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Integration of steroids analysis in serum using LC-MS/MS with full-automated sample preparation

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Introduction

Currently sample preparation for the detection of steroids in serum by liquid chromatography-mass spectrometry (LC-MS/MS) involves complex offline extraction methods such as solid phase extraction or liquid/liquid extraction, all of which require additional sample concentration and reconstitution in an appropriate solvent. These sample preparation methods are time-consuming, often taking one hour or more per sample, and are more vulnerable to variability due to analyst errors during manual preparation. Our approach is offering a high sensitivity steroid detection fully automated for multiple samples. It is using an automated sample preparation coupled to the detection capabilities of a high sensitivity triple stage quadrupole mass spectrometer, that requires no human intervention from loading the samples to obtaining the results.

Method

10 steroid hormones (cortisol, aldosterone, 11-deoxycortisol, corticosterone, 17-alpha-hydroxyl-progesterone (17-OHP), 4-androstene-3,17-dione (androstenedione), dehydroepiandrosterone (DHEA), dehydroepi-androsterone sulfate (DHEAS), progesterone and testosterone) in serum were verified using CHS™ MSMS Steroids Kit (PerkinElmer, USA).

Serum sample was loaded directly into the automated sample preparation system (CLAM-2000 Shimadzu, Japan). The CLAM-2000 was programmed to perform protein precipitation using acetonitrile followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2000 to the HPLC without human intervention for LC-MS/MS analysis.

Fig. 1 CLAM-2000 and LCMS-8060 system

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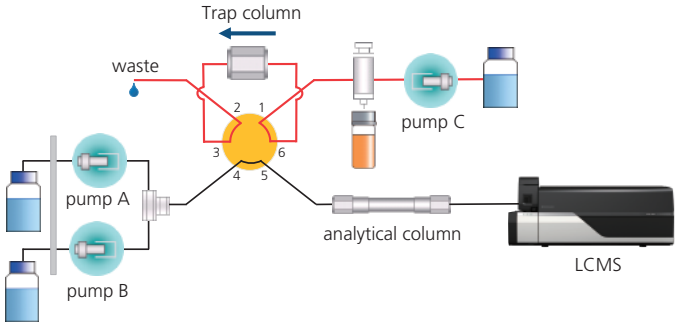
The treated samples were trapped using a MAYI-ODS column and then separated by Core-Shell Biphenyl HPLC column at 40 °C with a binary gradient system at a flow rate of 0.3 ml/min in 12 min.

Trap



Analysis

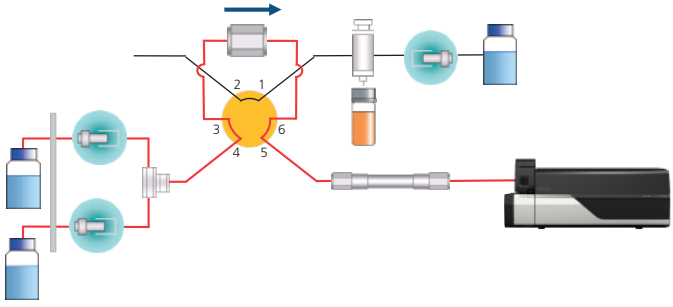
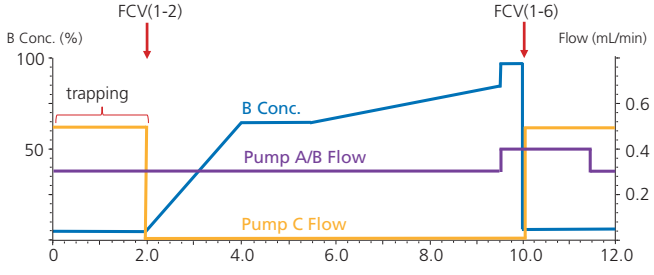


Fig. 2 Flow Diagram of Trapping system

Table 1 Analytical Condition

HPLC	
Mobile Phase A	: 1mM ammonium fluoride – water
Mobile Phase B	: Methanol
Mobile Phase C	: 10mM ammonium formate – water
Column temperature	: 40 °C
Analytical Column	: Kinetex Biphenyl (100mm L x 2mm I.D. , 2.6µm)
Guard Column	: MAYI-ODS column (5mm L x 2mm I.D.)
Injection Volume	: 30 µL
Gradient Program	:



Mass (LCMS-8060 triple quadrupole mass spectrometry)	
Ionization	: heated ESI
Nebulizing Gas Flow	: 3 L / min
Drying Gas Pressure	: 7 L / min
Heating gas flow	: 13 L/min
DL Temperature	: 120 °C
BH Temperature	: 450 °C
Interface Temperature	: 370 °C
MRM parameter	:

