PO-CON1629E

SHIMADZU
Excellence in Science

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

ASMS 2016 WP-486

Toshikazu Minohata, Daisuke Kawakami
Shimadzu Corporation, Japan

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

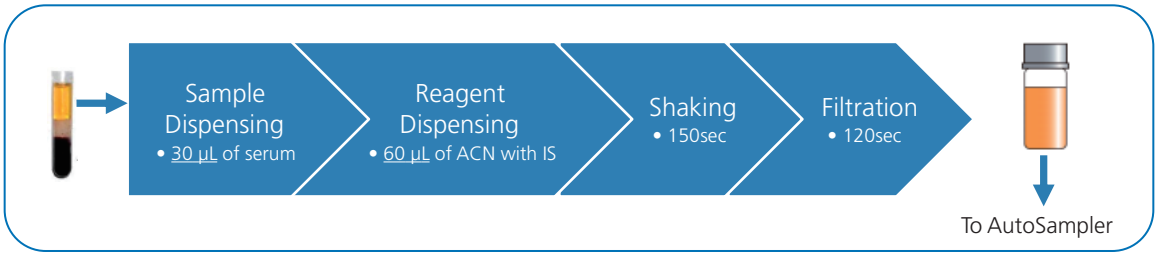
Introduction

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 1 hour or more per sample, and are more vulnerable to variability due to errors in manual preparation.
Our approach to offering a high sensitivity steroid detection method and timely, automated analysis of multiple samples is to use the automated sample preparation system coupled to the detection capabilities of a high-sensitivity triple stage quadrupole mass spectrometer.

Materials and Methods

10 steroid hormones (cortisol, aldosterone, 11-deoxycortisol, corticosterone, 17-OHP, androstenedione, DHEA, 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. 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.

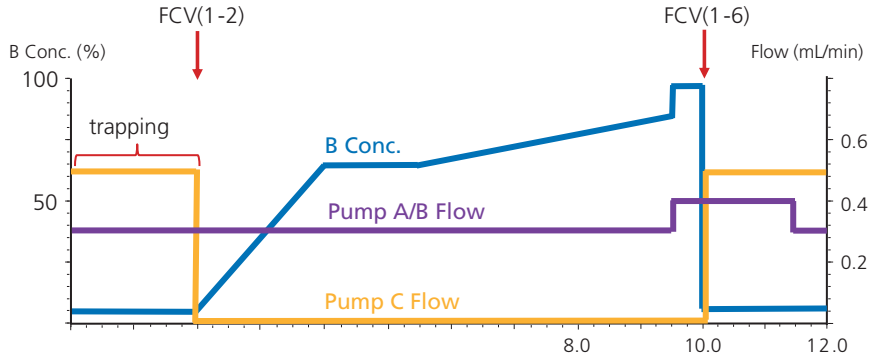
Fig.1 CLAM-2000 and LCS-8060 System



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

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 (50mm L x 2mm I.D. , 2.6µm)
Guard Column	: MAYI-ODS column (5mm L x 2mm I.D.)
Gradient Program	:



