	7,40%	2,98%	2,50%
Dihydrotestosterone	5,09%	1,43%	0,66%	9,64%	2,95%	1,83%
Androsterone	4,58%	1,80%	1,24%	10,56%	2,38%	1,63%
Estrone	5,72%	4,73%	1,75%	6,87%	8,18%	2,47%
Beta-Estradiol	3,37%	4,33%	4,78%	10,10%	7,15%	4,73%
Pregnenolone	4,46%	3,23%	2,23%	7,01%	5,94%	2,92%
17-OH-Pregnenolone	6,85%	3,48%	3,40%	6,53%	3,79%	2,56%
Progesterone	6,65%	1,28%	1,34%	11,30%	4,20%	1,48%
11-Deoxycorticosterone	4,90%	1,27%	1,42%	5,65%	1,87%	2,15%
Cortisone	5,16%	0,89%	0,92%	10,44%	4,28%	1,24%
21-Deoxycortisol	6,20%	2,77%	0,83%	6,87%	4,64%	2,30%

ANALYTE	R^2
17-OH-Progesterone	0,9986 ± 0,0003
Androstenedione	0,9982 ± 0,0003
DHEAS	0,9991 ± 0,0004
DHEA	0,9983 ± 0,0002
Testosterone	0,9984 ± 0,0003
Cortisol	0,9965 ± 0,0004
Corticosterone	0,9968 ± 0,0016
Aldosterone	0,9962 ± 0,0014
11-Deoxycortisol	0,9991 ± 0,0001
Dihydrotestosterone	0,9987 ± 0,0007
Androsterone	0,9989 ± 0,0004
Estrone	0,9978 ± 0,0006
Beta-Estradiol	0,9970 ± 0,0007
Pregnenolone	0,9982 ± 0,0009
17-OH-Pregnenolone	0,9981 ± 0,0010
Progesterone	0,9989 ± 0,0006
11-Deoxycorticosterone	0,9985 ± 0,0003
Cortisone	0,9975 ± 0,0005
21-Deoxycortisol	0,9969 ± 0,0006

ANALYTE	Accuracy intra-serie (Error %) Concentration		Accuracy inter-serie (Error %) Concentration	
	C1	C2	C1	C2
17-OH-Progesterone	5,73%	3,93%	7,17%	4,83%
Androstenedione	2,97%	4,16%	5,18%	5,14%
DHEAS	2,34%	1,55%	5,58%	3,08%
DHEA	5,70%	4,49%	9,16%	5,32%
Testosterone	6,25%	5,03%	7,33%	5,53%
Cortisol	5,43%	10,64%	6,13%	11,40%
Corticosterone	5,05%	3,54%	6,79%	5,84%
Aldosterone	5,89%	4,37%	7,55%	6,65%
11-Deoxycortisol	1,89%	1,64%	3,03%	2,58%
Dihydrotestosterone	5,05%	3,76%	6,54%	4,55%
Androsterone	1,85%	2,47%	3,42%	3,10%
Estrone	5,99%	2,61%	7,24%	3,18%
Beta-Estradiol	3,74%	1,07%	5,02%	2,59%
Pregnenolone	5,33%	1,75%	7,25%	2,95%
17-OH-Pregnenolone	8,50%	2,75%	8,19%	4,64%
Progesterone	3,22%	1,98%	4,62%	3,16%
11-Deoxycorticosterone	4,58%	4,14%	5,48%	4,69%
Cortisone	4,54%	8,61%	5,21%	9,45%
21-Deoxycortisol	2,90%	7,79%	4,06%	8,95%

