

# 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.



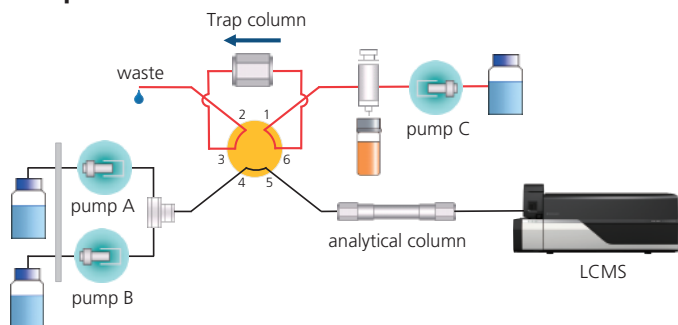
Fig. 1 CLAM-2000 and LCMS-8060 system



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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

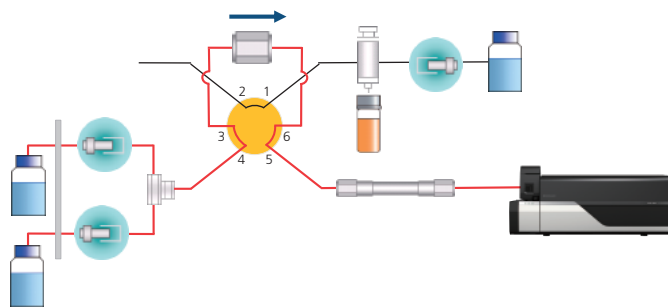


Fig. 2 Flow Diagram of Trapping system

Table 1 Analytical Condition

HPLC		Mass (LCMS-8060 triple quadrupole mass spectrometry)	
Mobile Phase A	: 1mM ammonium fluoride – water	Ionization	: heated ESI
Mobile Phase B	: Methanol	Nebulizing Gas Flow	: 3 L / min
Mobile Phase C	: 10mM ammonium formate – water	Drying Gas Pressure	: 7 L / min
Column temperature	: 40 °C	Heating gas flow	: 13 L/min
Analytical Column	: Kinetex Biphenyl (100mm L x 2mm I.D. , 2.6µm)	DL Temperature	: 120 °C
Guard Column	: MAYI-ODS column (5mm L x 2mm I.D.)	BH Temperature	: 450 °C
Injection Volume	: 30 µL	Interface Temperature	: 370 °C
Gradient Program	:	MRM parameter	:

MRM				
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	IS 363.40>121.10, 363.40>97.00	
MRM 4	+	Cortisol	IS 366.10>121.10, 366.10>97.10	
MRM 5	+	Aldosterone	IS 361.20>343.00, 361.20>315.20	
MRM 6	+	Aldosterone	IS 367.20>349.25, 367.20>331.10	
MRM 7	+	11-Deoxycortisol	IS 347.20>109.10, 347.20>97.05	
MRM 8	+	11-Deoxycortisol	IS 352.20>100.15, 352.20>113.05	
MRM 9	+	Corticosterone	IS 347.20>121.15, 347.20>97.15	
MRM 10	+	Corticosterone	IS 355.20>126.05, 355.20>337.00	
MRM 11	+	DHEA	IS 271.20>233.15, 271.20>213.20	
MRM 12	+	17-OHP	IS 331.10>97.00, 331.10>109.00	
MRM 13	+	17-OHP	IS 339.10>100.05, 339.10>113.10	
MRM 14	+	Testosterone	IS 289.10>97.15, 289.10>109.05	
MRM 15	+	Testosterone	IS 294.10>100.00, 294.10>113.05	
MRM 16	+	Androstenedione	IS 287.10>97.10, 287.10>109.15	
MRM 17	+	Androstenedione	IS 292.10>100.10, 292.10>113.05	
MRM 18	+	Progesterone	IS 315.20>97.05, 315.20>109.10	
MRM 19	+	Progesterone	IS 324.10>100.00, 324.10>113.00	
MRM 20	-	DHEAS <sub>neg</sub>	IS 367.10>97.10	
MRM 21	-	DHEAS <sub>neg</sub>	IS 373.10>98.00	
MRM 22	-	Aldosterone <sub>neg</sub>	IS 359.20>189.25, 359.20>331.35	
MRM 23	-	Aldosterone <sub>neg</sub>	IS 365.20>194.20, 365.20>337.20	

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## 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  $r^2 > 0.997$  for each curve. The reproducibility (N=3) at seven concentrations, including LLOQ of each compounds was excellent (CV<10%).

