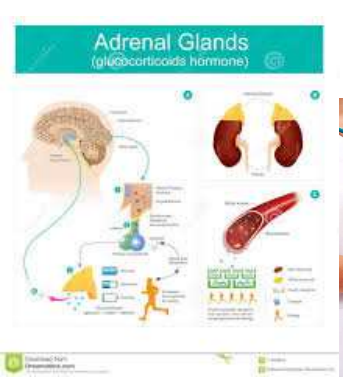
STEROID HORMONES in serum/plasma by LC/MS

pro training
ASSISTANCE



LC72410 Extractive Method - **LC 72310** non Extractive Method **-**

The ability to determine several parameters in a single run, makes mass spectrometry the method of choice for the analysis of **steroid hormones**.

19 steroid hormones in serum/plasma in a

SINGLE

chromatographic run of 12 minutes

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



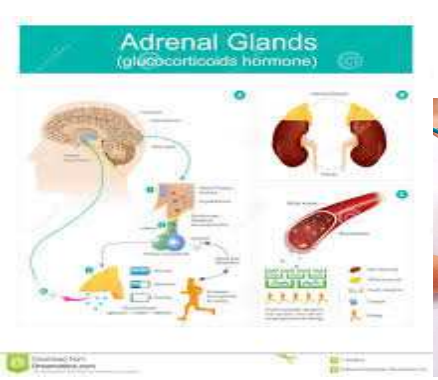
HIGHLIGHTS

LC72410

- 19 Clinically most relevant steroids covered in a single chromatographic run
- with SPE Clean Up Columns
- 1 analytical column
- reliable, robust and proven CE-IVD method
- high Sensitivity compared to other methods

	Linearity: (LLOQ-ULOQ)	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

- Method developed for **TOP Level** LC/MS triple quadrupole



HIGHLIGHTS

LC72310

- analysis of 19 steroid hormones in serum/plasma in a single chromatographic run
- a simple sample preparation procedure
- reliable, robust and proven CE-IVD method
- 1 analytical column for all parameters

Linearity: (LLOQ-ULOQ)	17-OH-Progesterone	0,026 – 570 ng/ml
	Androstenedione	0,016 – 1.060 ng/ml
	DHEAS	15 – 852.900 ng/ml
	DHEA	0,1 - 500 ng/ml
	Testosterone	0,006 - 260 ng/ml
	Cortisol	0,015 – 3.020 ng/ml
	Corticosterone	0,036 – 3.020 ng/ml
	Aldosterone	0,028 – 560 ng/ml
	11-Deoxycortisol	0,02 – 6.000 ng/ml
	Dihydrotestosterone	0,069 – 1.630 ng/ml
	Androsterone	0,13 – 2.200 ng/ml
	Estrone	0,011 - 510 ng/ml
	Beta-Estradiol	0,03 - 510 ng/ml
	Pregnenolone	0,06 – 64 ng/ml
	17-OH-Pregnenolone	0,06 - 250 ng/ml
	Progesterone	0,005 – 4.000 ng/m
	11-Deoxycorticosterone	0,012 – 22 ng/ml
	Cortisone	0,269 – 896,3 ng/ml
	21-Deoxycortisol	0,011 – 4,48 ng/ml

- Method developed for **Medium Level** LC/MS triple quadrupole

INTRODUCTION

HORMONES AND HORMONE ACTION MECHANISMS

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, i.e. 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).
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 divided, according to the site of production, adrenal and gonadal steroids, are included in this category as well as 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 in the steroid nucleus: 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 of androstane come from the nucleus, which contains 19 carbon atoms

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 passes through a series of enzymatic steps, mostly catalyzed by cytochrome P450 enzymes (cP450) localized within cells, and starts with the conversion of cholesterol to pregnenolone. This stage enzyme 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 activity by enzyme cP450.

Pregnenolone format 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, the zona reticularis of the adrenal glands and theca cells of the ovarian follicle and interstitium. 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. Although the enzymatic steps involved in the following metabolic fate androstenedione 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 and estrone and in turn estradiol. The interstitial cells also have the ability to turn testosterone into dihydrotestosterone. In contrast, in the case of peptide hormones, the secretion of steroids in circulation does not proceed through their 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, 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 equally with cortisol and progesterone. SHBG are globulins that bind with high affinity to testosterone, whereas estradiol is bound mainly 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.