ANALYTE	Concentration used to calculate Reproducibility and Accuracy Calibrator (ng/ml)				
	C Loq	C 1	C m	C 2	C s
17-OH-Progesterone	0,006	0,061	0,122	0,367	1,100
Androstenedione	0,009	0,089	0,178	0,533	1,600
DHEAS	6,790	67,901	135,802	407,407	1222,222
DHEA	0,044	0,444	0,889	2,667	8,000
Testosterone	0,007	0,133	0,267	0,800	2,400
Cortisol	0,444	4,444	8,889	26,667	80,000
Corticosterone	0,022	0,222	0,444	1,333	4,000
Aldosterone	0,014	0,028	0,056	0,167	0,500
11-Deoxycortisol	0,008	0,083	0,167	0,500	1,500
Dihydrotestosterone	0,007	0,069	0,139	0,417	1,250
Androsterone	0,111	0,222	0,444	1,333	4,000
Estrone	0,006	0,011	0,022	0,067	0,200
Beta-Estradiol	0,011	0,022	0,044	0,133	0,400
Pregnenolone	0,020	0,081	0,733	0,041	0,244
17-OH-Pregnenolone	0,061	0,122	0,244	0,733	2,200
Progesterone	0,020	0,407	0,815	2,444	7,333
11-Deoxycorticosterone	0,012	0,244	0,489	1,467	4,400
Cortisone	0,220	4,400	8,800	26,600	80,000
21-Deoxycortisol	0,010	0,020	0,040	0,130	0,400

Components of the kit (500 tests) :	All the reagents are stable 3 years at 2–8 °C except **Reagent A** and **Reagent C** that are stable at -20 °C.
Reagent A – Internal Standard Deuterated Solution, **10 x 1100 ul**	**Store at – 20 °C** **See Warnings**
Reagent B – Lysing Solution, **1 x 130 ml**	
Reagent C – Deproteinization Solution, **1 x 260 ml**	**Store at – 20 °C**
Reagent D – Conditioning Solution 1, **1 x 520 ml**	
Reagent E – Conditioning Solution 2, **1 x 520 ml**	
Reagent F – Conditioning Solution 3 and Wash 1, **1 x 520 ml**	
Reagent G – Wash Solution 2, **1 x 260 ml**	
Reagent H – Eluting Solution, **1 x 260 ml**	
Reagent I – Stabilizing Solution, **1 x 35 ml**	
Calibrator in plasma – Level 0, 8 x 1 ml	**Code LC72316 (packed separately – see data sheet)**
Calibrator in plasma – Level 1, 8 x 1 ml	**Code LC72316 (packed separately – see data sheet)**
Calibrator in plasma – Level 2, 8 x 1 ml	**Code LC72316 (packed separately – see data sheet)**
Calibrator in plasma – Level 3, 8 x 1 ml	**Code LC72316 (packed separately – see data sheet)**
Calibrator in plasma – Level 4, 8 x 1 ml	**Code LC72316 (packed separately – see data sheet)**
Calibrator in plasma – Level 5, 8 x 1 ml	**Code LC72316 (packed separately – see data sheet)**
Calibrator in plasma – Level 6, 8 x 1 ml	**Code LC72316 (packed separately – see data sheet)**
Reagent M1 – Mobile Phase M1, **5 x 500 ml**	
Reagent M2 – Mobile Phase M2, **5 x 500 ml**	
Clean up Columns, 500 pcs	

Minimum Instrumental equipment required:

LC/MS System with triple quadrupole ESI+/- according to molecules that you need to analyze
Centrifugal evaporator

Plasma Collection Procedure:

Dispense 3 ml of Whole Blood in a test tube with EDTA as anticoagulant. Centrifuge at 4000 rpm for 5 minutes. Collect the supernatant (plasma) and store it at - 20 °C for no longer than **1 month**.

Serum Collection Procedure:

Dispense 3 ml of venous blood into a tube for serum with or without gel. Centrifuge at 4000 rpm for 5 minutes. Separate the serum and store it at - 20 ° C for no longer than **1 month**.

