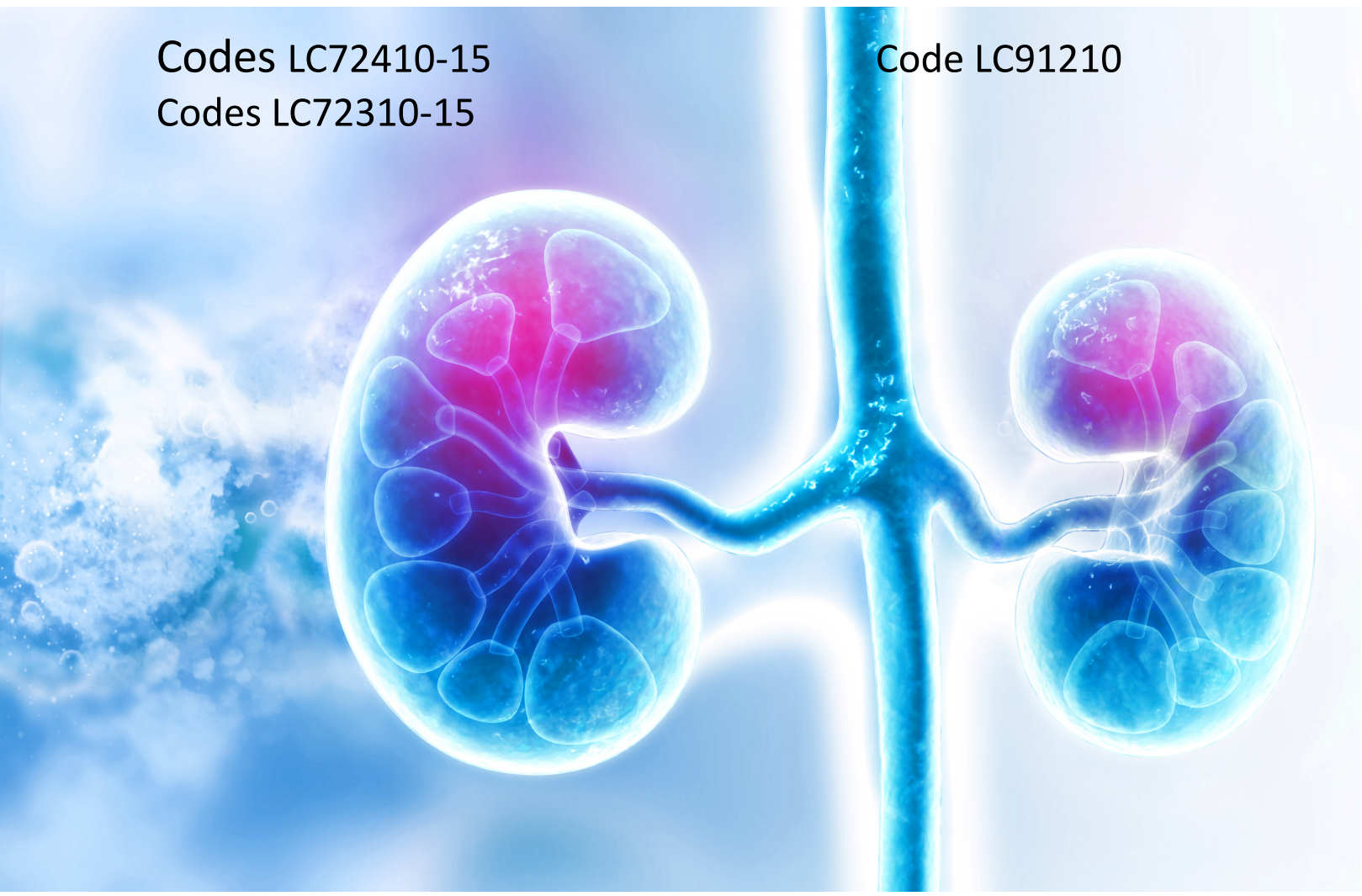
Steroid Hormones

in serum and in plasma by LC-MS/MS

in urine by LC-MS/MS



Codes LC72410-15
Codes LC72310-15

Code LC91210

eureka Lab Division

Special Clinical Chemistry

September 2021

BIOSYNTHESIS OF VARIOUS STEROID HORMONES

All these steroid hormones are synthesized from cholesterol through a common precursor steroid, pregnenolone, which is formed by the enzymatic cleavage of a 6-carbon side-chain of the 27-carbon cholesterol molecule, a reaction catalyzed by the cytochrome P450 side-chain cleavage enzyme (P450scc, CYP11A1) [1].

