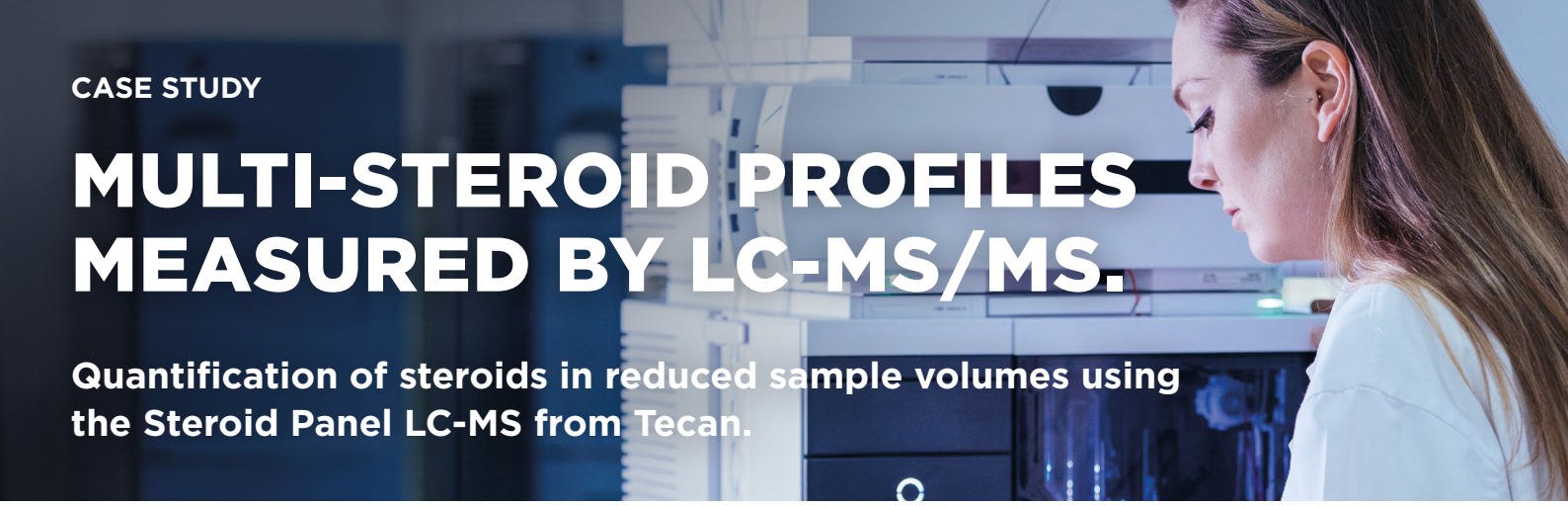

CASE STUDY

MULTI-STEROID PROFILES MEASURED BY LC-MS/MS.

Quantification of steroids in reduced sample volumes using the Steroid Panel LC-MS from Tecan.

Introduction.

Innovation in laboratories is ongoing at a rapid pace. Rethinking strategies is essential to staying competitive.[1] It is important to consider updating outdated methods with newly commercially available test kits and the latest advances in analytical equipment.[2] This ongoing trend of obtaining more information from less sample volume has resulted in Mass spectrometry taking a leading role in many laboratories[5,6]. This Case study describes the combination of the Steroid Panel LC-MS kit from Tecan and state-of-the-art UHPLC-MS[4] equipment to overcome the analytical challenge of quantifying steroids in small sample volumes.

Labor Berlin – Charité Vivantes GmbH has modified and tested the standardized Steroid Panel LC-MS kit for use with a reduced sample volume of only 100 µl. This approach enables access to a broader range of sample sources where only a small sample material is available. Simultaneously screening a full steroid profile also provides valuable information to characterize a complete picture of the status of the sample.[3]

Materials.

- Steroid Panel LC-MS (Tecan, 30220266)
- Stripped serum (In.Vent Diagnostica)
- 1290 Infinity II LC System (Agilent)
- QTRAP® 6500 LC-MS/MS System (AB SCIEX)
- MultiQuant™ Software (AB SCIEX)
- Steroid Tuning Mix 1 – 4 (Tecan, 30227628; 30227629; 30227630; 30227631)
- HPLC Column XBridge BEH C8 Column, 130 Å, 3.5 µm, 2.1 mm X 100 mm (Waters 186003048)

Methods.

The Steroid Panel LC-MS includes all the standards, reagents and consumables necessary to perform this assay. Only methanol and ultra-pure water have to be added. Lyophilized calibration standards in human serum are provided for reliability and stability. A modified solid phase extraction (SPE) method was used to extract 18 analytes from 100 µL serum samples. The extract was subsequently dissolved in 50 µL of reconstitution solution, and 20 µL of this mixture was injected into a 1290 Infinity II LC System coupled to a QTRAP 6500 LC-MS/MS System for analysis (Figure 1). LC-MS parameters and transitions were adapted from the Steroid Panel LC-MS instructions – and validated by Labor Berlin – for optimal performance on the QTRAP 6500 LC-MS/MS System. The IonDrive™ Turbo V source was operated in polarity switching mode, allowing simultaneous measurement of positive and negative MRM transitions in a single 10-minute run. The data was evaluated using MultiQuant Software, applying linear regression with 1/x weighting. The method was validated to ensure conformity with internal criteria, and steroid profiling was conducted on different samples. The standardized SPE protocol, as described in the Tecan Instruction for use, was modified in terms of a sample reduction from 250 µL to only 100 µL, with further reconstitution in only 50 µL, following one LC-MS/MS 20 µL injection.

