Simultaneous Quantification of 17 Steroids Using the Tecan Kit and Shimadzu 8060 LC-MS/MS Platform

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1. Introduction

Steroid hormones are essential regulators of metabolism, immune function, stress response, and reproduction. Accurate quantification of these hormones is critical for biomedical research, particularly in the investigation of endocrine disorders and metabolic diseases. While traditional immunoassays are widely used, they are often limited by cross-reactivity and a lack of multiplexing capability. These limits can compromise both specificity and throughput of steroids analysis. In contrast, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the gold standard for steroid quantification, providing superior specificity, sensitivity, and the ability to simultaneously measure multiple analytes in complex biological matrices. [1,2]

In this study, the analytical performance of a research-use-only steroid quantification kit was evaluated in combination with the Shimadzu Nexera X3 UHPLC and LCMS-8060NX platform for the simultaneous determination of 16 steroids and dexamethasone. Key evaluation criteria included intra-assay precision and trueness across three sample levels, with the goal of establishing the suitability of this workflow for clinical research applications in the field of steroidomics.

Figure 1: Overview of the analytical workflow: Tecan Resolvex A200 for sample preparation (left), Shimadzu Nexera LCMS-8060 system for analysis (center), and Tecan Steroid Panel LC-MS kit (right).

2. Material and Methods

Sample preparation was performed using the semi-automated Tecan® Resolvex® A200 platform, following the Tecan kit IFU (Tecan Cat. No. 30220266). The protocol includes solid-phase extraction (SPE) to ensure optimal recovery and ionization efficiency for all target analytes. The workflow consists of two LC-MS/MS runs: the first run quantifies 14 steroids and dexamethasone, while the second run quantifies two additional steroids, resulting in a total of 17 analytes. Calibrators and quality control samples are lyophilized in serum and reconstituted with LC-MS grade water prior to analysis. The Shimadzu LC-MS/MS system was operated in multiple reaction monitoring (MRM) mode, utilizing compound-specific transitions and collision energies. Data acquisition and quantification were conducted using LabSolutions software, with internal standard correction applied to all analytes (see Table 1).

Table 1: Overview of LC-MS/MS Setup and Conditions for Steroid Analysis.

MS-8060 Parameter	Value
Interface voltage	1 / -1 kV
Nebulizing Gas Flow	2.5 L/min
Heating Gas Flow	14 L/min
Interface Temperature	400 °C
DL Temperature	250 °C
Heat Block Temperature	450 °C
Drying Gas Flow	5 L/min
CID	230 kPa

Nexera X3 UHPLC	Details
LC-Moduls	Binary Pump
Column	C8 distributed by Tecan (Tecan Cat. No. 30215928)
Mobile Phase A	Water + ammonium fluoride (<5 mM)
Mobile Phase B	MeOH + ammonium fluoride (<5 mM)
Injection Volume	20 µL
Temperatures	Autosampler: 10 °C; Column oven: 40 °C

3. Results

3.1 Method Adaption on Shimadzu platform

Chromatographic separation was optimized to resolve analytes with similar structures and mass transitions, with particular attention to those known to be challenging, such as 11-deoxycortisol, 21-deoxycortisol, corticosterone, cortisol and cortisone, as well as 17-hydroxyprogesterone and 11-deoxycorticosterone [3]. Minimal changes were made to the gradients for run 1 (A) and run 2 (B) (see Figure 2) , and MRM transitions were carefully selected to achieve good separation between these analytes. The use of a C8 column distributed by Tecan, along with these optimizations, enabled robust resolution of 17 analytes, including the challenging pairs (see Figure 3 and Table 2). These optimizations provide efficiency, throughput, and analytical performance comparable to that of the original protocol.

