ANALYSIS OF 14 STEROID HORMONES IN HUMAN SERUM USING STEROID PANEL LC-MS ON THE KNAUER EUROSPHER II HPLC COLUMN.

Juliane Kramer[1], Giorgia Greco[1], Ayham Al Ahmad[2], Maike Arndt[2]

(1) KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin, Germany
(2) Tecan, IBL International, Flughafenstrasse 52A, 22335 Hamburg, Germany

AIM OF THE STUDY.

In the laboratory routine, continuous availability of supplies is essential. Consequently, in LC-MS analysis, it is crucial that a method is not only reliant on a specific chromatographic column to avoid creating dependencies. Therefore, this study aims to evaluate the extent to which the Steroid Panel LC-MS* (Cat# 30220266) from Tecan, originally developed on a C8 column (3.5 µm, 2.1 x 100 mm, Cat# 30215928), can also be utilized using a KNAUER Eurospher II 100-3 C8 100 x 2 mm HPLC column.
The impact of this transition on performance, particularly in terms of chromatographic separation, is presented in the following.

*For research use only. Not for use in diagnostic procedures.

Figure 1: KNAUER Eurospher II column

MATERIALS AND METHODS.

The Steroid Panel LC-MS is based on Solid Phase Extraction (SPE). After the clean-up, the extract is dried by nitrogen flow and afterwards analyzed by LC-MS. To guarantee baseline separation, two separate chromatographic runs are used. For the application on the KNAUER column, the LC method of the Steroid Panel LC-MS was solely adapted with regard to the flow rate in run 1: The flow rate was increased from 0.35 mL/min to 0.50 mL/min. The other parameters – for the LC, the mass spectrometer, as well as the sample preparation method – remain unchanged according to the Instruction for use. [1]
For method comparison, human serum samples from male and female subjects were used. [2]

Figure 2: Schematic Workflow of sample preparation of Steroid Panel LC-MS.

METHOD COMPARISON.

For the method comparison, 54 serum samples from female and male subjects were analyzed in a single determination for 14 analytes. If the values exceeded or fell outside the measuring range of the Steroid Panel LC-MS, the samples were excluded from the method comparison. After excluding outliers, more than 40 serum samples were included for each analyte to ensure a reliable data basis for the comparison study. The results show a great correlation between the different LC methods and columns used, resulting in r > 0.90 for all 14 analytes (s. Table 4).

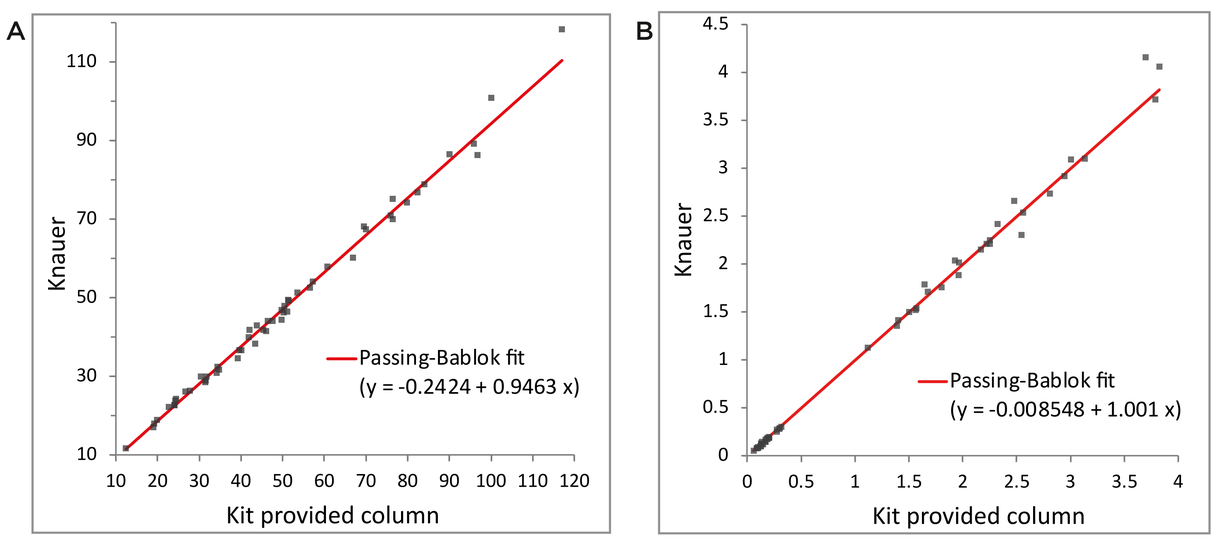

Figure 3: Method comparison based on Passing-Bablok fit for (A) Cortisol and (B) Testosterone.

Table 4: Method comparison based on at least 40 human serum samples.

Analyte	Passing-Bablok fit	r
11-Deoxycorticosterone	0.03143 + 0.796x	0.985
11-Deoxycortisol	0.03382 + 0.936x	0.987
17-OHP4	0.03297 + 0.8812x	0.978
21-Deoxycortisol	0.08507 + 0.76x	0.919
Aldosterone	1.030	0.909
Androstenedione	0.01758 + 0.9831x	0.998
Corticosterone	0.1158 + 0.9317x	0.998
Cortisol	-0.2424 + 0.9463x	0.996
Cortisone	0.1054 + 0.8712x	0.983
Dehydroepiandrosterone sulfate (DHEAS)	19.57 + 1x	0.983
Dihydrotestosterone (DHT)	-0.04298 + 1.215x	0.925
Estrone	0.0008983 + 1.053x	0.99
Progesterone	-0.01183 + 0.9462x	0.992
Testosterone	-0.008548 + 1.001x	0.997

CHROMATOGRAPHY.

