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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

Analysis of Steroids in Serum / Plasma Using RECIPE® ClinMass® LC-MS/MS Complete Kit System with Fully Automated Sample Preparation LC-MS/MS System

Ionela Regos[1], Yoshikawa, Kohei[2], Doriane Toinon[3] , Anja Grüning[3]
1 RECIPE® Chemicals + Instruments GmbH, 2 Shimadzu Japan, 3 Shimadzu Europa GmbH

User Benefits

- Full solution provided by Shimadzu and RECIPE®
- Fully automated sample preparation
- Verified method for RECIPE® ClinMass® LC-MS/MS Complete Kit Steroids in Plasma / Serum

■ Introduction

Steroid hormones are produced by the adrenal glands, testes or ovaries. They fulfil a wide range of physiological functions after binding to specific cell receptors of various target organs. Steroids are involved in the carbohydrate, lipid and protein metabolism, stress response, regulation of the water and mineral balance, reproductive processes and development of secondary sex characteristics.

Consequently, steroid hormones are also involved in a variety of diseases, like adrenal tumors, the Adrenogenital Syndrome (CAH), the Cushing´s Syndrome or the Polycystic Ovarian Syndrome.

In addition, synthetic steroids are used in medical treatment of acute and chronic diseases, e.g. gonadal dysfunction, allergies and suppression of immune responses.

Therefore, quantitative profiling of steroids is essential in diagnosis and evaluation of pathologic conditions and therapeutic responses.

RECIPE®'s fully validated analytical method provides the quantification of 8 steroids (Table 3) in serum/plasma using LC-MS/MS [1]. By addition of the Shimadzu CLAM (Clinical Laboratory Automated sample preparation Module) in front of the LC-MS/MS system (Figure 1) the required sample preparation could be fully automated which achieves results on a fast and high-precision analytical workflow.

To prove that the automated sample preparation leads to reliable results a method verification procedure was evaluated according to the CLSI Guidelines EP06, EP15-A3, EP17-A2.

Fig. 1 CLAM LCMS TQ

■ Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in clinical laboratories. For the described verification, a Shimadzu CLAM-2040 coupled with a Nexera X3 UHPLC system and a LCMS-8060 triple-quadrupole mass spectrometer with an ESI source was used.

Eight Steroids in serum were verified using the RECIPE® ClinMass® LC-MS/MS Complete Kit Steroids in Serum / Plasma (order no. MS12000). The ClinCal® Serum Calibrator Set lyophilised, for Steroids (order no. MS12013) and ClinChek® Serum Control lyophilised for Steroids (order no. MS12083) from RECIPE®, German were used.

Lyophilized, matrix-based calibrator and control samples were reconstituted, aliquoted and stored until use. Then the samples were loaded directly into the CLAM-2040. It was programmed to perform protein precipitation using Precipitant P including internal standards followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2040 to the LC without human intervention for LC-MS/MS analysis.

Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 10 min. Analytical conditions are listed in Table 1 and 2. The optimized MRM transitions are summarized in Table 3.

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060
Ionization	: Electrospray Ionization (ESI), positive
Interface Voltage	: 0.5 kV
DL Temp.	: 300 ˚C
Interface Temp.	: 400 ˚C
Nebulizing Gas	: 3 L/min
Drying Gas	: Off
Heat Block	: 400 ˚C
UHPLC	: Nexera X3 with SPE on-line
Column Oven	: 40 ˚C
Injection Volume	: 40.0 µL
Flow rate	: 0.5 mL/min
Time Programme	: Binary gradient

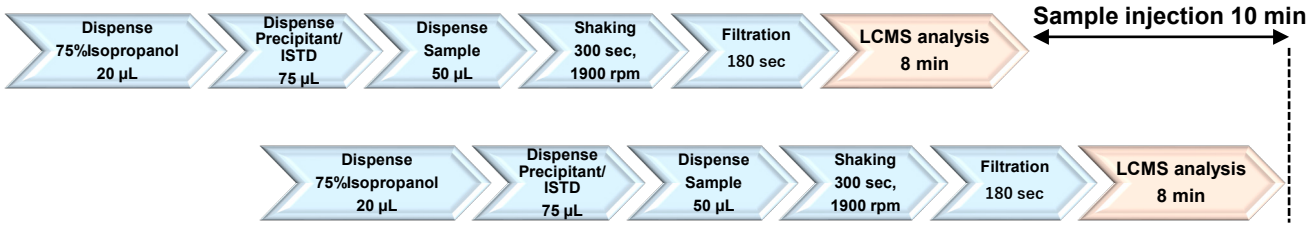
Table 2 Gradient

Time (min)	Pump Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
Initial	0.5	98	2
1.01	0.5	98	2
1.02	0.5	73	27
5.50	0.5	43	57
5.51	0.5	0	100
5.65	0.5	0	100
5.75	0.8	0	100
6.50	0.8	0	100
6.51	0.8	98	2
8	0.8	98	2