Injection Volume	: 30 µL

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

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

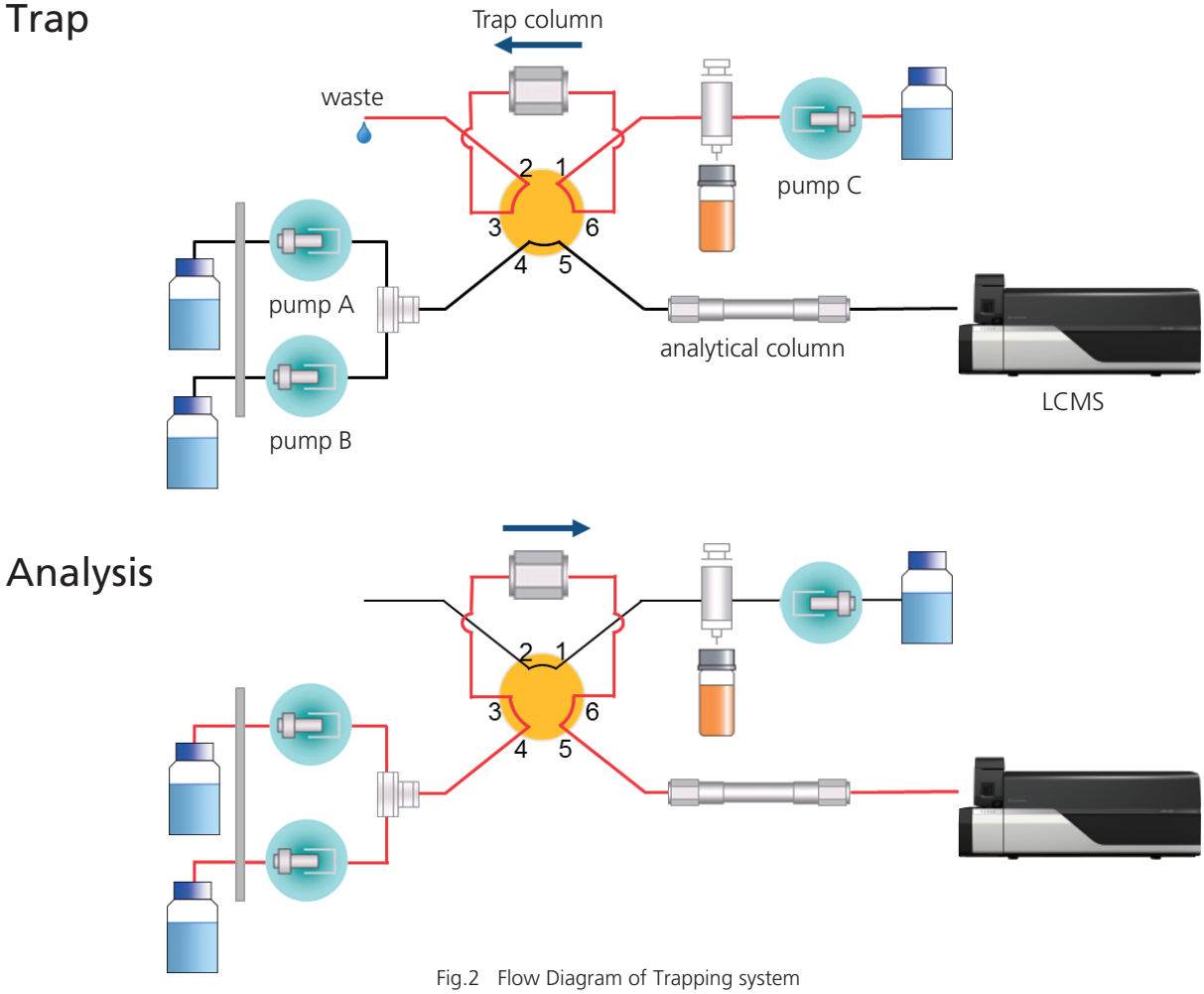


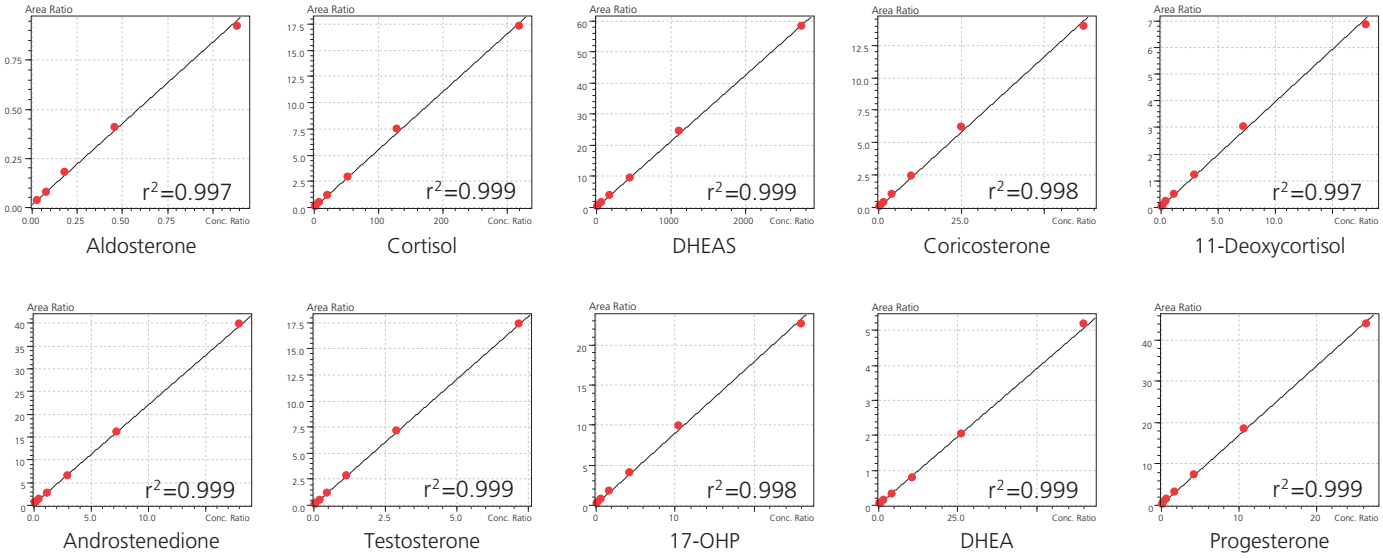
Fig.2 Flow Diagram of Trapping system