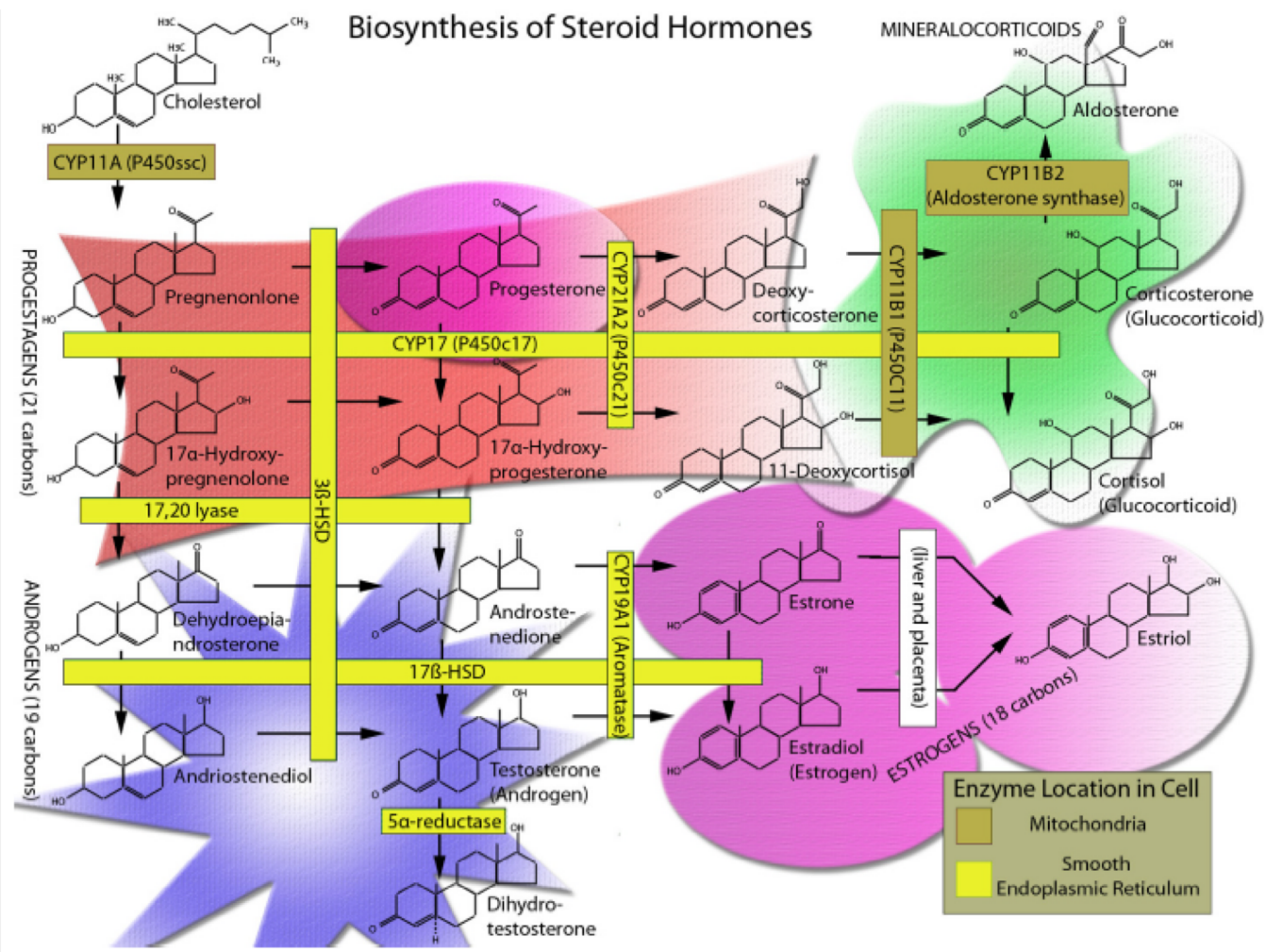


Fig. 1 Rapresentation of the pathway that underlies the biosynthesis of various steroid hormones. Taken from Hu *et al.* Nutrition & Metabolism 2010 [2]