Table 3 MRM transitions and isotope-labelled group

Analyte / IS	Quantifier MRM		Qualifier MRM		ISTD group
	Precursor (m/z)	Product (m/z)	Precursor (m/z)	Product (m/z)	
Androstenedione	286.9	97.0	109.1	286.9	1
Cortisol	363.1	121.1	97.0	363.1	2
Dehydroepiandrosterone-sulfate (DHEAS)	270.9	213.3	197.1	270.9	3
11-Deoxycortisol	346.9	97.0	109.1	346.9	4
21-Deoxycortisol	346.9	311.2	121.1	346.9	4
17-Hydroxyprogesterone	331.0	97.0	109.0	331.0	5
21-Hydroxyprogesterone	331.0	97.0	109.0	331.0	5
Testosterone	289.0	97.0	108.9	289.0	6
d7-Androstenedione	294.1	100.1			1
d4-Cortisol	367.2	121.2			2
d5-DHEAS	276.2	218.1			3
d5-11-Deoxycortisol	352.2	100.0			4
d8-17-Hydroxyprogesterone	339.2	100.1			5
d3-Testosterone	292.2	97.1			6

Fig. 2 Scheme fully automated sample preparation and analysis



■ Results

The trueness was determined by 4-fold analysis of three different quality control (QC) samples in a single analysis sequence. The results (precision in CV% and deviation from the target in % Bias) are summarized in Table 4. The acceptance criteria of CV<15% (<20% near LLOQ) and Bias ±20% were fulfilled.

To determine the intraday precision the three different levels of QC samples were prepared in 8-fold and analysed in a single analysis sequence. For the interday precision, the QC samples were prepared in 5-fold and analysed in a single analysis sequence on 3 days. The intra and interassay precision for each level is summarized in Table 5 and 6. The acceptance criteria of CV<15% (<20% near LLOQ) was fulfilled.

For determination of the linearity and the lower limit of quantification (LLOQ) several dilutions of ClinCal® Serum Calibrator Set lyophil. for Steroids, Level 0 - 6, (order no. MS12013, RECIPE®,Germany) were prepared in 3-fold and analyzed in a single analysis sequence.

The results for linearity evaluation and for the LLOQ are summarized in Table 7. The LLOQ was determined as the lowest concentration at which precision was < 20% and bias ±20%. The acceptance criteria for linearity are CV < 15% and bias ±15%.

Table 4 Trueness of measurement

Analytes	Sample	Target value [µg/L]	Measured value [µg/L]; Mean (n=4)	CV [%]	Bias [%]
Androstenedione	Control Sample, Level I	0.241	0.253	5.5	5.1
	Control Sample, Level II	0.680	0.709	10.6	4.2
	Control Sample, Level III	3.83	3.85	2.9	0.6
Cortisol	Control Sample, Level I	4.80	4.98	1.5	3.7
	Control Sample, Level II	13.7	13.9	1.6	1.5
	Control Sample, Level III	77.0	76.1	0.3	-1.2
DHEAS	Control Sample, Level I	79.2	87.0	3.0	9.8
	Control Sample, Level II	230	232	2.2	0.7
	Control Sample, Level III	1309	1249	2.1	-4.6
11-Deoxycortisol	Control Sample, Level I	0.228	0.224	1.5	-1.6
	Control Sample, Level II	0.672	0.647	1.6	-3.7
	Control Sample, Level III	3.83	3.57	2.0	-6.7
21-Deoxycortisol	Control Sample, Level I	0.222	0.231	5.7	3.8
	Control Sample, Level II	0.628	0.630	2.3	0.4
	Control Sample, Level III	3.55	3.36	2.4	-5.3
17-Hydroxyprogesterone	Control Sample, Level I	0.242	0.247	2.9	2.1
	Control Sample, Level II	0.659	0.681	3.4	3.3
	Control Sample, Level III	3.71	3.62	2.1	-2.4
21-Hydroxyprogesterone	Control Sample, Level I	0.414	0.421	5.1	1.8
	Control Sample, Level II	1.18	1.19	4.6	0.5
	Control Sample, Level III	6.70	6.51	1.2	-2.8
Testosterone	Control Sample, Level I	0.186	0.193	4.3	3.5
	Control Sample, Level II	0.504	0.500	5.4	-0.8
	Control Sample, Level III	2.74	2.54	3.0	-7.3