Synthetic steroids used in therapy usually do not contract bond with transport proteins and are therefore able to exert biological effects immediate. 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 and determine 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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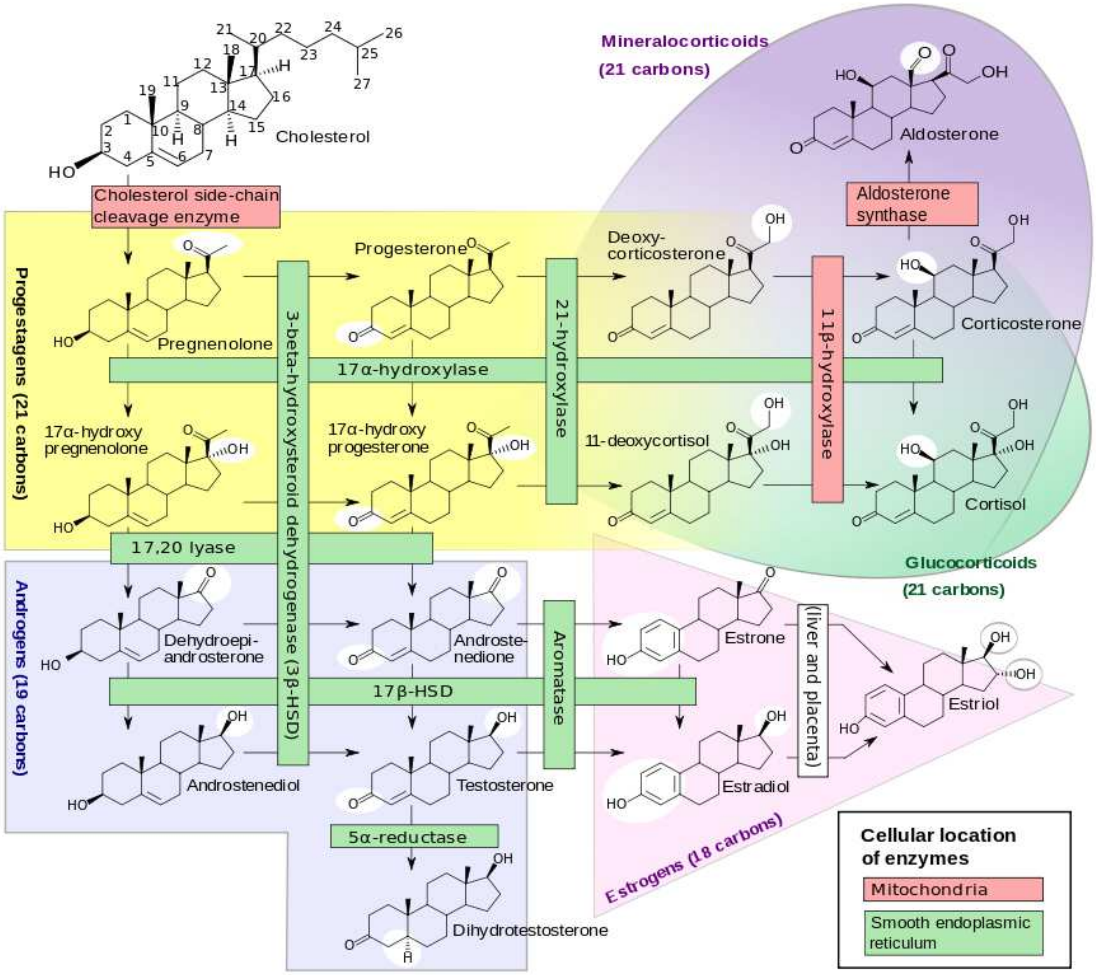
Biosynthetic pathway diagram



PHYSIOPATHOLOGY

Steroid hormones control numerous processes, so the disorders charged by the biosynthesis of this class of substances can cause serious compromisions and troubles in the organism and therefore cause serious diseases. The following are some of these pathologies.

Corticol Hypersecretion/Cushing's syndrome

Cushing syndrome occurs when your body is exposed to high levels of the hormone cortisol for a long time. Cushing syndrome, sometimes called hypercortisolism, may be caused by the use of oral corticosteroid medication. The condition can also occur when your body makes too much cortisol on its own.

Too much cortisol can produce some of the hallmark signs of Cushing syndrome — a fatty hump between your shoulders, a rounded face, and pink or purple stretch marks on your skin. Cushing syndrome can also result in high blood pressure, bone loss and, on occasion, type 2 diabetes.

