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Application News

LCMS-8060 Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of 20 Steroid Hormones and High-Sensitivity Analysis of Estrogens

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User Benefits

- Simultaneous analysis of 20 steroid hormones in 15 minutes
- Derivatization of estrogens for high-sensitivity analysis

■ Introduction

Steroid hormones are a group of biomolecules characterized by having a steroid skeleton in their molecular structure and are involved in the control mechanisms of a wide range of physiological phenomena, including metabolism, nerve transmission, gene expression, reproduction, blood pressure, and vascular permeability. In recent years, the approach of analyzing multiple steroid hormones simultaneously and analyzing them as profiles has been attracting attention as it has brought new findings in the fields of regenerative medicine and carcinogenesis. The ability to easily measure steroid hormone profiles is expected to accelerate research that will lead to the elucidation of the mechanisms of various diseases and therapeutic methods.

Immunoassay techniques, such as ELISA and RIA, are widely used for quantitation of steroid hormones, but they are not suitable for profile measurements because they require reagent kits and sample volumes for each measurement item. Immunoassays also carry the risk of inaccurate results due to antibody cross-reactivity, which has led to the development of highly specific steroid hormone quantification methods using LC/MS/MS for research applications. However, since steroid hormones in biological samples are a mixture of extremely low concentrations of some steroids, such as estrogen, and high concentrations of others, various innovations are required to establish simultaneous analysis even with LC/MS/MS methods that excel in quantitative dynamic range.

This article introduces an example of simultaneous determination of 20 steroid hormones in human blank serum matrix using the LC/MS/MS Method Package for Steroid Hormones (Figs. 1 and 2). This method package includes a sample preparation procedure and analytical conditions that enable the quantification of 20 steroid hormones.