Normal Range (ng/ml)	
17-OH-Progesterone	< 1,7
Androstenedione	< 2,0
DHEAS	148,0 – 3.852,0
DHEA	M: 1,8 – 12,5 F: 1,3 – 9,8
Testosterone	M: 2,9 – 8,6 F: < 1,7
Cortisol	46,0 – 207,0
Corticosterone	1,2 – 20,5
Aldosterone	0,025 – 0,229
11-Deoxycortisol	< 0,9
Dihydrotestosterone	M: 0,25 – 0,75 F: 0,05 – 0,30
Androsterone	0,20 – 0,80
Estrone	M: 0,01 – 0,06 F: 0,017 – 0,20 (premenopause) 0,007 - 0,04 (postmenopause)
Beta-Estradiol	M: < 0,05 F: 0,05 – 0,145 (follicular) 0,112 – 0,443 (ovulation) 0,05 – 0,241 (luteal) < 0,059 (postmenopause)
Pregnenolone	< 1,3
17-OH-Pregnenolone	< 7,3
Progesterone	M: < 0,13 F: < 9,4
11-Deoxycorticosterone	< 0,5
Cortisone	7,1 – 27,9
21-Deoxycortisol	< 0,12

N.B.: the values shown refer to ADULTS subjects.

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ANALYTICAL PROCEDURE

STEP 1 :

Dilute the **Reagent A** – Internal Standard deuterated Solution 1:1 with water HPLC grade. Prepare a suitable volume considering the number of samples of the session (40 µl per sample).

STEP 2 : Preparation of samples, calibration and control

In eppendorf tubes dispense in sequence:

	Calibrator	**Sample**	**Controls**
Calibrator	400 µl		
Sample		400 µl	
Controls			400 µl
Reagent A – Internal Std deuterated Sol **(previously diluted)**	40 µl	40 µl	40 µl
Vortex for 5 seconds			
Reagent B – Lysing Solution	250 µl	250 µl	250 µl
Vortex for 20 seconds			
Reagent C – Deproteinization Sol. (-20°C)	500 µl	500 µl	500 µl

Vortex for 45 seconds

Centrifuge at 14.000 rpm for 10 minutes

STEP 3 : Conditioning of Clean-up columns

- Drop 1 ml of **Reagent D** – Conditioning Solution 1 into Clean-up Columns
  Discharge the eluate
  - Flux drop by drop (1 ml/min ca) without drying up the stationary phase

  Or

  - Centrifuge at 2000 rpm for 1 minute

- Drop 1 ml of **Reagent E** – Conditioning Solution 2 into Clean-up Columns
  Discharge the eluate
  - Flux drop by drop (1 ml/min ca) without drying up the stationary phase

  Or

  - Centrifuge at 2000 rpm for 1 minute

- Drop 0.5 ml of **Reagente F** – Conditioning Solution 3 and Wash 1 into Clean-up Columns
  Discharge the eluate
  - Flux drop by drop (1 ml/min ca) without drying up the stationary phase

  Or

  - Centrifuge at 2000 rpm for 1 minute

STEP 4 : Load of Samples, Calibrators and Controls

- Transfer all the surnatant of samples, calibrators and controls, prepared in STEP 2, into Clean-up Columns
  Discharge the eluate
  - Flux drop by drop (1 ml/min ca) without drying up the stationary phase

Or

  - Centrifuge at 2000 rpm for 1 minute

STEP 5 : Wash of Clean-up Columns

- Drop 0.5 ml of **Reagente F** – Conditioning Solution 3 and Wash 1 into Clean-up Columns
  Discharge the eluate
  - Flux drop by drop (1 ml/min ca) without drying up the stationary phase

Or

  - Centrifuge at 2000 rpm for 1 minute

- Drop 0,5 ml of **Reagent G** – Wash Solution 2 into Clean-up Columns
  Discharge the eluate
  - Flux drop by drop (1 ml/min ca) without drying up the stationary phase

Or

  - Centrifuge at 2000 rpm for 1 minute and then at 4000 rpm for 1 minute

STEP 6 : Elution
- Place the Clean-up Columns in the collection tubes
- Drop 0,5 ml of **Reagent H** – Eluting Solution into Clean-up Columns
  Collect the eluate
  - Flux drop by drop (1 ml/min ca) and dry the stationary phase with an air flow

Or

  - Centrifuge at 2000 rpm for 1 minute and then at 4000 rpm for 1 minute

STEP 7 : Dry the eluate in the collection tubes with centrifugal evaporator or nitrogen flow evaporator or equivalent system (T max 55°C)

STEP 8 : Pour into the collection tubes containing the samples, the calibrators and the dried controls, 60 µl of **Reagent I** - Stabilizing Solution.