MAJOR STEROIDS AND THEIR CLINICAL RELEVANCE

Steroidogenic Tissues	Trophic Hormone	Steroids(s)	Physiological Functions
Ovary			
Granulosa cells	FSH	Estradiol	Estrogen, a principal female sex steroid, required for growth and ovulation, responsible for secondary female sex characteristics, regulator of cardiovascular physiology, bone integrity and neuronal growth
Luteinized Granulosa/ luteal Cells	LH	Progesterone	A progestin, required for follicular growth and ovulation, responsible for changes associated with luteal phase of the menstrual cycle, essential for the establishment and maintenance of early pregnancy
Theca-interstitial Cells	LH	Testosterone Androstenedione	Androgens, precursors for estrogens, transported into granulosa cells, where they are converted into estardiol and other estrogens by aromatase (CYP19A1) enzyme
Testis			
Leydig cells	LH	Testosterone	The most prevalent male sex hormone (androgen); testosterone and its biologically active form, dihydrotestosterone (DHT) are necessary for normal spermatogenesis and development, responsible for secondary sex characteristics, responsible for increased muscle mass, sexual function, body hair and decreased risk of osteoporosis
Adrenal gland			
Z. glomerulosa Cells	ACTH, K^+ Angiotensin II	Aldosterone	The principal mineralocorticoid, raises blood pressure and fluid volume, enhances sodium reabsorption in the kidney, sweat gland, stomach and salivary gland and also enhances excretion of potassium and hydrogen ions from the kidney.
Z. glomerulosa Cells	ACTH	Cortisol	The dominant glucocorticoid in humans (in rodents, the major glucocorticoid is corticosterone), elevates blood pressure and Na^+ uptake, involved in stress adaptation, regulates carbohydrate, protein and lipid metabolism nearly opposite to that of insulin, influences inflammatory reactions and numerous effects on the immune system.
Z. reticularis Cells	ACTH POC-derived peptide Other factors	Androstenedione DHEA DHEA-sulfate	The function of adrenal androgens is not well understood, except that they contribute to the maintenance of secondary sex characteristics, may also be involved in the regulation of bone mineral density, muscle mass and may beneficial actions against type 2 diabetes and obesity
Placenta	Peptide growth Factors, cAMP	Progesterone Estrogens	Maintenance of pregnancy
Brain			
Neurons, Glial cells Purkinje cells	Neurotransmitters Neuropeptides	Progesterone Estradiol, DHEA, ALLO, THDOC	Neurosteroids are implicated in various processes such as proliferation, differentiation, activity and survival of nerve cells and a variety of neuronal functions including control and behavior, neuroendocrine and metabolic processes.

Fig. 2 The table describes major steroids and the biological processes they are involved into. Taken from Hu *et al.* Nutrition & Metabolism 2010 [2]

CLINICAL ABNORMALITIES OF STEROID HORMONES

Primary hyperaldosteronism (PA)

Some studies have revealed that 2% of patients with hypertension is affected by primary hyperaldosteronism. Such disease is a common curable form of arterial hypertension and it is mostly caused by an aldosterone-producing adenoma that is also known as Conn's syndrome [1]. High plasma levels of aldosterone are not the unique feature of PA. In fact, low levels of renin are observed as well. As consequence, the heart, arterial wall and kidney functions will also be impaired [3].

Adrenal insufficiency

Low production of cortisol can be due to the impaired acitivity of adrenal grands which leads to a clinical condition called primary adrenal insufficiency.

Alternatively, it can also be due to the deficiency of corticotropin-releasing hormone or corticotropin (secondary or tertiary adrenal insufficiency).

An important remark is that primary adrenal insufficiency (**Addison's disease**) is characterized by deficiency of cortisol and aldosterone. In secondary and tertiary adrenal insufficiency, Angiotensin II stimulates the adrenal grands to produce aldosterone. However, cortisol lacks as well [1].

One of the most common symptoms of Addison's disease is dark tanning and freckling of the skin. However, it also occurs in unexposed areas like the gums.