A

Time Shimadzu RT (min)	B conc (%)
0	41
1.2	50
6	59
6.7	88
9	88
9.1	43
10	43
11	43

B

Time Shimadzu RT (min)	B conc (%)
0	45
0.95	45
1.4	70
5.4	95
5.9	95
5.91	45
7.5	45

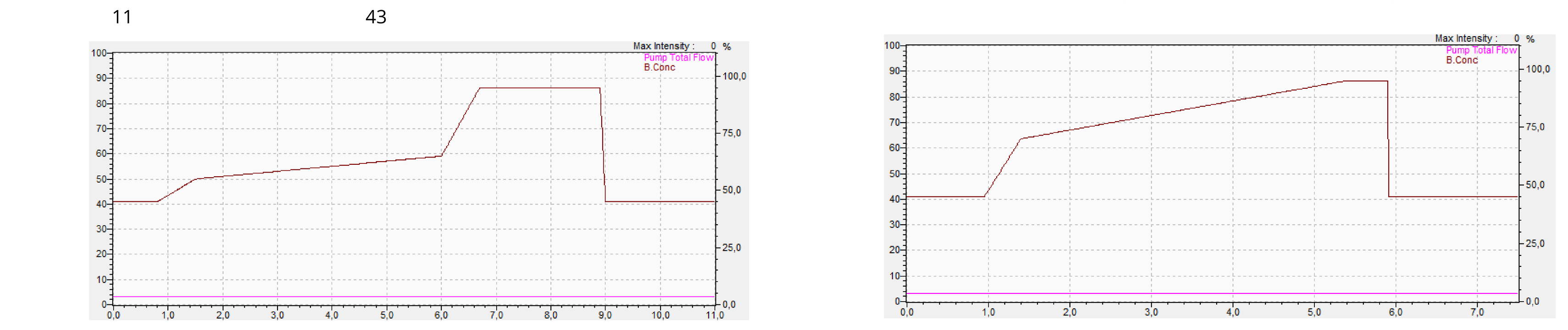

Figure 2. Shimadzu Gradient Profiles of Run1 (A) and Run 2 (B) After Adaption to the Shimadzu Platform.

1.Taylor, A.E., Keevil, B.G., & Huhtaniemi, I.T. (2015). Mass spectrometry and immunoassay: how to measure steroid hormones today and tomorrow. European Journal of Endocrinology, 173(2), D1–D12.
2.Vogeser, M., & Parhofer, K.G. (2007). Liquid chromatography tandem-mass spectrometry (LC-MS/MS)–technique and applications in endocrinology. Experimental and Clinical Endocrinology & Diabetes, 115(09), 559-570.
3.Zhu, Y., Li, Y., Wang, Y., Wang, Y., & Wang, Y. (2022). Simultaneous determination of 11 endogenous corticosteroids in human plasma by liquid chromatography–tandem mass spectrometry for the diagnosis of adrenal diseases. Frontiers in Endocrinology, 13(9), 940314. https://doi.org/10.3389/fendo.2022.940314.