Fig. 1 LC/MS/MS Method Package for Steroid Hormones

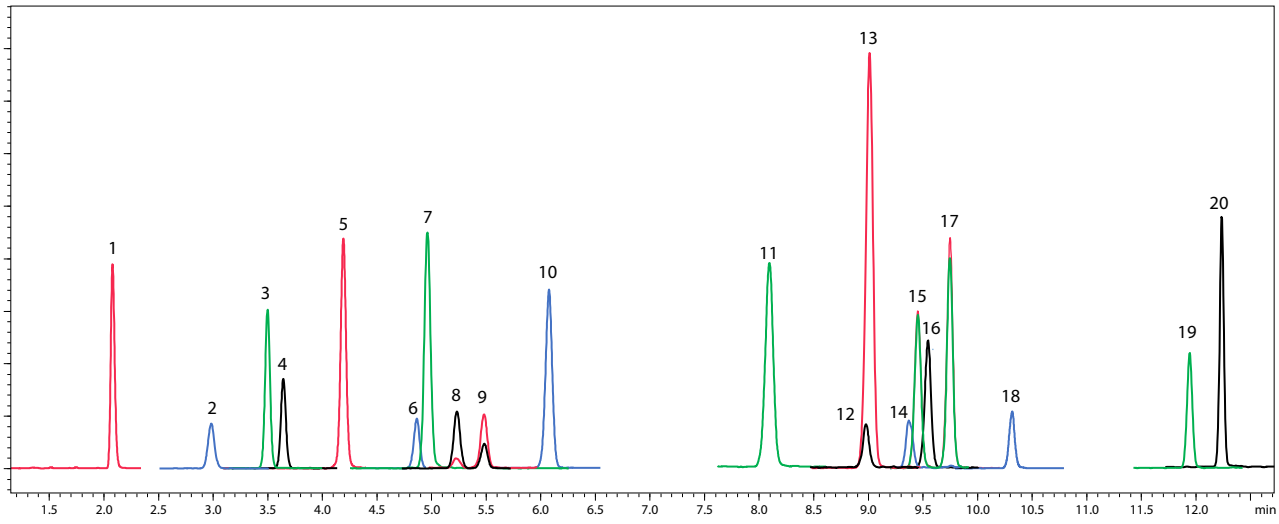

Fig. 2 Chromatogram of 20 Steroid Hormones Analyzed Simultaneously using LC/MS/MS Method Package for Steroid Hormones
1. Estriol (Derivatized), 2. Aldosterone, 3. Cortisol, 4. Cortisone, 5. Estradiol (Derivatized), 6. Dehydrocorticosterone, 7. Estrone (Derivatized),
8. 21-Deoxycortisol, 9. Corticosterone, 10. 11-Deoxycortisol, 11. Androstenediol, 12. 17-Hydroxypregnenolone, 13. Testosterone,
14. Dehydroepiandrosterone, 15. Deoxycorticosterone, 16. Androstenedione, 17. 17-Hydroxyprogesterone,
18. Dihydrotestosterone, 19. Pregnenolone, 20. Progesterone

■ Sample Preparation and Analytical Conditions

A standard-spiked serum used for quantitative evaluations was prepared by adding 20 steroid hormones and 19 stable isotopes as internal standards to a steroid-eliminated blank serum (Golden West Diagnostics, Catalog#: MSG3000, Mass Spect Gold Human Serum, Ultra-Low Hormones & Steroids, Lipid Free). Mixed standard samples without serum were also prepared for the calibration curves (0.5, 1, 5, 10, 50, 100, 500 pg/mL and 1, 5, 10 ng/mL of standard samples). After preparing each sample, quantitative analysis by LC/MS/MS was performed to calculate the accuracy and precision (CV).

Sample Preparation of Calibration Samples and Standard-Spiked Serum (patent pending: 2023-015154)

The sample preparation flow is shown below (Fig. 3). Calibration samples or the standard-spiked serum were loaded onto a solid-phase column and estrogen and non-estrogen fractions were eluted separately. The estrogen fraction was then subjected to derivatization using a derivatization reagent (2-Fluoro-1-methylpyridinium *p*-Toluenesulfonate) (Fig. 4). After derivatization, both fractions were mixed and subjected to LC/MS/MS.

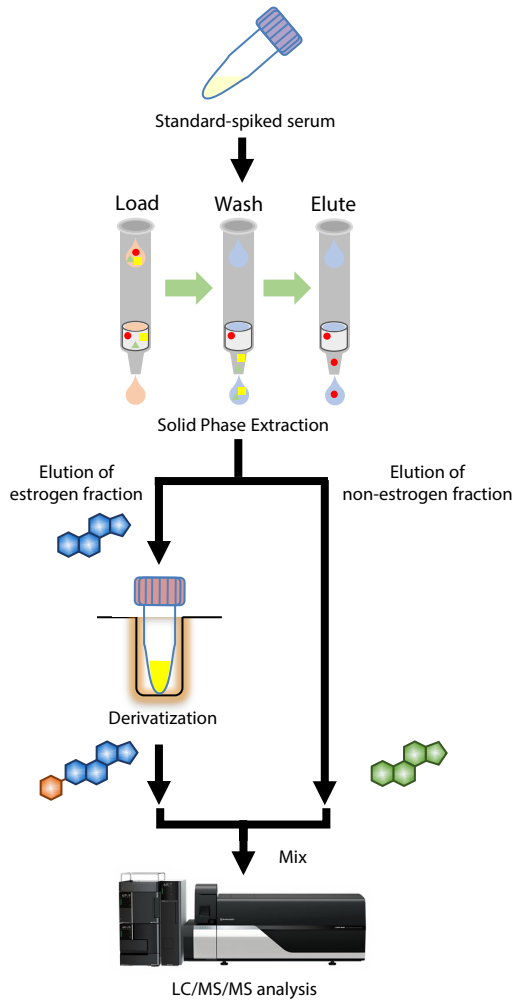

Fig. 3 Workflow of Sample Preparation

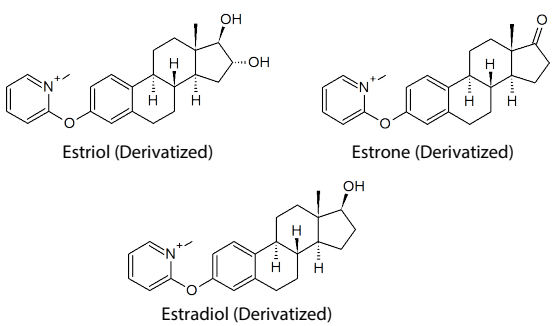

Fig. 4 Derivatized Estrogens

LC/MS/MS Analysis Conditions

LC/MS/MS analysis conditions are shown in Table 1.