A baseline separation of analytes with comparable m/z ratios is necessary to ensure adequate performance and reliable quantification.[2] This is essential for the analytes 17-OH-progesterone (17-OHP4) and 11-deoxycorticosterone. These analytes share the same fragment ion for both the qualifier and the quantifier in this method.
Since the transitions for cortisone and cortisol share the same fragment ion for the quantifier, the separation of these analytes is crucial as well. In addition, corticosterone, 11-deoxycortisol and 21-deoxycortisol can cause interferences, due to comparable transitions. The baseline separation is shown in Figure 4, forming the basis for reliable quantification of these steroids.

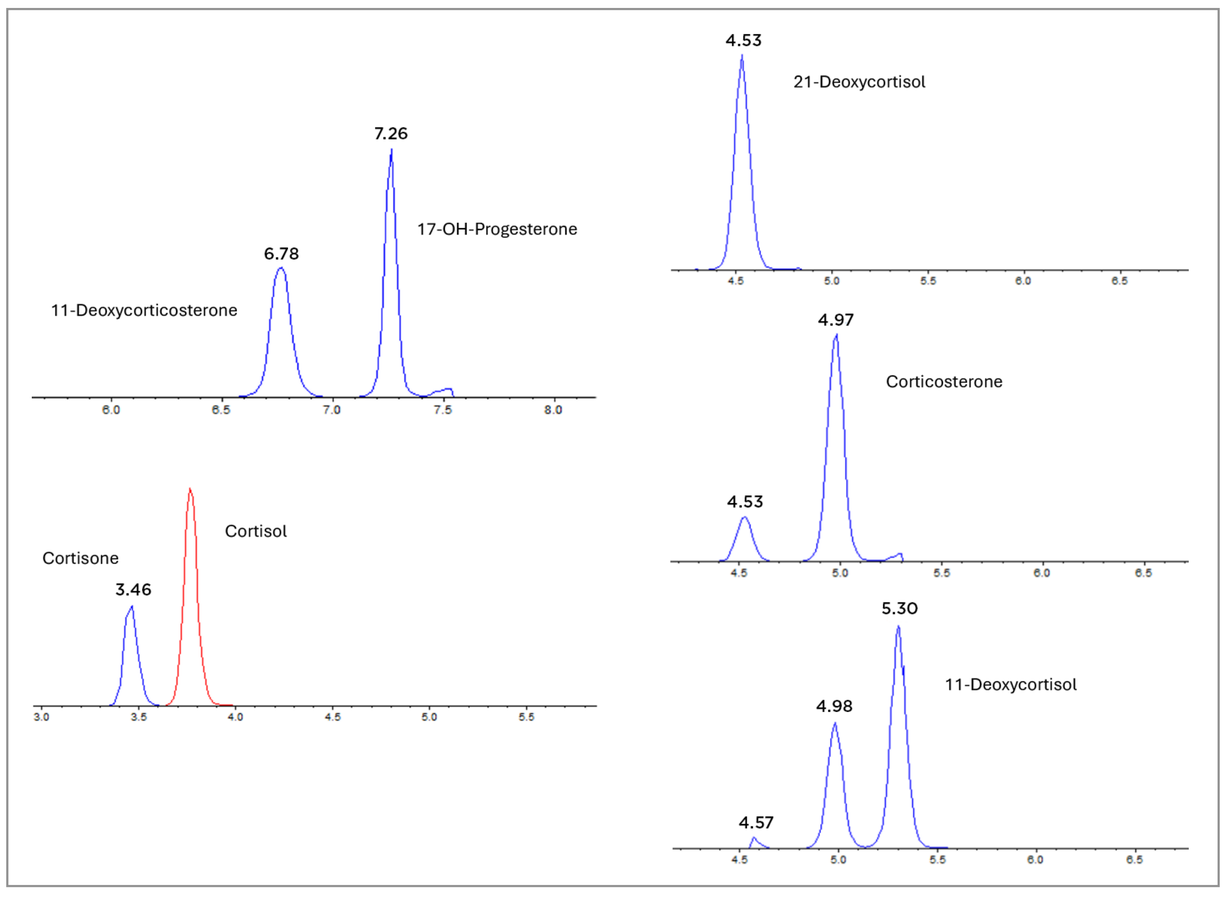

Figure 4: Chromatographic separation on KNAUER Eurospher II column at a concentration of Calibrator C and D, respectively.

CONCLUSION.

The comparison shows that both columns are suitable for the reliable quantification of 14 steroids with the Steroid Panel LC-MS. This study thus provides the foundation for future validations and implementations in the laboratory, ensuring the reliability of the supply chain by using two possible columns.

[1] Tecan, Steroid Panel LC-MS, Instruction for Use (IFU), Version 2024-10, see https://ibl-international.com/media/mageworx/downloads/attachment/file/3/0/30220266_ifu_ww_en_steroid_panel_lc-ms_ruo_2024-10_sym9.pdf.
[2] CLSI guideline EP09c, Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd Edition, June 2018.

TECAN.
SCALING HEALTHCARE INNOVATION GLOBALLY