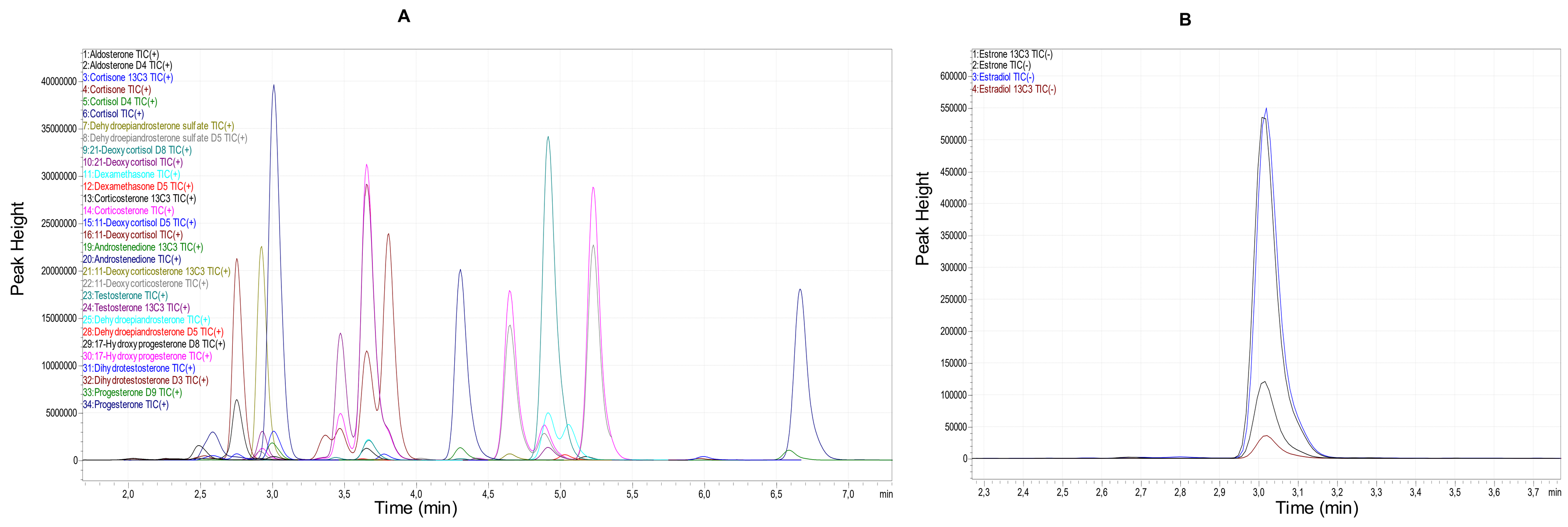

Figure 3: MRM trace overlay of 17 analytes detailing the elution profile. The data shown were acquired using calibrator F. (A = Run 1, B = Run 2).

A

Compound name	RT (min)	Quantifier m/z	Quantifier CE (V)	Qualifier m/z	Qualifier CE (V)
11-Deoxycorticosterone 13C3	4,62	334,2>112,2	-28	334,2>100,1	-27
11-Deoxycorticosterone	4,62	331,2>109,2	-27	331,2>97,2	-22
11-Deoxycortisol D5	3,76	352,2>113	-29	352,2>100,2	-30
11-Deoxycortisol	3,76	347,2>97	-27	347,2>109	-27
17-Hydroxyprogesterone D8	5,14	339,2>100	-26	339,2>113	-30
17-Hydroxyprogesterone	5,14	331,2>97,2	-25	331,2>109	-26
21-Deoxycortisol D8	3,39	355,2>319,1	-16	355,2>301,3	-19
21-Deoxycortisol	3,39	347,2>311,1	-16	347,2>121,2	-25
Aldosterone D4 (+)	2,44	365,1>347,3	-17	365,1>300,3	-20
Aldosterone (+)	2,44	361,1>343,2	-17	361,1>299,2	-21
Androstenedione 13C3	4,28	290,1>100,2	-22	290,1>112,2	-24
Androstenedione	4,28	287,2>97,2	-22	287,2>109,1	-23
Corticosterone 13C3	3,61	350,2>332,1	-15	350,2>124,2	-23
Corticosterone	3,61	347,1>329,3	-14	347,1>121,2	-23
Cortisol D4 (+)	2,96	367,2>121,1	-24	367,2>77	-55
Cortisol (+)	2,96	363,1>121,2	-24	363,1>91,1	-55
Cortisone 13C3	2,72	364,2>166,2	-24	364,2>105	-42
Cortisone	2,72	361,1>163,3	-23	361,1>91,1	-55
Dehydroepiandrosterone D5	5,01	294,2>258,5	-13	294,2>218,2	-20
Dehydroepiandrosterone	5,01	289,2>271,4	-9	289,2>253,2	-13
Dehydroepiandrosterone sulfate D5	3,03	276,2>258,4	-13	276,2>97,1	-26
Dehydroepiandrosterone sulfate	3,03	271,2>253,3	-13	271,2>91,1	-54
Dexamethasone D5	3,58	398,2>378,3	-8	398,2>360,1	-13
Dexamethasone	3,58	393,1>373,3	-10	393,1>355,2	-12
Dihydrotestosterone D3	5,93	294,2>258,4	-16	294,2>91,2	-51
Dihydrotestosterone	5,93	291,2>255,3	-15	291,2>159,2	-21
Progesterone D9	6,56	324,2>100,2	-24	324,2>113,2	-26
Progesterone	6,56	315,2>109,2	-24	315,2>97,2	-22
Testosterone 13C3	4,90	292,1>112,2	-25	292,1>100,2	-22
Testosterone	4,90	289,1>109,2	-24	289,1>97,2	-22