Table 5 Intraassay results [CV%]

Analytes	Sample	Measured value [µg/L] Mean (n=8)	CV [%]
Androstenedione	Control Sample, Level I	0.251	4.8
	Control Sample, Level II	0.708	7.1
	Control Sample, Level III	3.86	2.2
Cortisol	Control Sample, Level I	4.95	1.6
	Control Sample, Level II	13.9	1.3
	Control Sample, Level III	75.9	0.7
DHEAS	Control Sample, Level I	86.6	3.3
	Control Sample, Level II	233	2.1
	Control Sample, Level III	1237	2.0
11-Deoxycortisol	Control Sample, Level I	0.223	2.1
	Control Sample, Level II	0.643	1.6
	Control Sample, Level III	3.65	6.3
21-Deoxycortisol	Control Sample, Level I	0.223	6.8
	Control Sample, Level II	0.617	3.5
	Control Sample, Level III	3.43	6.5
17-Hydroxyprogesterone	Control Sample, Level I	0.245	3.8
	Control Sample, Level II	0.678	2.5
	Control Sample, Level III	3.64	2.2
21-Hydroxyprogesterone	Control Sample, Level I	0.422	3.5
	Control Sample, Level II	1.195	3.4
	Control Sample, Level III	6.48	2.2
Testosterone	Control Sample, Level I	0.192	3.5
	Control Sample, Level II	0.486	5.5
	Control Sample, Level III	2.54	2.6

Table 6 Interassay results [CV%]

Analytes	Sample	Measured value [µg/L] Mean (n=5)	CV [%]
Androstenedione	Control Sample, Level I	0.235	10.2
	Control Sample, Level II	0.678	7.2
	Control Sample, Level III	3.82	5.4
Cortisol	Control Sample, Level I	4.93	2.6
	Control Sample, Level II	13.7	2.6
	Control Sample, Level III	75.2	2.1
DHEAS	Control Sample, Level I	83.1	4.6
	Control Sample, Level II	232	3.0
	Control Sample, Level III	1270	3.2
11-Deoxycortisol	Control Sample, Level I	0.230	3.8
	Control Sample, Level II	0.653	3.6
	Control Sample, Level III	3.65	3.1
21-Deoxycortisol	Control Sample, Level I	0.210	6.6
	Control Sample, Level II	0.608	4.6
	Control Sample, Level III	3.42	4.3
17-Hydroxyprogesterone	Control Sample, Level I	0.241	4.2
	Control Sample, Level II	0.656	3.6
	Control Sample, Level III	3.59	2.1
21-Hydroxyprogesterone	Control Sample, Level I	0.397	6.6
	Control Sample, Level II	1.141	4.6
	Control Sample, Level III	6.38	4.3
Testosterone	Control Sample, Level I	0.193	4.4
	Control Sample, Level II	0.504	4.7
	Control Sample, Level III	2.65	3.8

Table 7 Linearity evaluation, including LLOQ / LOD and CV and Bias at the LLOQ

Analytes	Linear Range [µg/L]	R^2	LLOQ [µg/L]	LOD [µg/L]	CV (%)	Bias (%)
Androstenedione	0.0400 – 20.5	0.997	0.0400	0.0133	4.2	3.2
Cortisol	0.593 – 590	0.999	0.593	0.198	4.2	0.1
DHEAS	13.4 – 10480	0.999	13.4	4.47	11.6	-3.9
11-Deoxycortisol	0.0545 – 30.2	0.996	0.0545	0.0182	6.0	-13.6
21-Deoxycortisol	0.109 – 28.4	0.994	0.109	0.0363	2.5	-13.1
17-Hydroxyprogesterone	0.0650 – 28.8	0.998	0.0650	0.0217	4.4	9.1
21-Hydroxyprogesterone	0.125 – 52.8	0.998	0.125	0.0417	10.9	-9.5
Testosterone	0.0505 – 14.4	0.996	0.0505	0.0168	3.5	5.7

■ Conclusion

The ClinMass® LC-MS/MS Complete Kit for Steroids in Serum / Plasma (order no. MS12000) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

All 8 analytes passed the acceptance criteria for accuracy (trueness, precision) and linearity.

■ References

1. Instruction Manual, ClinMass® LC-MS/MS Complete Kit Steroids in Serum / Plasma, RECIPE ® Chemicals + Instruments GmbH

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Shimadzu Corporation
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