

## Background

The response to ACTH Test (Synacthen®) is a very useful for the screening of steroidogenesis enzymatic deficiency. With the development of steroid quantification by LC-MS/MS more specific than most of immunoassays, the determination of reference value is required at basal and under stimulation time.

## Objectives

Determination of references values for 6 steroids in serum by LC-MS/MS method and after Synacthen Test:

- 21-deoxycortisol (21DF),
- 11-deoxycortisol (11OH),
- Deoxycorticosterone (DOC),
- Corticosterone (Cortico),
- delta4 androstenedione (Delta4),
- 17-hydroxyprogesterone (17OHP)

using the same extraction and chromatography

## Materials and Methods

### Extraction of the Samples and calibration curve

-according to SLE method after addition of deuterium internal standard.

**Steroid quantification** : HPLC1290® Agilent Technology + mass spectrometer triple quadrupole 6460 ® Agilent technology.

This method was validated according to the Norm (linear response, CV less than 10% for the repeatability, less than 15% for the reproducibility).

The limit of Quantification (nmol/L)

21-DF	Cortico	11-OH	Delta 4	DOC	OHP
0.125	0.125	0.135	0.125	0.125	0.125

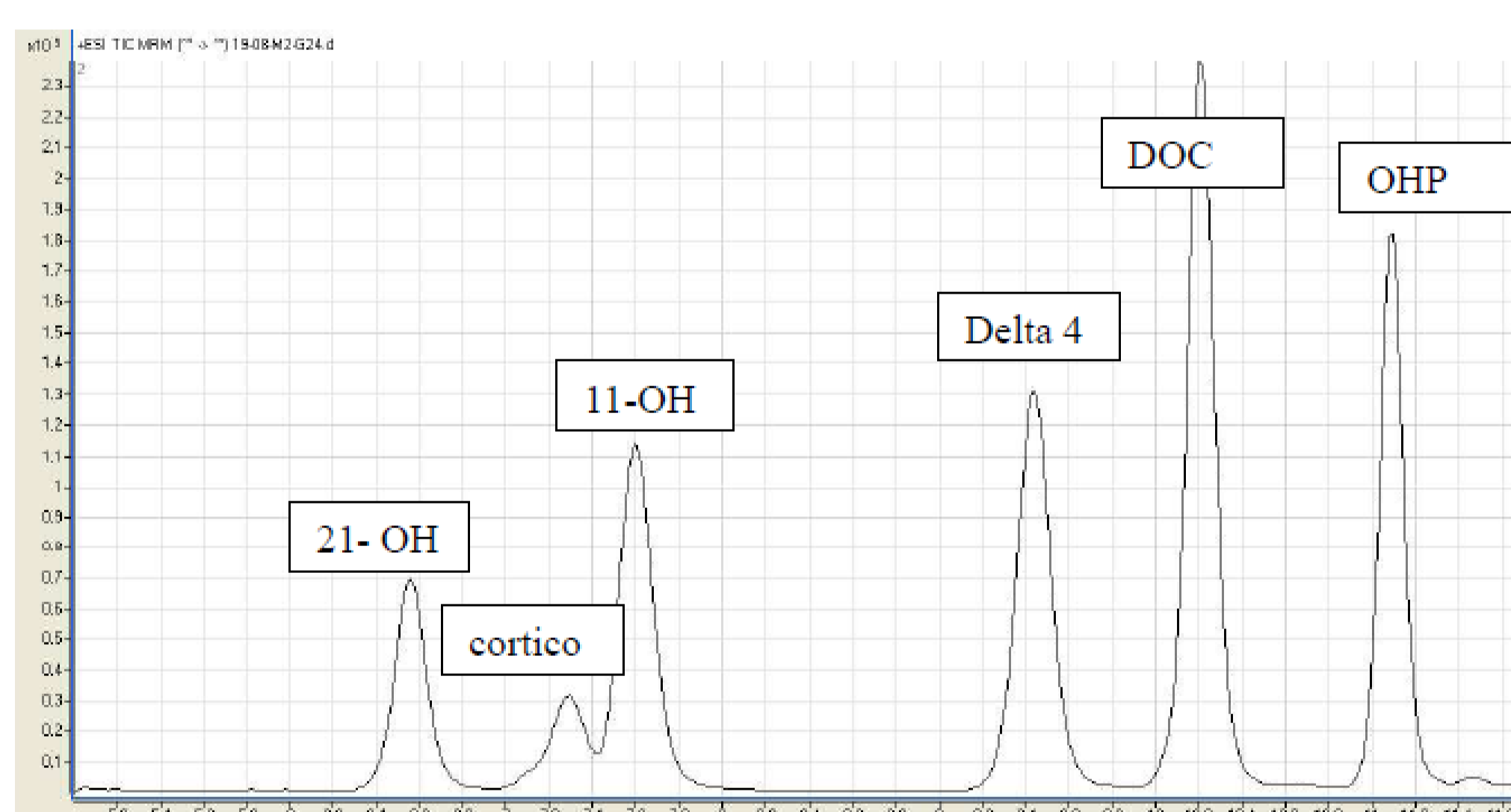
**Reference values** were performed for these 6 steroids after Synacthen® (T0, T60) in a cohort of patients previously studied in radioimmunoassay for 17OHP and 21DF and genetic status (normal, heterozygous, non- classical form) for mutation of *CYP21A2* confirmed by sequencing.

In the normal group (normal response to Synacthen® determined in radioimmunoassay), steroids were quantified at T0 and T60 min (peak of stimulation for 17OHP and 21DF) for 55 patients.

## Results

Normal values of 6 steroids after **Synacthen® (T0, T60)** for 55 patients

	21 DF nmol/L	Cortico nmol/L	11-OH nmol/L	Delta 4 nmol/L	DOC nmol/L	OHP nmol/L
<b>T0</b>						
médiane	<0.130	6.833	0.6	2.79	0.125	1.43
1-3° quartile (min-max)	(0.13-0.80)	(3.88-10.62)	(0.4-1.72)	(1.26-4.43)	(0.13-0.14)	(0.87-2.79)
		(0.80-59.45)	(0.13-12.4)	(0.436-8.931)	(0.13-0.984)	(0.268-6.34)
<b>T60</b>						
médiane	0.422	52.82	2.84	3.80	0.374	3.74
1-3° quartile (min-max)	(0.13-2.02)	(46.4-68.32)	(3.02-4.16)	(2.24-5.69)	(0.26-0.76)	(3-6.46)
		(16.2-97.3)	(1.06-29.4)	(0.57-10.9)	(0.123-1.40)	(1.32-8.85)



Chromatography method separated the followed steroids

**After ACTH test, 21DF (<0.6nmol/L) exclude heterozygous** for a mutation of the *CYP21A2* gene. All non classical form for the 21-hydroxylase deficiency have a value of **17OHP under ACTH test superior to 40 nmol/L** confirming our previous studies (Tardy et al. Hormon Research 2005;64,p41).

## Conclusions

An evaluation of normal values for these 6 steroids may be useful to diagnose affected parents and prevent risk of Congenital Adrenal Hyperplasia for children. The utilization of LC-MS/MS method showed a reliable, sensitive and specific method to detect disorders of steroid biosynthesis when multiple précurseurs were high.