CLINICAL ABNORMALITIES OF STEROID HORMONES

Cushing's syndrome

Administration of high doses of glucocorticoids for long periods of time can lead to a disease called Cushing's syndrome (CS).

It has been defined as rare syndrome since it affects about 40–70 individuals per million every year, especially women whose age ranges between 30 and 50 years [1].

Cushing syndrome which is caused by endogenous alteration is not so frequent (up to 0.7–2.4 per million population per year).

It can be distinguished as adrenocorticotropic hormone (ACTH)-dependent (80–85% of cases) or ACTH-independent (15–20%) [4].

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia (CAH) is a hormone-related disease due to an enzymatic activity deficiency in the adrenal cortex. As consequence, cortisol production is reduced and production of corticotropin raises [1].

METHODS OF MEASUREMENT IN CLINICAL LABORATORIES

Status of the art

Many scientific works performed by using immunoassays shed light on their cross-reactivity as cause of lack of specificity.

The College of American Pathologists Proficiency Testing Program for the year 2002 (Y-survey) pointed out that the antibodies available on the market for the determination of steroids by using immunoassays show non-specificity [1].

For instance, urine contains cortisol in conjugated and free form as well as its metabolites. These may lead to cross reactivity in the immunoassays.

Variations between immunoassays and mass spectrometry looks more evident in patients with Cushing's syndrome as indicated in the Figure 3 [5].

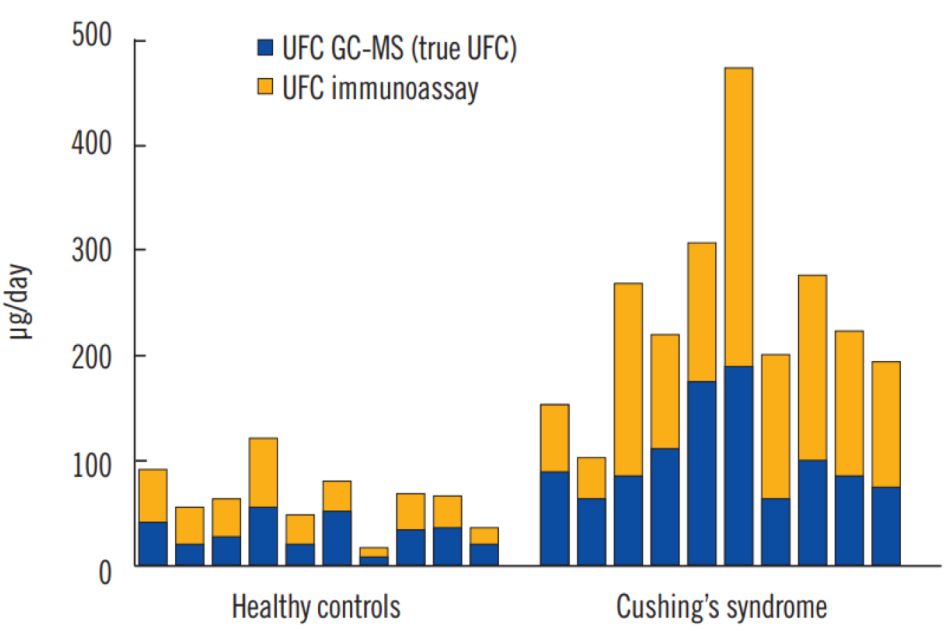


Fig. 3 Graphic showing the influence of immunoassays on the determination of urinary free cortisol (UFC) in healthy individuals vs people affected by **Cushing's syndrome** .

Taken from Casals, *et al.* Annals of Laboratories medicine-2020 [5].

Immunoassays present other disadvantages such as the lack of recovery correction, incapacity to discriminate steroids that are administrated from endogenous ones and high bias and variations among different producers [5].

TECHNIQUE OF CHOICE

Liquid chromatography-mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) overcome the limitations of immunoassays because provide specificity for the determination of molecules in a matrix and simplify the sample preparation avoiding the derivatization [1].