Vortex for 30 seconds

Transfer all the supernatant in vials with polypropylene insert

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

INJECTION :

- Inject 15 µl of solution in LC system (5-20 µl, according to instrumental sensitivity)

STEROID HORMONES - Warnings

REAGENT A – INTERNAL STANDARDS DEUTERATED SOLUTION:

Solution contains: ***Aldosterone-D7, Cortisol-D4, Testosterone-D3, Estradiol-D5, Pregnenolone-D4, DHEA-D6, DHEAS-D5, 17-OH-Progesterone 13C3. Store at – 20 °C.***		
Ionization:	Hormones:	Internal Standard used for calibration:
Positive	**ANDROSTENEDIONE**	***Testosterone-D3***
Positive	**DHEAS**	***DHEAS-D5***
Positive	**DHEA**	***DHEA-D6***
Positive	**TESTOSTERONE**	***Testosterone-D3***
Positive	**DIHYDROTESTOSTERONE**	***Testosterone-D3***
Positive	**ANDROSTERONE**	***Testosterone-D3***
Positive	**ESTRONE**	***DHEA-D6***
Positive	**BETA-ESTRADIOL**	***Beta-Estradiol-D5***
Positive	**PROGESTERONE**	***Cortisol-D4***
Positive	**11-DEOXYCORTICOSTERONE**	***Testosterone-D3***
Positive	**CORTICOSTERONE**	***Testosterone-D3***
Positive	**ALDOSTERONE**	***Aldosterone-D7***
Positive	**11-DEOXYCORTISOL**	***Cortisol-D4***
Positive	**PREGNENOLONE**	***Pregnenolone-D4***
Positive	**17-OH-PREGNENOLONE**	***Testosterone-D3/Pregnenolone-D4***
Positive	**CORTISOL**	***Cortisol-D4***
Positive	**17-OH-PROGESTERONE**	***17-OH-Progesterone-13C3***
Positive	**CORTISONE**	***Cortisol-D4***
Positive	**21-DEOXYCORTISOL**	***Cortisol-D4***
In case of low sensitivity for the DHEAS and/or ALDOSTERONE		
Negative	**DHEAS**	***DHEAS-D5***
Negative	**ALDOSTERONE**	***Aldosterone-D7***

COLUMN CONDITIONING

Install a new analytical column Poroshell 120 EC C18 (50 x 2,1 mm, 1,9 um), thermostated at 55 °C Disconnect the detector and flux a solution of Mobile Phase M1 : Mobile Phase M2 (20 : 80 v/v) set flow at 400 ul / minute for 10 minutes. **Don't recycle the washing solutions**. Condition further on the column at the flow of Mobile Phase M1 : Mobile Phase M2 (20 : 80 v/v) at flow of 400 ul / minute for 10 minutes. Then, flux Mobile Phase M1 : Mobile Phase M2 (80 : 20 v/v) set flow at 400 µl / min, for 5 minutes. And, flux Mobile Phase M1 : Mobile Phase M2 (65 : 35 v/v set flow at 400 µl / min, for 10 minutes. **Three injections of HPLC grade water before proceeding with the analytical series.**

It's NOT possible to make analysis at recycling phase.

If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux a solution of Mobile Phase M1 : Mobile Phase M2 (80 : 20 v/v) set flow at 400 µl / minute for 5 minutes discharge it, then flux Mobile Phase M1 : Mobile Phase M2 (10 : 90 v/v) set flow at 400 µl / minute, for 15 minutes. The column must be stored in a solution of Mobile Phase M1 : Mobile Phase M2 (10 : 90 v/v)

INJECTION NEEDLE WASHING

H_2O:ACN:IPA 33:33:33 + 0.1% Formic Ac.