Treatments for Cushing syndrome can return your body's cortisol production to normal and noticeably improve your symptoms. The earlier treatment begins, the better your chances for recovery.

Adrenal cortical insufficiency

Addison's disease, also called adrenal insufficiency, is an uncommon disorder that occurs when your body doesn't produce enough of certain hormones. In Addison's disease, your adrenal glands, located just above your kidneys, produce too little cortisol and, often, too little aldosterone.

Addison's disease occurs in all age groups and both sexes, and can be life-threatening.

Treatment involves taking hormones to replace those that are missing.

In children, adrenal function is commonly impaired after long-term treatment which corticosteroids such as for asthma.

This is an important clinical problem requiring tests, although there is no agreement on which tests should be performed. Morning plasma cortisol, urine free cortisol and cortisol response to ACTH are often used.

Urine free cortisol is, however, a very poor test of adrenal suppression and measurements of total cortisol metabolites is much better.

Congenital adrenal hyperplasia (CAH) is a common cause of primary adrenal insufficiency that can be missed.

Newborn screening based on determinations of blood spot 17-hydroxyprogesterone concentrations should pick up the 21-hydroxylase deficiency.

Other rare causes of adrenal insufficiency include infarction or haemorrhage and adrenal hypoplasia.
Later adrenal destruction by metastases, sarcoidosis, histoplasmosis and amyloidosis may be found. Patients may have few or no symptoms of adrenal cortical insufficiency until they suffer a physical stress such as trauma, surgery or infection when they present with tiredness, weakness, lethargy, anorexia, nausea, weight loss, dizziness and hypoglycaemia.

Adrenal insufficiency is seen in disorders of cortisol production. Congenital adrenal Hyperplasia in children often presents with symptoms due to excess or deficiency of sex steroids.

Mineralcorticoid excess

Primary aldosteronism is a type of hormonal disorder that leads to high blood pressure.
Your adrenal glands produce a number of essential hormones.

One of these is aldosterone, which balances sodium and potassium in your blood.
In primary aldosteronism, your adrenal glands produce too much aldosterone, causing you to lose potassium and retain sodium. The excess sodium in turn holds on to water, increasing your blood volume and blood pressure.Diagnosis and treatment of primary aldosteronism are important because people with this form of high blood pressure have a higher risk of heart disease and stroke.
Also, the high blood pressure associated with primary aldosteronism may be curable.
Options for people with primary aldosteronism include medications, lifestyle modifications and surgery.
Common conditions causing the overproduction of aldosterone include:

- A benign growth in an adrenal gland (aldosterone-producing adenoma) - a condition also known as Conn's syndrome
- Overactivity of both adrenal glands (idiopathic hyperaldosteronism) In rare cases, primary aldosteronism may be caused by:
- A cancerous (malignant) growth of the outer layer (cortex) of the adrenal gland (adrenal cortical carcinoma) A rare type of primary aldosteronism called glucocorticoid-remediable aldosteronism that runs in families and causes high blood pressure in children and young adults

Adrenogenital syndromes

Congenital adrenal hyperplasia is the term that together with adreno-genital syndromes is commonly used to describe a group of autosomal recessive disorders due to the lack of one of the 5 enzymes involved in the synthesis of cortisol in the adrenal cortex.
The most common enzyme defect is that affecting the enzyme 21-hydroxylase and determines more than 90-95% of the congenital adrenal hyperplasias.