MRM	1	+	DHEAS 271.20>213.20, 271.20>197.10
MRM	2	+	DHEAS IS 277.10>219.20, 277.10>203.10
MRM	3	+	Cortisol 363.40>121.10, 363.40>97.00
MRM	4	+	Cortisol IS 366.10>121.10, 366.10>97.10
MRM	5	+	Aldosterone 361.20>343.00, 361.20>315.20
MRM	6	+	Aldosterone IS 367.20>349.25, 367.20>331.10
MRM	7	+	11-Deoxycortisol 347.20>109.10, 347.20>97.05
MRM	8	+	11-Deoxycortisol IS 352.20>100.15, 352.20>113.05
MRM	9	+	Coricosterone 347.20>121.15, 347.20>97.15
MRM	10	+	Coricosterone IS 355.20>125.05, 355.20>337.00
MRM	11	+	DHEA 271.20>253.15, 271.20>213.20
MRM	12	+	17-OHP 331.10>97.00, 331.10>109.00
MRM	13	+	17-OHP IS 339.10>100.05, 339.10>113.10
MRM	14	+	Testosterone 289.10>97.15, 289.10>109.05
MRM	15	+	Testosterone IS 294.10>100.00, 294.10>113.05
MRM	16	+	Androstenedione 287.10>97.10, 287.10>109.15
MRM	17	+	Androstenedione IS 292.10>100.10, 292.10>113.05
MRM	18	+	Progesterone 315.20>97.05, 315.20>109.10
MRM	19	+	Progesterone IS 324.10>100.00, 324.10>113.00
MRM	20	−	DHEAS_neg 367.10>97.10
MRM	21	−	DHEAS_neg IS 373.10>98.00
MRM	22	−	Aldosterone_neg 359.20>189.25, 359.20>331.35
MRM	23	−	Aldosterone_neg IS 365.20>194.20, 365.20>337.20

Result and discussion

We evaluated this system using calibrator and control serum spiked with 10 steroids contained in the kit and carried out concurrent analysis over a range of concentrations for each steroid: cortisol (1.51-320 ng/mL), aldosterone (0.03-1.14 ng/mL), 11-deoxycortisol (0.08-18 ng/mL), corticosterone (0.29-62 ng/mL), 17-OHP (0.12-26 ng/mL), androstenedione (0.08-18 ng/mL), DHEA (0.31-65 ng/mL), DHEAS (12.9-2750 ng/mL), progesterone (0.12-26.5 ng/mL) and testosterone (0.03-7.2 ng/mL). The calibration curves that were generated had linear regression values of r2 >0.997 for each curve. The reproducibility (N=3) at seven concentrations, including LLOQ of each compounds was excellent (CV<10%).

