STEROID PANEL LC-MS BY TECAN.

Exploring some essential issue for sexual steroids determination.

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AIM OF THE STUDY.

Tecan offers the "Steroid Panel LC-MS" kit for the simultaneous determination of 17 steroids and dexamethasone in serum. Interesting features of this kit are the limited volume of serum required (250 µL), the automated and simple sample preparation, and the high sensitivity in particular for sexual steroids testosterone and estradiol with the possibility to determine also estrone. In our laboratory at Andrology, Female Endocrinology and Gender Transition Unit of Azienda Universitaria Ospedaliera Careggi (Florence, Italy), we presently employ an LC-MS/MS Lab Developed Test (LDT) for steroid hormones, which is intended to be substituted with a commercially available kit in the forthcoming time. The essential requirements for sexual hormones in which we are interested are high sensitivity and accuracy for testosterone at low concentrations (females and children) and for estradiol at low concentrations (men and postmenopausal women) together with the possibility to quantitate estrone (adults). The Steroid Panel LC-MS kit from Tecan could have suitable performance. Still, before adopting this new method it is imperative to ascertain that the crucial analytical concerns mentioned above can be satisfactorily addressed.

MATERIAL AND METHODS.

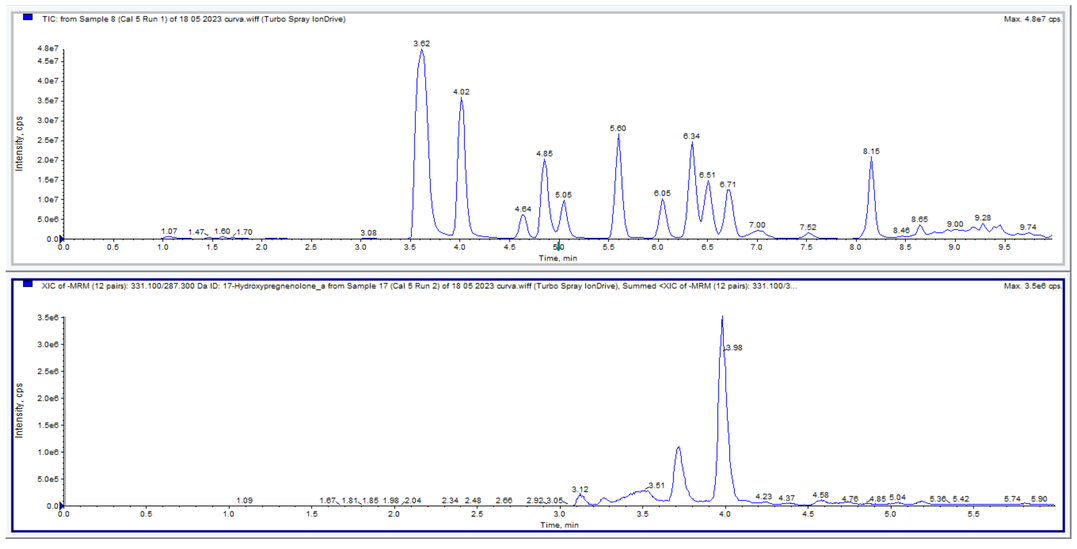
The Steroid Panel LC-MS kit from Tecan (CatNo. 30191875) including the quantitative analysis of 17 steroids and dexamethasone was used. Calibration range and retention time for all the included steroids are reported in table 1.

Total Ion chromatograms of the two runs are shown in figure 1. After sample preparation, 20 µL of the extract was injected into the system: Liquid Chromatography (LC, 1260 Infinity II LC System, Agilent, Santa Clara, USA) coupled with Mass Spectrometer (MS, Triple Quadrupole 6500 System, Sciex, Framingham, USA). The data were acquired and evaluated using Sciex Software (Analyst and MassQuant).

Table 1. Calibration range of the measured analytes.