The synthesis of adrenal steroid hormones is a complex process that starting from cholesterol allows the synthesis of cortisol, aldosterone and sex hormones.
Cholesterol undergoes a series of enzymatic transformations in successive stages and each synthesis product represents the substrate for the synthesis of another compound and so on. It is therefore a real cascade of reactions in which different enzymes are involved.
The enzyme 21-hydroxylase (also called CYP21 or P450c21) belongs to the cytochrome P-450 category and, at the intracellular level, is localized in the endoplasmic reticulum. It catalyzes the conversion of 17-hydroxy progesterone (17OH-P) to 11-deoxycortisol, a precursor of cortisol, and of progesterone to deoxycorticosterone, a precursor of aldosterone. (Book a genetic test for this deficit).
In the event that there is a partial or total deficit in the functionality of the enzyme the patient who carries the defect is not able to efficiently synthesize an adequate amount of cortisol; therefore, the low cortisol values produced are not able to exert the negative feedback on the hypothalamus and on the pituitary; this causes an increased production of CRH and ACTH, which, in turn, lead to hyperstimulation of the cortico-adrenal cortex.Despite the hyperstimulation of the adrenal gland, however, the enzyme block remains the same and can not be overcome; indeed, just as a result of adrenal hyperstimulation there is an accumulation of those precursors of cortisol which, in the biosynthetic sequence of adrenal hormones, are placed upstream of the enzymatic defect. The accumulated precursors, therefore, not being able to continue along the way that should lead them towards the synthesis of cortisol, are diverted to other biosynthetic pathways that instead lead to the production of male sex hormones (testosterone).
This may result in an over-synthesis of sex hormones and, consequently, the appearance of signs of hyperandrogenism (virilization) which, in female infants, can manifest, already at birth, with genital ambiguity while in males it is clinically manifested only in a subsequent period, or in the first years of life, through a pathological increase in the speed of growth. At the same time, due to the lack of cortisol synthesis, an insufficient production of aldosterone can be added, which can cause, in the most serious cases, a concomitant hydroelectrolytic imbalance with hypovolaemia and shock.

Hypogonadism

Hypogonadism is a condition in which the testicles or ovaries do not produce enough hormones.
In both men and women hypogonadism can be primary, that is due to a malfunction of the testicles or ovaries, or central, that is due to a malfunction of the hypothalamus or pituitary gland.
Correcting this problem appropriately is essential to avoid complications such as infertility and, in the case of man, impotence, osteoporosis and weakness.
Fortunately, in many cases it is a condition that responds well to treatment.

What are the causes of hypogonadism?
The forms of primary hypogonadism may be caused by some autoimmune diseases, genetic diseases (such as Turner's syndrome and Klinefelter's syndrome) or development, infections, liver or kidney disorders, radiation exposure or surgery.

Central hypogonadism may be caused by bleeding, some drugs (including steroids and opioids), genetic problems (such as Kallmann's syndrome, a disturbus that makes men lose their sense of smell), infections, nutritional deficiencies, excess iron, radiation therapy, significant and at the same time rapid weight loss (eg associated with anorexia nervosa), surgery, trauma or tumors (such as craniopharyngeoma in children and prolactinoma in adults).

A very frequent cause in the male is the hypogonadism of the late-onset adult, ie the gradual reduction of testosterone levels from age 40 (unlike the woman who has a sudden blockage of ovarian function around the age of 50). If not appropriately diagnosed and treated, adult hypogonadism reduces longevity and quality of life.

What are the symptoms of hypogonadism?
In girls, hypogonadism is associated with the absence of menstruation and, in some cases, problems with breast development and height. During puberty can also be accompanied by hot flashes, hair loss and low libido. In boys, however, hypogonadism can increase chest volume, reduce beard and hair, lead to loss of muscle mass and cause sexual problems.
In the case of brain tumors these symptoms can include headaches and loss of vision and symptoms of other hormonal deficiencies (eg hyperthyroidism).

In general, the diagnosis of hypogonadism involves the assessment of FSH and LH hormone levels, estrogen (in women) and testosterone (in men).

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a hormonal disorder common among women of reproductive age. Women with PCOS may have infrequent or prolonged menstrual periods or excess male hormone (androgen) levels. The ovaries may develop numerous small collections of fluid (follicles) and fail to regularly release eggs.

Signs and symptoms of PCOS often develop around the time of the first menstrual period during puberty. Sometimes PCOS develops later, for example, in response to substantial weight gain.

Signs and symptoms of PCOS vary. A diagnosis of PCOS is made when you experience at least two of these signs:

- **Irregular periods.** Infrequent, irregular or prolonged menstrual cycles are the most common sign of PCOS. For example, you might have fewer than nine periods a year, more than 35 days between periods and abnormally heavy periods.
- **Excess androgen.** Elevated levels of male hormone may result in physical signs, such as excess facial and body hair (hirsutism), and occasionally severe acne and male-pattern baldness.
- **Polycystic ovaries.** Your ovaries might be enlarged and contain follicles that surround the eggs. As a result, the ovaries might fail to function regularly.

PCOS signs and symptoms are typically more severe if you're obese.