Results

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%).

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.

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



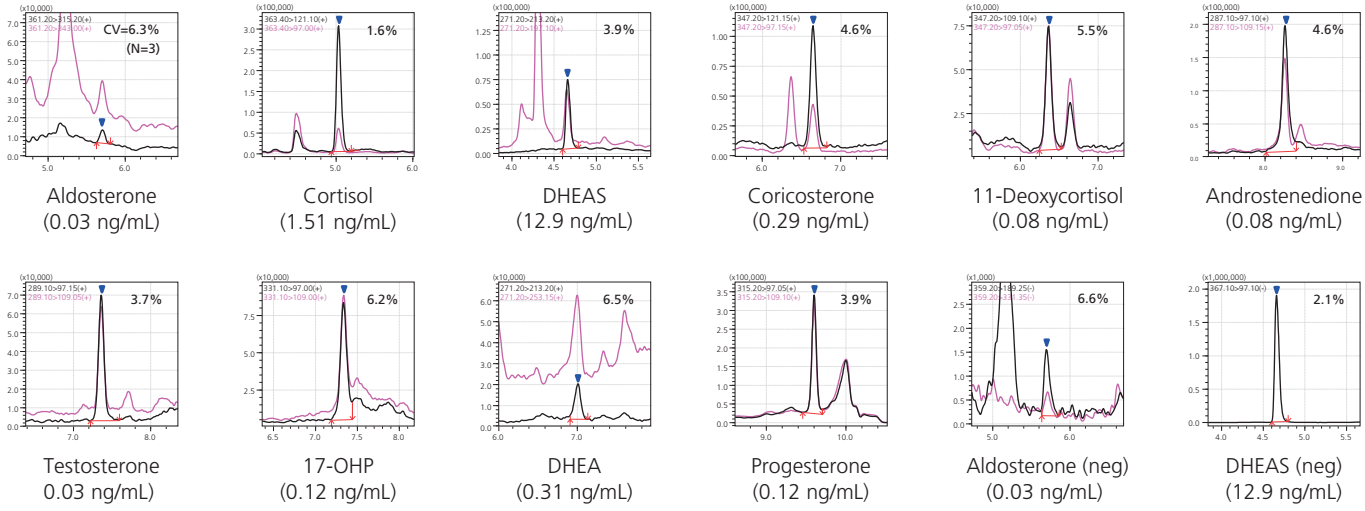
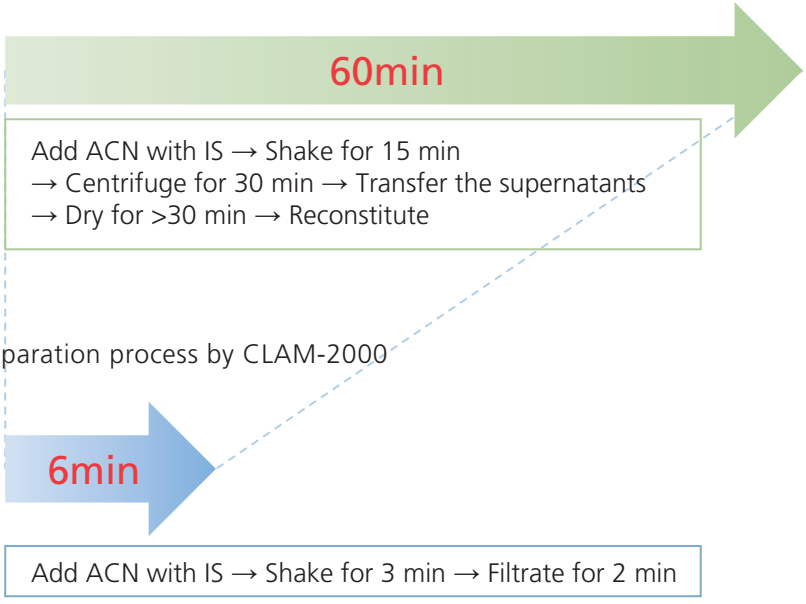