B

Compound name	RT (min)	Quantifier m/z	Quantifier CE (V)	Qualifier m/z	Qualifier CE (V)
Estradiol 13C3	3,03	274,2>148,1	40	274,2>186,2	42
Estradiol	3,03	271,3>145,1	39	271,3>183,2	41
Estrone 13C3 (-)	3,02	272,2>148,1	37	272,2>146,1	55
Estrone (-)	3,02	268,9>145,1	37	268,9>143,0	54

Table 2: Overview of Target Analytes, Retention Times, and MRM Transitions (A = Run 1, B = Run 2)

3.2 Linearity

Calibration curves for all analytes demonstrated good linearity, with R^2 values >0.99 for vast majority of analytes. Such as for relevant parameter like 17-OHP4 R^2 was 0.998, for testosterone 0.999, and for cortisol 0.999. All analytes were fitted using a linear model with either 1/C or 1/C² weighting, as appropriate.

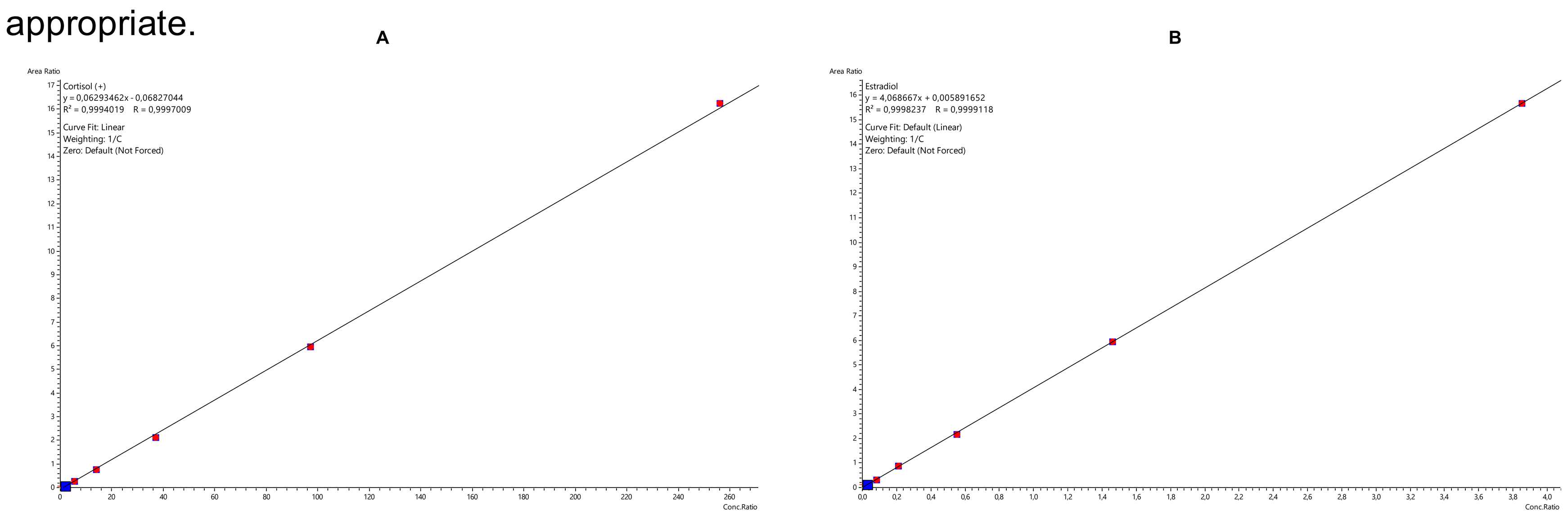

Figure 4. Calibration curves for (A) cortisol and (B) estradiol.
Both analytes were fitted using a linear model with 1/C weighting showing excellent linearity with R^2 > 0.999.

3.3 Trueness and precision

The adapted Tecan kit demonstrated strong analytical performance across all analytes and quality control levels. Precision testing with 15 replicates per QC level showed %RSD values below 20% for all analytes, indicating excellent repeatability (e. g., 17-OHP4: 3.9%/3.7%, testosterone: 1.9%/3.2%, cortisol: 4.5%/8.7% for high/low QC). Trueness was assessed at three sample levels (S1, S2, S3, with most analytes falling within ±20% of target values (e. g., 17-OHP4: 0.2–6.2%, testosterone: -5.4–13.0%, cortisol: 4.5–7.2%). These results confirm the method's reliability and suitability for clinical research applications (See Table 3 and 4).

Analyte	Truness S1	S2	S3
Progesterone	-4.3%	16.5%	-9.0%