PARAMETERS SET ON TRIPLE QUADRUPOLE SCIEX 4500

Parameters ESI +

Curtain Gas (CUR): 25
Collision Gas (CAD): 9
Ion Spry Voltage: (IS): 5500 V
Temperature (TEM): 600°C
Ion Source Gas 1 (GS1): 50
Ion Source Gas 2 (GS2): 60

Parameters ESI - (to optimized DHEAS and/or Aldosterone in case of low sensitivity in ESI + mode)

Curtain Gas (CUR): 30
Collision Gas (CAD): 8
Ion Spry Voltage: (IS): -4500 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	65	35	400
0.50	65	35	400
2.00	55	45	400
7.80	29.5	70.5	400
7.90	2	98	400
9.90	2	98	400
10.0	65	35	400
12.0	65	35	400

Fragmentations (optimized on TRIPLE QUADRUPOLE SCIEX 4500)

ANALYTE	***RT***	**Q1**	**Q3**
Aldosterone - 1 **+**	2.25	361.2	315.1
Aldosterone - 1 **-**	2.25	359.1	188.9
Cortisone - 1	2.66	361.2	163.0
Cortisone - 2	2.66	361.2	121.0
Cortisol - 1	2.96	363.1	121.0
Cortisol - 2	2.96	363.1	115.1
21-Deoxycortisol - 1	3.64	347.2	311.1
21-Deoxycortisol - 2	3.64	347.2	269.2
Corticosterone - 1	3.87	347.1	121.1
Corticosterone - 2	3.87	347.1	107.0
11-Deoxycortisol - 1	4.06	347.1	97.0
11-Deoxycortisol - 2	4.06	347.1	109.0
β-Estradiol - 1	4.60	255.1	159.1
β-Estradiol - 2	4.60	255.1	144.0
Estrone - 1	4.62	271.2	133.0
Estrone - 2	4.62	271.2	157.0
Androstenedione - 1	4.63	287.1	109.0
Androstenedione - 2	4.63	287.1	97.0
11-Deoxycorticosterone - 1	5.01	331.1	97.0
11-Deoxycorticosterone - 2	5.01	331.1	109.0
Testosterone - 1	5.05	289.1	97.0
Testosterone - 2	5.05	289.1	109.0
DHEA - 1	5.43	289.1	213.1
DHEA - 2	5.43	289.1	253.0
17-OH-Progesterone - 1	5.53	331.0	97.0
17-OH-Progesterone - 2	5.53	331.0	109
17-OH-Pregnenolone - 1	5.57	297.2	105.0
17-OH-Pregnenolone - 2	5.57	297.2	145.0
Dihydrotestosterone (DHT) - 1	6.54	291.1	255.2
Dihydrotestosterone (DHT) - 2	6.54	291.1	159.2
Progesterone - 1	6.86	315.1	97.0
Progesterone - 2	6.86	315.1	109.0
Androsterone - 1	7.20	273.1	159.2
Androsterone - 2	7.20	273.1	147.0
Pregnenolone - 1	7.58	299.1	159.2
Pregnenolone - 2	7.58	299.1	131.1
DHEAS - 1 +	3.75	271.2	133.0
DHEAS - 2 +	3.75	271.2	157.0
DHEAS - 1 -	3.75	367.0	133.0
Aldosterone-D7 **+**	2.25	368.2	322.1

Aldosterone-D7 **-**	2.25	366.0	194.1
Cortisol-D4	2.96	367.1	121.0
B-Estradiol-D5	5.39	260.2	161.0
Testosterone-D3	5.05	292.2	97.1
DHEA-D6	5.43	277.2	259.2
17-OH-Progesterone-13C3	5.53	334.1	112.1
Pregnenolone-D4	7.58	303.2	285.2
DHEAS-D5 +	3.74	276.2	138.0
DHEAS-D5 -	3.74	374.0	100.0