Reduction of bone density

Serum testosterone concentration decreases gradually with aging. Thus, the concept of late-onset hypogonadism has gained increasing attention in recent years. The symptoms of late-onset hypogonadism are easily recognizable and therefore also include the decrease in bone mineral density resulting in an increased risk of fracture. However, the greatest risk was observed in the conditions in which both testosterone and estradiol levels are reduced, suggesting a synergistic effect between the two hormones.

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TECHNICAL FEATURES LC 72410 Extractive Method

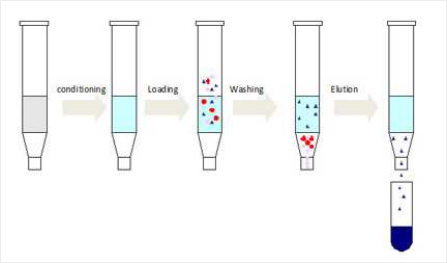
ANALYTE	Concentration used to calculate Reproducibility and Accuracy				
	Calibrator (ng/ml)				
	C Loq	C 1	C m	C 2	C s
17-OH-Progesterone	0,006	0,061	0,122	0,367	1,100
Androstenedione	0,009	0,089	0,178	0,533	1,600
DHEAS	6,790	67,901	135,802	407,407	1222,222
DHEA	0,044	0,444	0,889	2,667	8,000
Testosterone	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

ANALYTE	Reproducibility intra-serie (CV%)			Reproducibility inter-serie (CV %)		
	Concentration			Concentration		
	C Loq	Cm	Cs	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%

ANALYTICAL PROCEDURE LC72410

PROTEIN PRECIPITATION

SPE EXTRACTION



DRYING

INJECTION

TECHNICAL FEATURES LC 72310

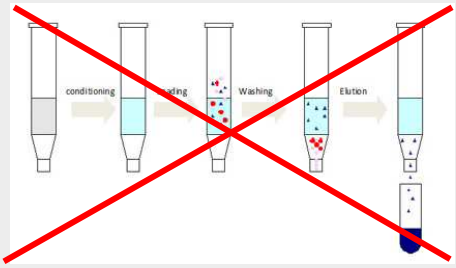
ANALYTE	Concentration used to calculate Reproducibility and Accuracy				
	Calibrator (ng/ml)				
	C Low	C1	Cm	C2	Cs
17-OH-Progesterone	0,061	0,122	0,367	1,10	5,50
Androstenedione	0,089	0,178	0,533	1,60	8,0
DHEAS	68,037	136,074	408,222	1.224,67	6.123,33
DHEA	0,448	0,896	2,689	8,067	40,333
Testosterone	0,133	0,266	0,799	2,398	11,99
Cortisol	4,481	8,963	26,889	80,667	103,333
Corticosterone	0,222	0,444	1,333	4,0	20,0
Aldosterone	0,028	0,055	0,166	0,499	2,493
11-Deoxycortisol	0,084	0,167	0,501	1,503	7,517
Dihydrotestosterone	0,069	0,139	0,416	1,247	6,233
Androsterone	0,222	0,445	1,335	4,004	20,02
Estrone	0,011	0,022	0,066	0,199	0,997
Beta-Estradiol	0,044	0,067	0,133	0,4	1,998
Pregnenolone	0,733	1,10	1,833	2,20	11,0
17-OH-Pregnenolone	0,733	1,10	1,833	2,20	11,0
Progesterone	0,407	0,815	2,444	7,333	36,667
11-Deoxycorticosterone	0,244	0,489	1,467	4,4	22,0
Cortisone	4,481	8,963	26,889	80,667	403,333
21-Deoxycortisol	0,022	0,045	0,134	0,403	2,017