Fig.3 Calibration Curves (L1-L7) and MRM Chromatograms (L1) of 10 Steroids

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

- Traditional sample preparation (protein precipitation)



- Automated sample preparation process by CLAM-2000

Fig.4 Comparison with a time required for sample preparation

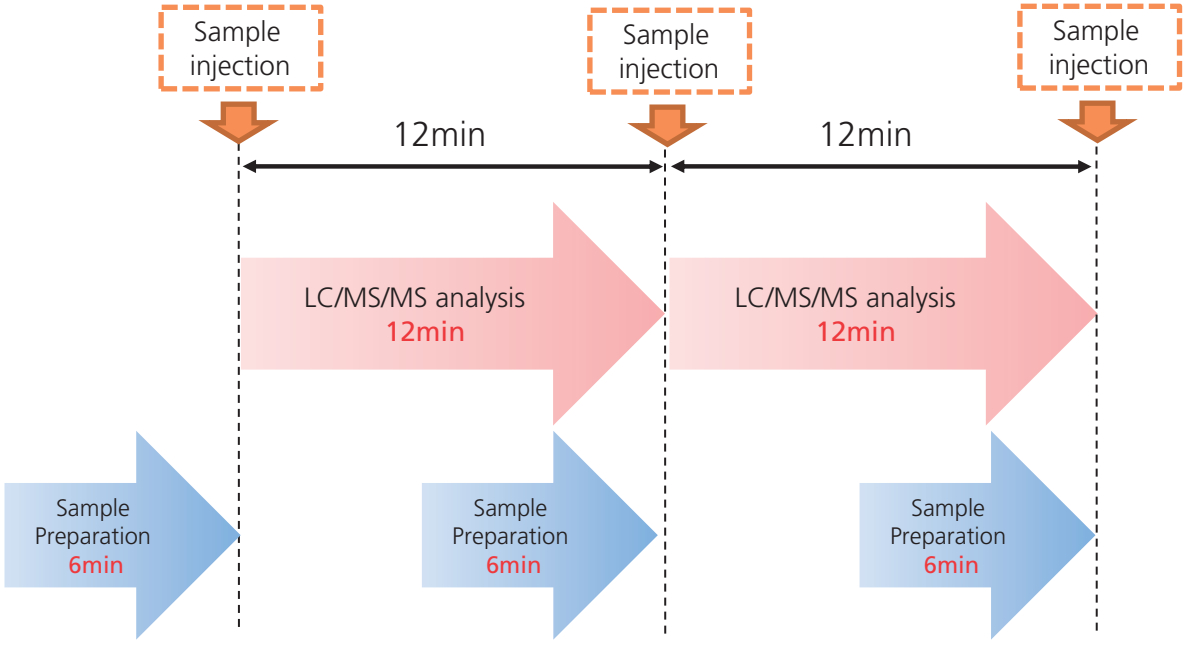


Fig.5 Analytical Flow with Parallel Processing

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

Conclusions

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

Disclaimer: CLAM-2000 and LCMS-8060 are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

64th ASMS Conference on Mass Spectrometry & Allied Topics, June 5-9, 2016, San Antonio, TX

First Edition: May, 2016

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/