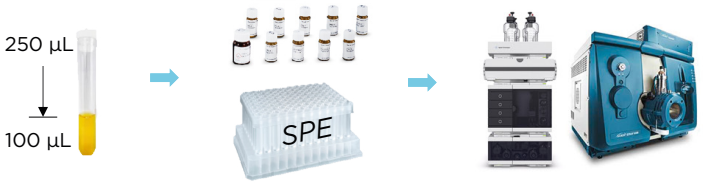

Figure 1: Graphical representation of the three components needed to perform a successful assay.

Results.

Validation.

Testing was performed according to internal criteria based on *Accuracy (trueness and precision) of measurement methods and results* (ISO 5725-4:2020) and *Chemical analysis – decision limit, detection limit and determination limit under repeatability conditions – terms, methods, evaluation* (DIN 32645:2008-11). The modified SPE protocol was run over a series of eight days. Further experiments were performed to determine the lower limit of quantification (LLOQ), recovery and matrix effects (Table 1). Recovery and matrix studies were performed by spiking stripped serum samples with

replicates (n = 6) of low and high concentrations of each analyte, without internal standard correction (Equations 1 and 2). The linear correlation coefficient of calibration, R^2, was above 0.99, and quality control, intra and inter-day accuracy and precision variance over six days were below 15% for all analytes. The LLOQ for each analyte was below the lowest standard kit calibrator, Calibrator A. Overall, the recovery and matrix effects were within the acceptable ranges – between 50% and 90%, and 80% and 120%, respectively (Table 1).

Table 1: Data representing the calibration range, LLOQ, recovery and matrix effects for each analyte. *Results may vary for non-stripped serum samples.

Nr.	Analyte name	Calibration range [ng/mL]	LLOQ [ng/mL]	Re-covery [%]*	Matrix effect [%]*
1	11-Deoxycorticosterone	0.04 – 5.13	0.03	78.41	106.56
2	11-Deoxycortisol	0.10 – 12.82	0.02	79.77	114.10
3	17-Hydroxypregnenolone	0.30– 38.47	0.09	70.47	97.53
4	17-Hydroxyprogesterone	0.10 – 12.82	0.03	76.75	113.02
5	21-Deoxycortisol	0.10 – 12.82	0.02	85.91	81.93
6	Aldosterone	0.10 – 4.59	0.01	62.95	87.30
7	Androstenedione	0.10 – 12.82	0.04	81.98	113.29
8	Corticosterone	0.30 – 38.47	0.08	80.91	93.92
9	Cortisol	2.00 – 256.5	0.14	84.60	107.73
10	Cortisone	0.50 – 64.1	0.33	75.03	83.16
11	Dehydroepiandrosterone	1.00 – 45.9	0.72	51.57	99.92
12	Dehydroepiandrosterone Sulfate	50.00 – 6412	13.59	67.17	99.92
13	Dexamethasone	0.50 – 64.12	0.45	81.42	95.75
14	Dihydrotestosterone	0.15 – 1.57	0.10	72.79	106.88
15	Estradiol	0.03 – 3.85	0.01	83.00	110.30
16	Estrone	0.01 – 1.28	0.01	78.01	107.85
17	Progesterone	0.10 – 12.82	0.03	63.05	99.56
18	Testosterone	0.04 – 13.42	0.02	77.47	100.04

$$1)\ \text{Matrix effect} = \frac{\text{area(spiked extract)}}{\text{area(standard)}} * 100$$

$$2)\ \text{Recovery} = \frac{\text{area(spiked sample)}}{\text{area(spiked extract)}} * 100$$

Equations: Calculation of **1)** matrix effect and **2)** recovery.

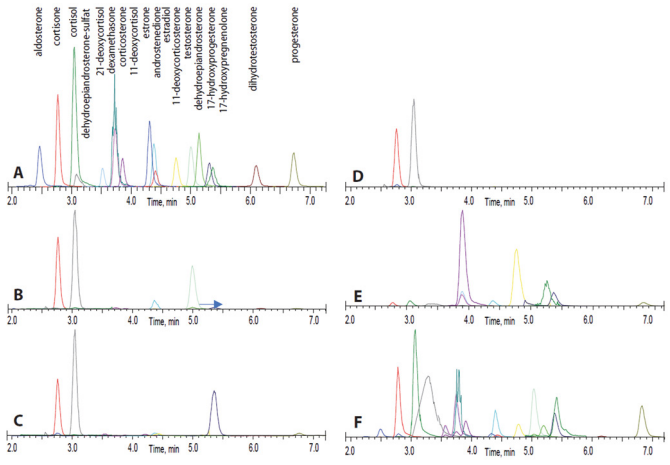

Figure 2: Example steroid Chromatograms. **A)** System stability check; **B-F)** hormone measurements of different samples show distinct hormone accumulation; Example: C shows an increase in 17-hydroxyprogesterone; **Chromatograms have been enhanced for comprehensive visualization.

Conclusions.

Combining the Steroid Panel LC-MS kit with state-of-the-art technology enabled the sample volume to be reduced from 250 µL to 100 µL while still obtaining excellent results. Reducing the sample volume enables the screening of a broader range of samples. The use of a commercially available kit also ensures consistent quality in every measurement, helping to avoid deviations and, therefore, saving costs and time in the long run. Keeping up with LCMS and its technological advantage is essential for labs to stay competitive in a fast-moving scientific world.

Abbreviations.

ISO	International Organization for Standardization
LLOQ	Lower limit of quantification
SPE	Solid phase extraction
UHPLC-MS	Ultra High-Performance Liquid Chromatography – Mass Spectrometry

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