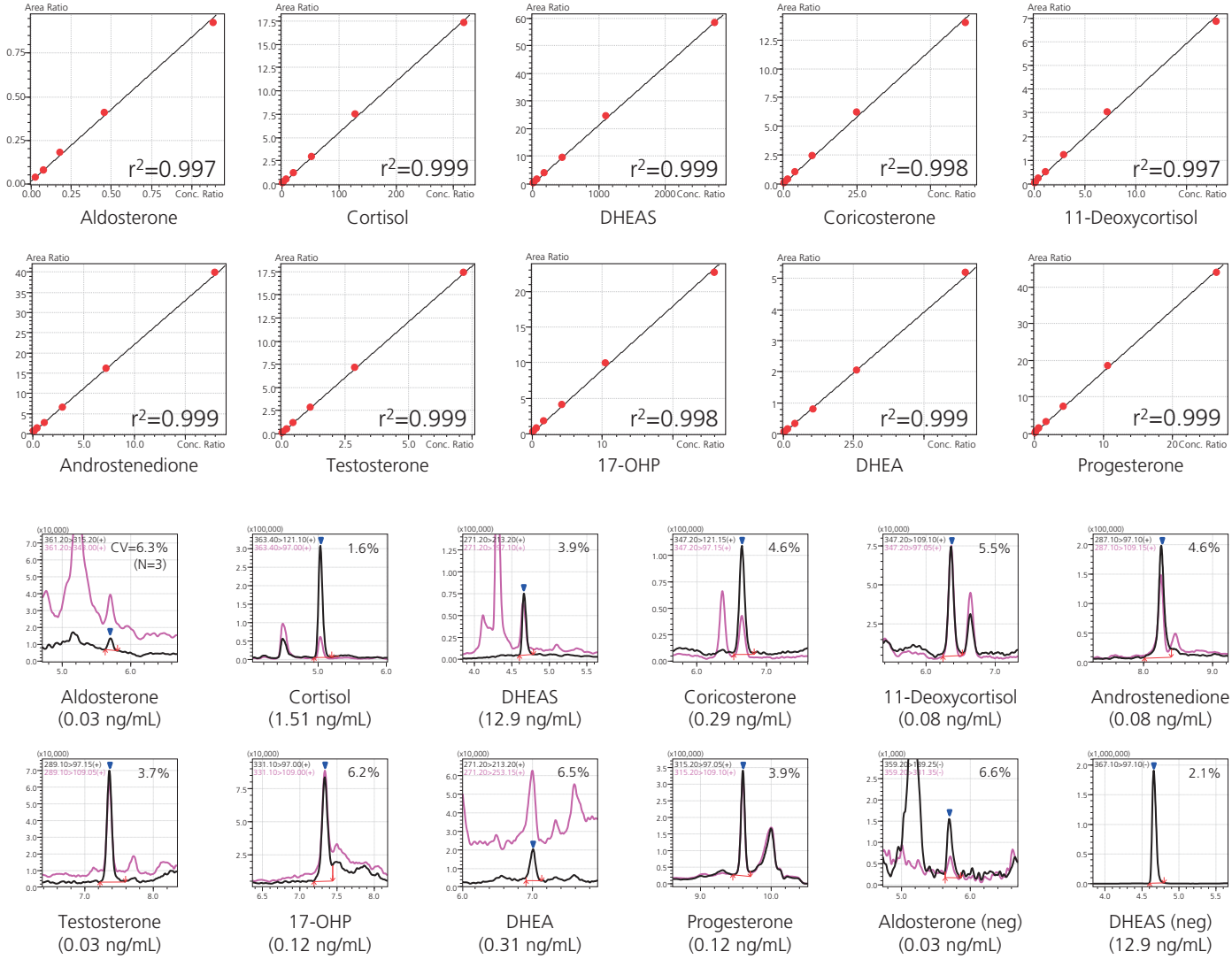


Fig. 3 Calibration Curves (L1-L7) and MRM Chromatograms (L1) of 10 Steroids

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We found that the sample preparation time was reduced from 60 minutes to 6 minutes by the automated system. Thus sample preparation and LC-MS/MS analysis can be performed in parallel to accelerate throughput.

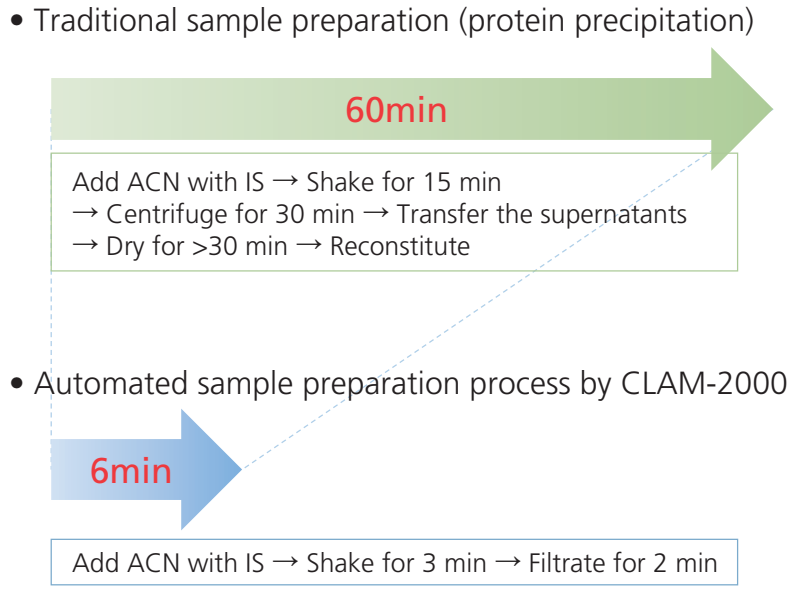

Fig. 4 Comparison with a time required for sample preparation

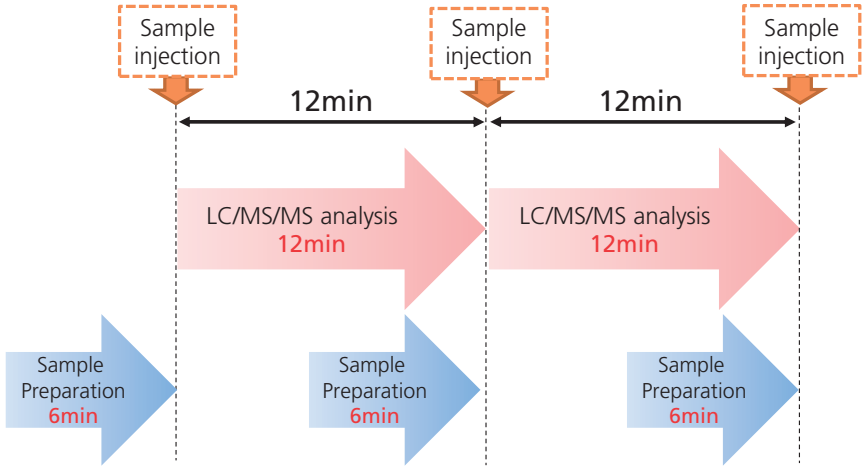

Fig. 5 Analytical Flow with Parallel Processing

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Conclusion

We completed steroid analysis using the automated sample preparation system coupled to LC-MS/MS. The results shows the capability of the system for large sample set analyses with improved accuracy and precision by eliminating human error associated with manual sample handling.

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