ANALYTE	Reproducibility intra-serie (CV%)			Reproducibility inter-serie (CV %)		
	Concentration			Concentration		
	C Low	Cm	Cs	C Low	Cm	Cs
17-OH-Progesterone	3,22%	2,32%	1,29%	8,39%	5,84%	2,69%
Androstenedione	3,76%	4,22%	2,49%	12,08%	9,13%	8,22%
DHEAS	2,47%	2,10%	1,17%	6,16%	2,93%	3,27%
DHEA	3,03%	6,14%	4,22%	9,92%	10,71%	7,13%
Testosterone	5,20%	2,16%	3,25%	6,92%	8,90%	6,18%
Cortisol	1,24%	1,04%	1,26%	3,78%	3,73%	7,47%
Corticosterone	6,72%	3,13%	2,49%	8,25%	10,81%	10,45%
Aldosterone	5,35%	7,48%	2,75%	15,51%	9,02%	3,75%
11-Deoxycortisol	3,46%	1,99%	2,40%	13,58%	10,20%	6,51%
Dihydrotestosterone	4,62%	3,90%	4,21%	9,06%	10,51%	8,55%
Androsterone	4,77%	3,26%	4,01%	10,35%	4,10%	6,78%
Estrone	4,07%	10,46%	4,23%	11,04%	12,50%	10,44%
Beta-Estradiol	8,85%	7,25%	4,73%	15,81%	14,84%	7,23%
Pregnenolone	8,49%	4,74%	5,84%	9,06%	5,88%	12,12%
17-OH-Pregnenolone	7,85%	4,92%	4,83%	8,86%	9,55%	9,58%
Progesterone	3,82%	4,47%	5,22%	7,49%	5,21%	6,05%
11-Deoxycorticosterone	12,33%	1,95%	3,97%	11,84%	8,55%	8,96%
Cortisone	2,35%	2,87%	2,08%	7,01%	3,60%	3,92%
21-Deoxycortisol	5,82%	1,56%	1,50%	9,96%	3,46%	2,40%