Table 1 LC/MS/MS Analysis Conditions

System:	Nexera™ X3
Column:	HALO ES-C18, 100 mm × 3 mm I.D., 2.7 µm
Mobile Phases:	A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Mode:	Gradient elution (15 min)
Flowrate:	0.4 mL/min
Injection Volume:	10 µL
System:	LCMS-8060
Ionization:	ESI (Positive)
Mode:	MRM
Nebulizing Gas Flow:	3 L/min
Drying Gas Flow:	10 L/min
Heating Gas Flow:	10 L/min
Interface Temp.:	400 °C
DL Temp.:	300 °C
Heat Block Temp.:	500 °C
Interface Voltage:	0.5 kV

■ Quantitative Analysis Using LC/MS/MS Method Package for Steroid Hormones

Table 2 shows the results of the determination of steroid hormone concentrations in the standard-spiked serum (n=3). Using LC/MS/MS Method Package for Steroid Hormones, good quantification results were obtained (70 % < Accuracy < 130 %, CV < 20 %). Fig. 5 shows the calibration curve for derivatized estrogens and the chromatogram of derivatized estrogens in the standard-spiked serum. The respective LOQs for the derivatized estrogens were 1 pg/mL for Estradiol, 0.5 pg/mL for Estrone, and 100 pg/mL for Estriol.

Table 2 Steroid Hormones Quantified in Standard-Spiked Serum (n=3) (20 Compounds)

#	Compounds	Spiked (pg/mL)	Measured (pg/mL)	Accuracy %	CV
1	Aldosterone	750	830	110	9.1
2	Cortisol	750	775	103	2.2
3	Cortisone	750	789	105	2.7
4	Dehydrocorticosterone	750	804	107	6.0
5	21-Deoxycortisol	750	700	93	3.7
6	Corticosterone	200	258	129	17.4
7	11-Deoxycortisol	200	231	116	12.6
8	Androstenediol	2000	1436	72	5.2
9	Testosterone	750	811	108	1.9
10	17-Hydroxypregnenolone	2000	2260	113	9.4
11	Dehydroepiandrosterone	750	720	96	6.7
12	Androstenedione	750	810	108	2.1
13	Deoxycorticosterone	200	203	102	17.9
14	17-Hydroxyprogesterone	750	790	105	5.7
15	Dihydrotestosterone	750	824	110	3.1
16	Pregnenolone	750	942	126	4.3
17	Progesterone	750	818	109	2.4
18	Estradiol (Derivatized)	200	195	98	0.6
19	Estrone (Derivatized)	200	235	117	1.2
20	Estriol (Derivatized)	200	195	98	16.8