Moreover, LC/MS represents a valid structural characterization technique in the clinical laboratories for the determination of steroid hormones since it allows to standardize the analytical procedure in clinical diagnostics to reduce analytical intra- and interlaboratory variability. As result, LC/MS provides greater accuracy than immunoassays. Many studies show that MS is the most suited method for the quantification of UFC. In fact, when UFC was determined by immunoassay, the results were overestimated by two fold circa in comparison to those obtained by mass spectrometry and they were dependent on the presence of the metabolites that cross-react. In the light of this, MS confirms to be the most accurate and reliable method for the determination of steroid hormones [5].

Eureka Lab Division

Based on such scientific founding, Eureka Lab Division has developed diagnostic kits for routine determination of 19 steroid hormones in human **serum** and **plasma** by LC-MS/MS. Moreover, Eureka has also developed a kit to analyze steroid hormones in **urine**. This choice is due to the clinical need to evaluate hormone production independently on circadian rhythms. This allows to eliminate interpretative error linked to the time of sampling.

Eureka kits represent a valid tool to analyze and quantify steroid hormones with:

Accuracy, Reproducibility, Specificity

STEROID HORMONES IN SERUM/PLASMA BY LC-MS/MS

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

LC72310 (100 TESTS)
LC72315 (500 TESTS)

NON-EXTRACTIVE METHOD

PROTEIN PRECIPITATION + INTERNAL STANDARD

CENTRIFUGATION AND DILUTION

INJECTION INTO LC-MS/MS

TECHNICAL REQUIREMENTS (LC72310-15)

Minimum instrumental equipment required:

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

TECHNICAL ADVANTAGES (LC72310-15)

Analyte	Sensitivity (LLOD) ng/ml
17-OH-Progesterone	0.006
Androstenedione	0.005
DHEAS	5.0
DHEA	0.03
Testosterone	0.002
Cortisol	0.005
Corticosterone	0.012
Aldosterone	0.009
11-Deoxycortisol	0.006
Dihydrotestosterone	0.02
Androsterone	0.04
Estrone	0.003
β-Estradiol	0.01
Pregnenolone	0.02
17-OH-Pregnenolone	0.02
Progesterone	0.001
11-Deoxycorticosterone	0.006
Cortisone	0.08
21-Deoxycortisol	0.003

Analyte	Min. conc. analysable (LLOQ) ng/ml
17-OH-Progesterone	0.02
Androstenedione	0.016
DHEAS	15
DHEA	0.1
Testosterone	0.006
Cortisol	0.015
Corticosterone	0.036
Aldosterone	0.028
11-Deoxycortisol	0.02
Dihydrotestosterone	0.069
Androsterone	0.13
Estrone	0.011
β-Estradiol	0.03
Pregnenolone	0.06
17-OH-Pregnenolone	0.06
Progesterone	0.005
11-Deoxycorticosterone	0.012
Cortisone	0.269
21-Deoxycortisol	0.011

STEROID HORMONES IN SERUM/PLASMA BY LC-MS/MS

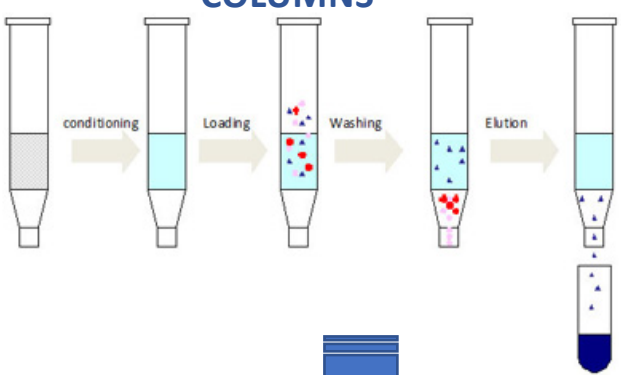
17-OH-Progesterone, 11-Deoxycorticosterone, 11-Deoxycortisol, 17-OH-Pregnenolone, 21-Deoxycortisol, Aldosterone, Androstenedione, Androsterone, Corticosterone, Cortisol, Cortisone, Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone sulfate (DHEAS), Dihydrotestosterone, Estradiol, Estrone, Pregnenolone,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

EXTRACTIVE METHOD

LC72410 (100 TESTS)
LC72415 (500 TESTS)

PROTEIN PRECIPITATION + INTERNAL STANDARD

SPE EXTRACTION ON CLEAN UP COLUMNS



CENTRIFUGATION AND DILUTION

INJECTION

TECHNICAL REQUIREMENTS (LC72410-15)