ANALYTICAL PROCEDURE LC72310

PROTEIN PRECIPITATION

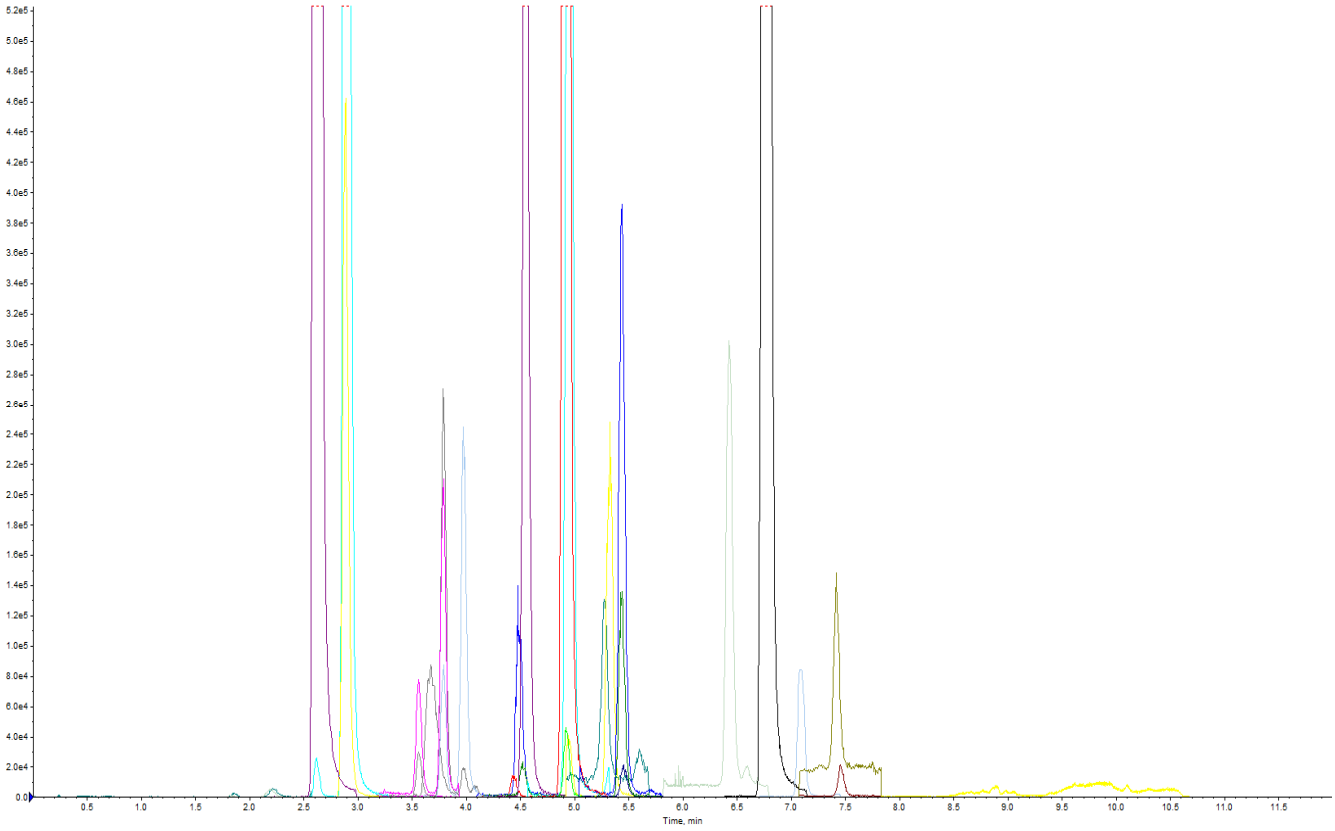
SPE EXTRACTION



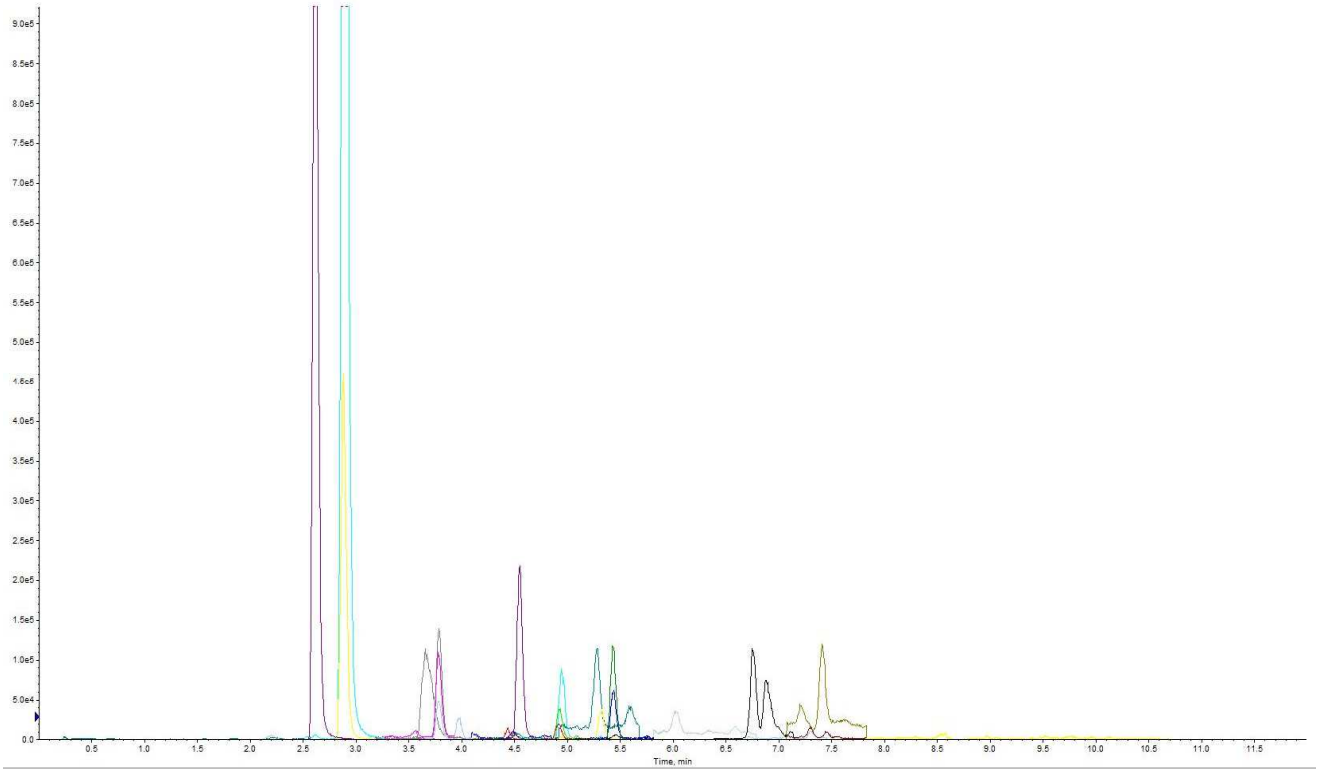
DRYING

INJECTION

CALIBRATOR CHROMATOGRAM



SAMPLE CHROMATOGRAM



TO SUMMARIZE….

The eureka kit

"Steroid hormones in serum/plasma by LC/MS"

enables

- To analyze**19** most relevant steroid hormones in serum/plasma in a **single** chromatographic run of 12 minutes
- To use a simple sample preparation procedure
- To reach high sensitivity compared to other methods
- To run the analysis on medium/high level LC/MS triple quadrupole

eureka kit

Produced by

eureka Lab Division