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC72316	Calibrator in plasma for Steroid Hormones	7 x 2 x 1 ml
LC72319	Control in plasma for Steroid Hormones – Levels 1, 2 and 3	3 x 5 x 1 ml
SK72410	Starter kit for Steroid Hormones	27 x 1 ml
Z699675902	Analytical Column Poroshell 120-EC C 18 (50 x 2.1 mm. 1.9 um)	1 Pc
S29057U	Glass vials of 2 ml with screw cap	1 x 100 Pcs
S24717	Polypropylene Insert 100 ul for vials of 2 ml	1 x 100 Pcs
S7344223	Corning Tubes mm 13x100	1 x 250 Pcs

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STEROID HORMONES IN SERUM/PLASMA

(Reference Chromatogram)

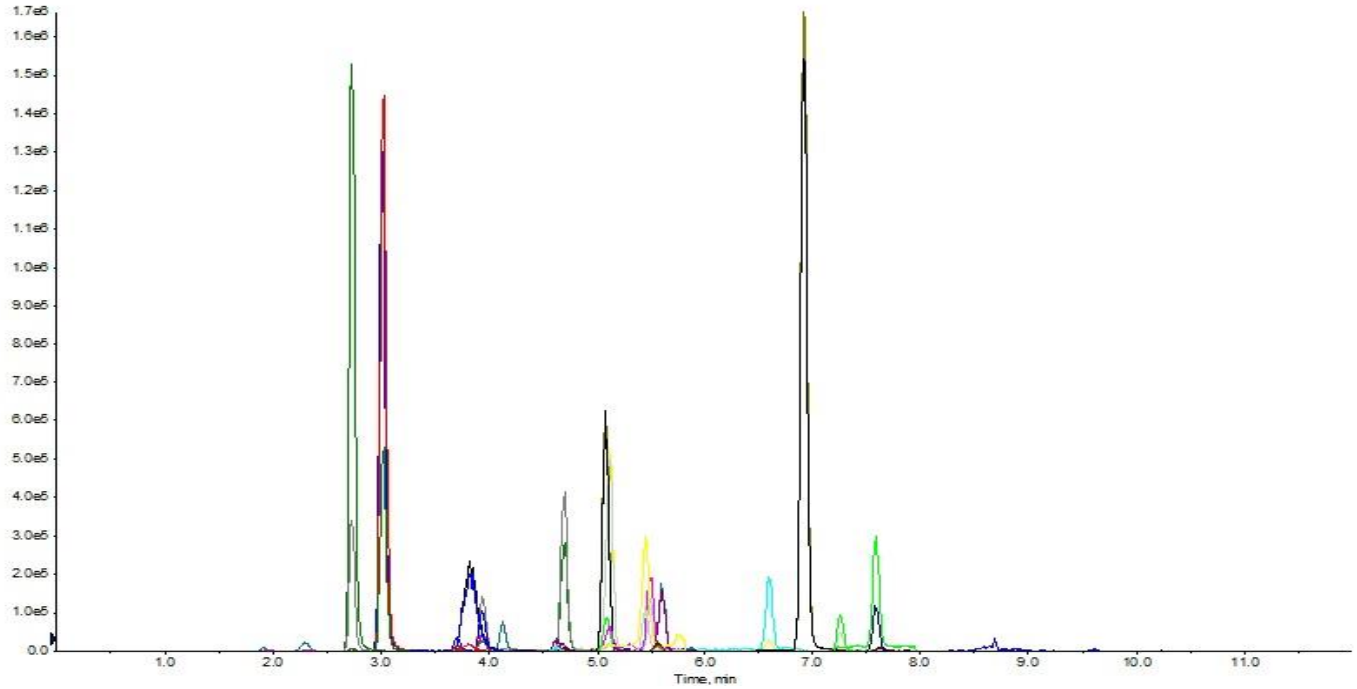

Fig.1 Extract Ion Chromatogram (Positive Ions) – Serum/Plasma Hormones