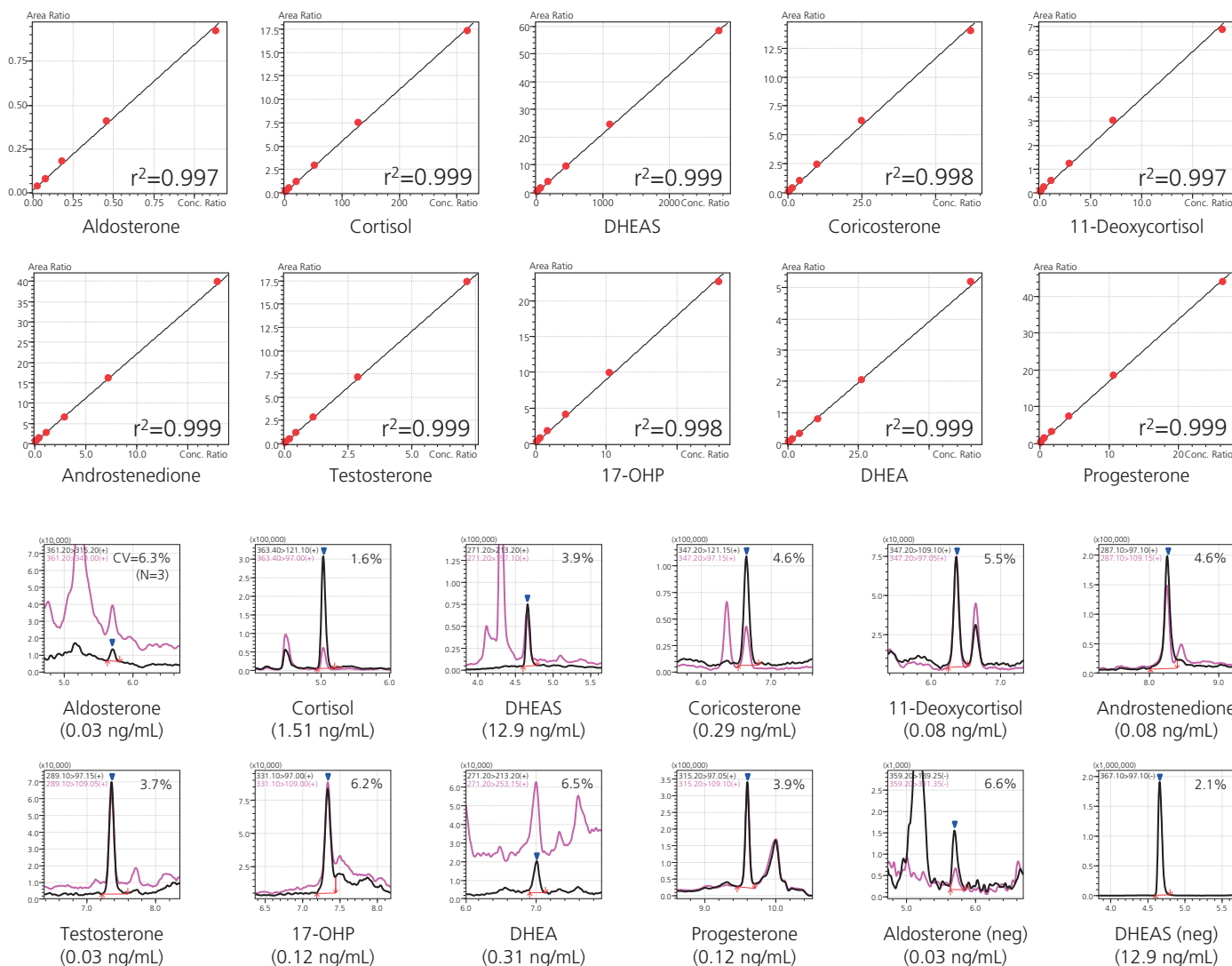
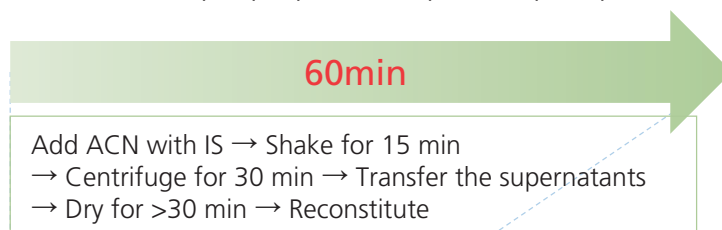


Fig. 3 Calibration Curves (L1-L7) and MRM Chromatograms (L1) of 10 Steroids

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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.

- Traditional sample preparation (protein precipitation)



- Automated sample preparation process by CLAM-2000

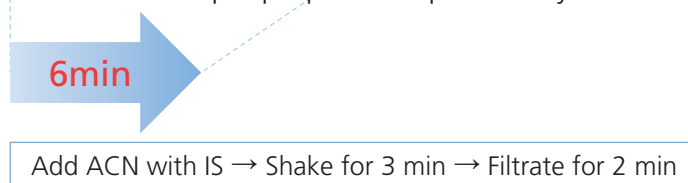


Fig. 4 Comparison with a time required for sample preparation

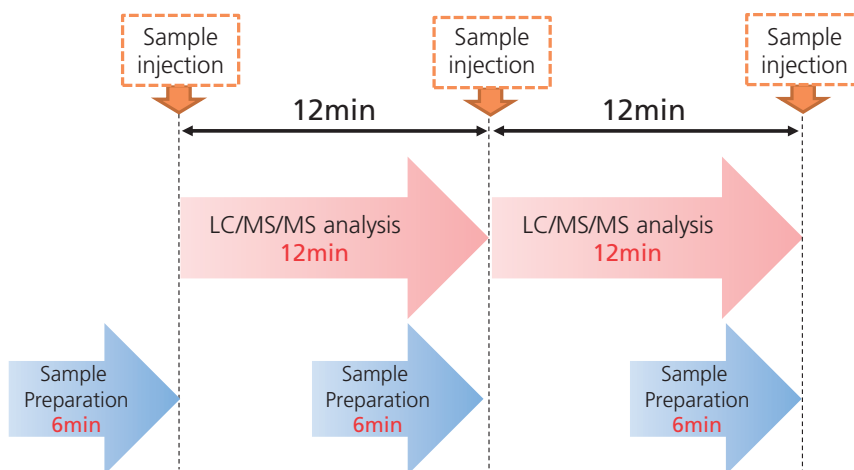


Fig. 5 Analytical Flow with Parallel Processing

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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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