Analyte	Retention time [min]	CalA [ng/mL]	CalF [ng/mL]
11-deoxycorticosterone	6.02	0.04	5.13
11-deoxycortisol	5.03	0.10	12.8
17-hydroxyprogesterone	6.69	0.10	12.8
21-deoxycortisol	4.83	0.10	12.8
aldosterone	3.07	0.10	4.59
androstenedione	5.58	0.10	12.8
corticosterone	4.84	0.30	38.5
cortisol	4.00	2.00	257
cortisone	3.63	0.50	64.1
dexamethasone	4.89	0.50	64.1
dehydroepiandrosterone	6.50	1.00	45.9
dehydroepiandrosterone sulfate	3.62	50.0	6412
dihydrotestosterone	7.51	0.15	1.57
progesterone	8.14	0.10	12.8
testosterone	6.32	0.04	13.4
17-hydroxypregnenolone	3.98	0.30	38.5
estradiol	3.72	0.03	3.9
estrone	3.70	0.01	1.3

Figure 1: Total Ion Chromatograms



TESTOSTERONE ACCURACY.

Accurate and precise measurement of testosterone at low concentration is essential for the management of various aspects of women's health, including hormonal balance, reproductive health, the diagnosis and management of polycystic ovary syndrome (PCOS), bone health, cardiovascular risk assessment, sexual function, and the maintenance of fair competition in sports.

Therefore, the accuracy of testosterone measurements with the Steroid Panel LC-MS kit was checked by analyzing third-party internal quality controls (TP) in triplicate (table 2) and an external quality control (UK-NEQAS steroid hormones for male and female testosterone, see table 3).

Table 2. Testosterone precision and accuracy based on internal quality controls run in triplicate in three different days.

Testosterone	c (measured) [nmol/L]	c (target value) [nmol/L]	Accuracy [%]	CV% interassay
Control 1 TECAN	0.87	0.94	92.8	12.4
Control 2 TECAN	31.51	30.57	103.1	7.8
Control 1 TP	0.73	0.72	101.5	4.9
Control 2 TP	5.08	5.27	96.4	6.1

Table 3. Testosterone accuracy based on external standard material from UK-NEQAS.

Testosterone	c (measured) [nmol/L]	c (target value) [nmol/L]	Accuracy [%]
VEQ UK-NEQAS 1A Female	0.88	0.88	100.2
VEQ UK-NEQAS 1B Female	2.22	2.43	91.4
VEQ UK-NEQAS 1C Female	0.96	1.07	89.5
VEQ UK-NEQAS 2A Female	1.36	1.39	98.1
VEQ UK-NEQAS 2B Female	0.59	0.62	95.5
VEQ UK-NEQAS 2C Female	2.31	2.33	99.2
VEQ UK-NEQAS 1A Male	10.04	11.0	91.2
VEQ UK-NEQAS 1B Male	10.91	11.8	92.5
VEQ UK-NEQAS 1C Male	18.24	22.2	82.2
VEQ UK-NEQAS 2A Male	8.02	9.1	88.1
VEQ UK-NEQAS 2B Male	17.1	19.8	86.4
VEQ UK-NEQAS 2C Male	19.07	22.0	86.7

ESTRADIOL ACCURACY.

The precise and accurate quantification of low concentrations of estradiol is imperative for the comprehensive management of diverse facets of post-menopausal women's health. Additionally, it is increasingly garnering attention in relation to several aspects of men's health, encompassing hormonal equilibrium and reproductive well-being. Therefore, the accuracy of estradiol measurements with the Steroid Panel LC-MS kit was checked by analyzing third-party internal quality controls (TP) in triplicate (table 4).

Table 4. Estradiol precision and accuracy based on internal quality controls run in triplicate in three different days.

Estradiol	c (measured) [nmol/L]	c (target value) [nmol/L]	Accuracy [%]	CV% interassay
Control 1 TECAN	0.57	0.52	109.2	5.9
Control 2 TECAN	9.67	9.81	98.6	5.9
Control 1 TP	0.35	0.29	121.9	7.1
Control 2 TP	1.54	1.49	103.2	3.7

SUMMARY OF GENERAL PERFORMANCE.

At a first use, this kit shows

- Good overall reproducibility for testosterone and estradiol (sample recovery and chromatographic performances)
- A simple, reliable and automated sample preparation protocol
- Claimed stability in IFU is fitting (perfectly) to our needs
- Precision and accuracy of all the other steroids need be evalued to have a complete view of the performance of this kit.

CONCLUSION.

The performance of the Steroid Panel LC-MS met our first goals, especially regarding accuracy for the two main sexual steroids. For validation purposes, precision and accuracy will be evaluated on all the other steroids. Moreover, LoD, LoQ and linearity will be assessed and a study in order to verify the comparability with the existing LDT will be performed in the future on a large number of real samples.

On our homepage you will find further information on the Steroid Panel LC-MS, including three interesting videos.

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