DHT	-0.5%	4.1%	6.2%
17-OHP4	0.2%	1.3%	6.2%
DHEA	3.1%	0.7%	-19.5%
Testosterone	13.0%	1.6%	-5.4%
11-deoxycorticosterone	2.4%	1.0%	0.6%
Estradiol	-1.3%	4.5%	7.9%
androstenedione	10.6%	5.3%	2.3%
Estron	-1.8%	3.5%	5.8%
11-deoxycortisol	1.0%	-2.5%	6.3%
Dexamethasone	-7.3%	-0.1%	11.8%
Corticosterone	-1.5%	1.2%	7.6%
21-deoxycortisol	-18.9%	16.2%	0.9%
DHEAS	0.9%	10.1%	-18.7%
Cortisol	7.0%	7.2%	4.5%
Cortisone	6.3%	0.2%	1.9%
Aldosterone	-3.6%	-3.1%	0.8%

Table 3. Trueness of the adapted method:
Percent deviation from assigned values at three QC levels (S1–S3) for each analyte.

Analyte	Precision in % QC H	QC L
Progesterone	14.2%	13.8%
DHT	2.9%	6.6%
17-OHP4	3.9%	3.7%
DHEA	2.8%	3.8%
Testosterone	1.9%	3.2%
11-deoxycorticosterone	2.7%	3.1%
Estradiol	2.4%	3.4%
androstenedione	2.4%	4.0%
Estron	2.4%	2.8%
11-deoxycortisol	2.1%	5.4%
Dexamethasone	9.0%	6.5%
Corticosterone	3.3%	5.0%
21-deoxycortisol	10.5%	17.2%
DHEAS	13.9%	13.3%
Cortisol	4.5%	8.7%
Cortisone	3.9%	4.3%
Aldosterone	6.4%	8.6%

Table 4. Precision (CV%) of adapted method:
Coefficient of variation (CV%) at high and low QC levels for each analyte.

4. Conclusion

The Steroid Panel LC-MS kit, combined with the Shimadzu platform, enables high-throughput, multiplexed steroid analysis in two runs. This standardized workflow minimizes operator variability and improves reproducibility. The Shimadzu LC-MS/MS system provides high sensitivity and selectivity, allowing reliable quantification of a broad steroid panel, even at low concentrations. The adaptation of the kit to the Shimazu platform ensures precision and accuracy, making it ideal for clinical research and studies of endocrine pathways or disease mechanisms.