Minimum instrumental equipment required:

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

TECHNICAL ADVANTAGES (LC72410-15)

Analyte	Sensitivity (LLOD) ng/ml	Min conc analyzable (LLOQ) ng/ml
17-OH-Progesterone	0.002	0.006
Androstenedione	0.003	0.009
DHEAS	2.0	6.79
DHEA	0.015	0.044
Testosterone	0.002	0.007
Cortisol	0.15	0.444
Corticosterone	0.008	0.022
Aldosterone	0.005	0.014
11-Deoxycortisol	0.003	0.008
Dihydrotestosterone	0.002	0.007
Androsterone	0.04	0.111
Estrone	0.002	0.006
Beta-Estradiol	0.005	0.011
Pregnenolone	0.008	0.02
17-OH-Pregnenolone	0.02	0.061
Progesterone	0.007	0.02
11-Deoxycorticosterone	0.005	0.012
Cortisone	0.1	0.22
21-Deoxycortisol	0.005	0.01

INTERNAL STANDARDS DEUTERATED SOLUTION (LC72310-15 and LC72410-15)

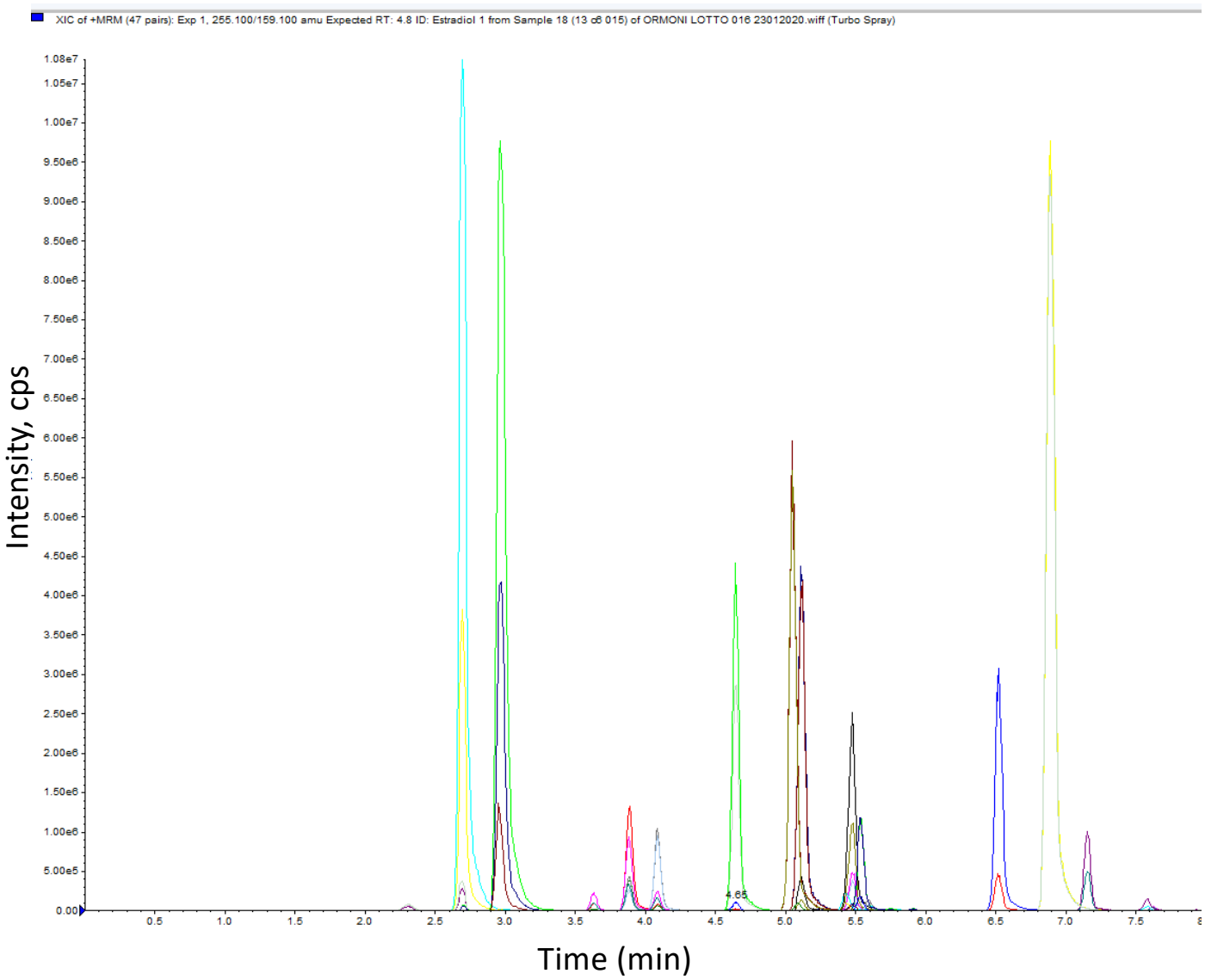
The solution contains Aldosterone-D7, Cortisol-D4, Testosterone-D3, β-Estradiol-D5, Pregnenolone-D4, DHEA-D6, DHEAS-D5, 17-OH-Progesterone $^{13}C3$. To be stored at .