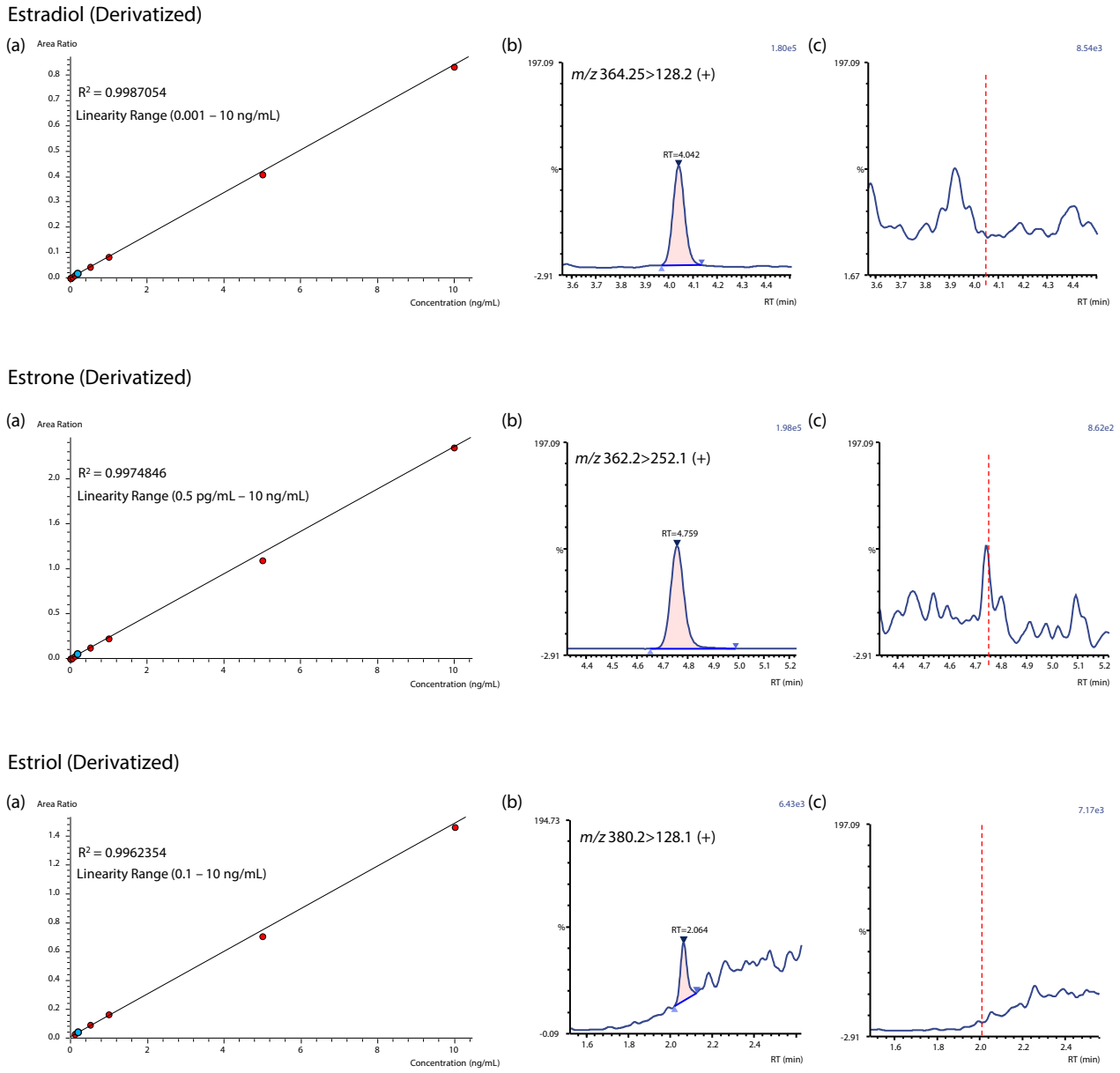

Fig. 5 Calibration Curves and Chromatograms of Derivatized Estrogens
(a) Calibration Curves, (b) Chromatograms of Standard-Spiked Serum (Estrogens were Spiked at 200 pg/mL per Sample), (c) Chromatograms of Blank Serum

■ Conclusion

Quantitative analysis of 20 steroid hormones in human blank serum matrix was performed using LC/MS/MS Method Package for Steroid Hormones. The analysis took 15 minutes, and good quantitative results were obtained for all the components. For the derivatized estrogens, Estradiol, Estrone, and Estriol showed LOQs of 1 pg/mL, 0.5 pg/mL, and 100 pg/mL, respectively, so high-sensitivity analysis was achieved. LC/MS/MS Method Package for Steroid Hormones enables simultaneous determination of steroid hormones in serum.

■ Acknowledgment

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