Labor Berlin

COMPREHENSIVE MULTI-STEROID PROFILING FROM 100 µL SAMPLE VOLUMES USING AN LC-MS KIT.

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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 Rethinking strategies is essential to staying competitive.[1] It is important to consider updating outdated methods with newly commercially available test kits and the latest advances in analytical equipment.[2] The ongoing trend of obtaining more information from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 equipment to overcome the analytical challenge of quantifying steroids in small sample volumes.[4] Labor Berlin – Charite Vivantes GmbH has modified and tested the standardized Steroid Panel LC-MS kit for use with a reduced sample volume of only 100 µL.

EFFICIENCY AND ADAPTATION.

Simultaneously screening a full steroid profile provides valuable information to characterize a complete picture of the status of the sample.[3] Laboratories strive to increase efficiency and adapt to the growing demand for high-throughput analysis. There is a clear trend toward obtaining more data from ever smaller sample volumes, which is especially important in situations where only small amounts of sample material are available. Mass spectrometry has become the method of choice for multi-analyte steroid profiling due to its high sensitivity, specificity, and multiplexing capabilities. In this study, we present the application of the Tecan Steroid Panel LC-MS kit (Cat.no. 30220266) in combination with UHPLC-MS/MS instrumentation, enabling the simultaneous quantification of 18 relevant steroids from as little as 100 µL of serum. This workflow demonstrates how advanced analytical technologies can be leveraged to maximize data output while minimizing sample consumption. The ability to analyze comprehensive steroid panels from limited material opens up new possibilities for research, quality control, and method development in various laboratory settings. Furthermore, the streamlined process supports laboratories in meeting the increasing expectations for robust, reproducible, and efficient analytical solutions, ensuring that even challenging sample types can be processed with confidence and precision.

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

Key workflow adaptation were implemented to improve sensitivity, throughput, and data quality. The main differences between the original and adapted protocols are summarized below (Table 1).

Table 1: Key Workflow adaptations.

Parameter	Original Protocol	Adapted Protocol
Sample Volume	250 µL	100 µL
Reconstitution Volume	100 µL	50 µL
LC-MS/MS System	QTRAP 5500 LC-MS/MS	QTRAP 6500 LC-MS/MS
Polarity Mode	Polarity switching (run 1) and run 2 (ESI-)	Polarity switching (only one run)
Run Time	10 min (run1) and 6 min (run 2)	10 min

RESULTS.

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 2). 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. The linear correlation coefficient of calibration, R^2, was above 0.99, and quality control, intra- and interday 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 2).

Table 2: Validation data representing the calibration range, LLOQ, recovery and matrix effects for each analyte.

No.	Analyte name	Calibration range [ng/mL]	LLOQ [ng/mL]	Recovery [%]*	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

*Results may vary for non-stripped serum samples.

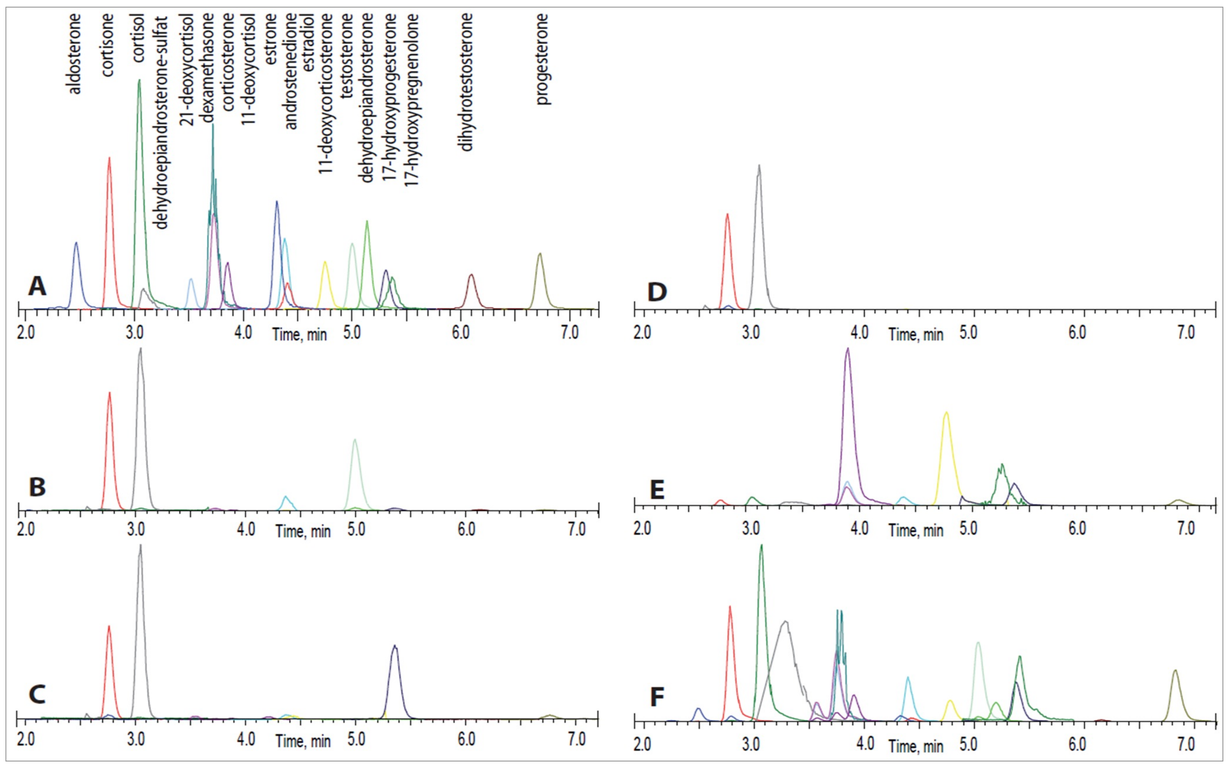

Figure 2: Example steroid Chromatograms. A) System stability check; B-F) hormone measurements of different samples show distinct hormone accumulation; Example: C shows an increase in 17-hydroxyprogesterone; *Chromatograms have been enhanced for comprehensive visualization.

CONCLUSION.

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.

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

Reference

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Disclaimer: The combined use of reagents, process script and instrument has to be validated individually on site by each laboratory.

TECAN.