Ionization	Hormones	Internal Standard
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-$^{13}C3$
Positive	CORTISONE	Cortisol-D4
Positive	21-DEOXYCORTISOL	Cortisol-D4

In case of low sensitivity for the DHEAS and/or ALDOSTERONE

Ionization	Hormones	Internal Standard
Negative	DHEAS	DHEAS-D5
Negative	ALDOSTERONE	Aldosterone-D7

REFERENCE CHROMATOGRAM



Calibrator in plasma for steroid hormones (LC72310).

According to an increasing retention time: Aldosterone, Cortisone, Cortisol, 21 Deoxycortisol, DHEAS, Corticosterone, 11-Deoxycortisol, β-estradiol, Estrone, Androstenedione, 11 Deoxycorticosterone, Testosterone, DHEA, 17-OH Progesterone, 17-OH Pregnenolone, Dihydrotestosterone, Progesterone, Androsterone, Pregnenolone.

STEROID HORMONES IN URINE BY LC-MS/MS

(Aldosterone, Cortisol, Cortisone, Estrone, β-estradiol)

liquid-liquid extraction

LC91210 (100 TEST)

Add **Reagent A** (Deproteinization Solution) and **Reagent B** (Deuterated IS Solution) to calibrator, sample and control.

Vortex and centrifuge

Collect the supernatant and add Reagent C (Extraction Solution) to it.

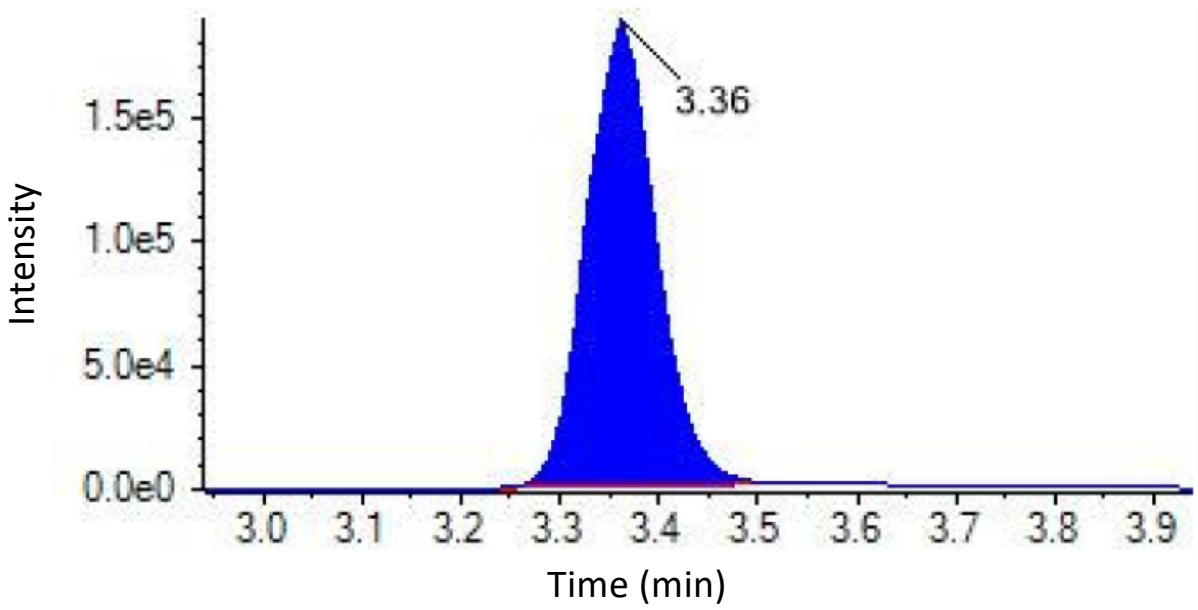
Vortex and centrifuge

Take 800 µl of the lower phase and dispense it into eppendorf tubes

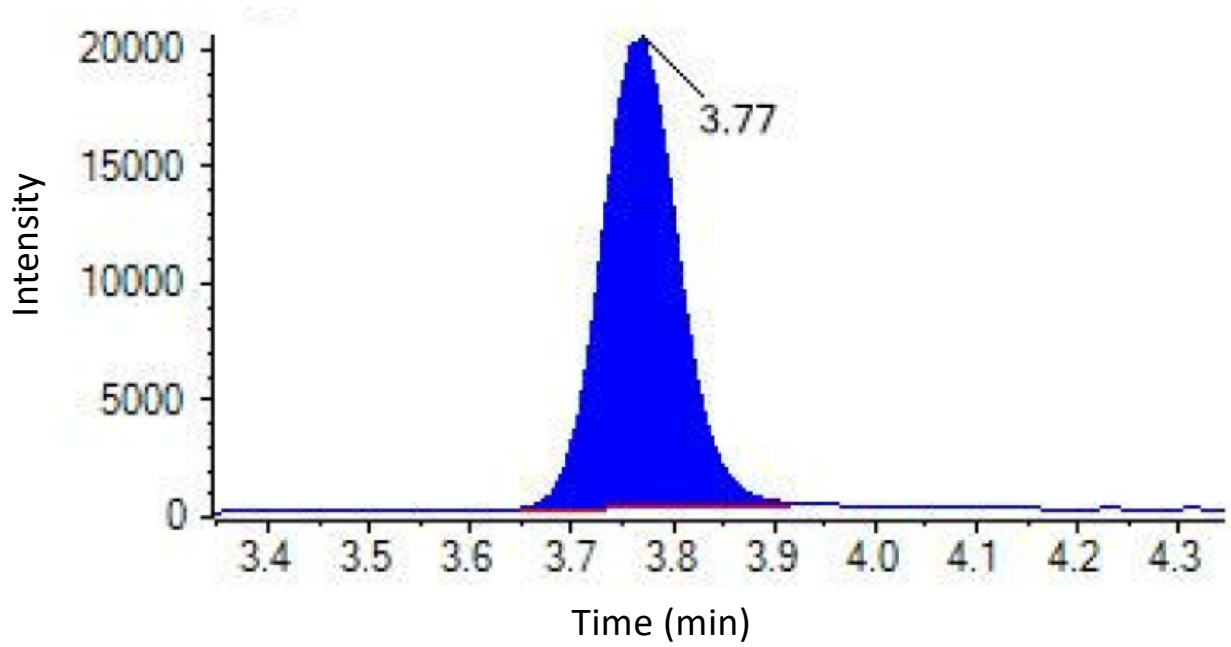
Add Reagent D and Reagent E in sequence

INJECTION

REFERENCE CHROMATOGRAM



Control in urine- Level 3
RT 3.36 min Cortisone 300 pg/ml
trans. 361.2→ 163.0



Calibrator in urine (Cortisol-D4)
RT 3.77 min

THE ADVANTAGES OF THE EUREKA KITS

Eureka kits for the determination of steroid hormones in serum/plasma or urine applied to the LC-MS/MS

guarantee

- ✓ Straightforward and Quick Sample Preparation
- ✓ Robust, Fast and Reliable CE-IVD methods
- ✓ Multiparametric run of 19 steroid hormones, with option to be Automatized
- ✓ 1 analytical column
- ✓ High sensitivity and great accuracy
- ✓ Method with or w/o SPE extraction
- ✓ Single run of only 12 minutes
- ✓ Time and Cost Optimization
- ✓ Stability for 3 years
- ✓ Post sales service and training courses

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