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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer

High-Sensitivity Analysis of Cortisol in Human Blood Serum Using LCMS-8060NX Triple Quadrupole Mass Spectrometer

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User Benefits

- Quantitative analysis of cortisol with an extremely low concentration value in blood serum is possible by the LC/MS/MS method optimized for high-sensitivity analysis.
- This method can be proposed as a total solution which includes sample preparation.
- High device robustness is realized because the IonFocus™ unit introduces ions efficiently into the MS.

■ Introduction

Cortisol is secreted from the adrenal cortex and is an essential hormone for maintaining life, as its functions include promoting the metabolism of various nutrients such as proteins, carbohydrates, and fats, an anti-inflammatory action, and an immunosuppressive action. Measurement of cortisol is important as a diagnostic index of the pituitary function and the adrenal cortex function. Although immunoassay methods such as the electrochemical luminescence immunoassay method (ECLIA) and the chemiluminescence immunoassay method (CLIA) are used in measurements, cross-reactivity with other steroids and steroid-type drugs is a concern. Therefore, this article introduces a quantitative analysis method using LC/MS/MS, which provides high sensitivity and selectivity.

In order to verify the applicability of this method to monitoring of cortisol decrease in blood serum, in this experiment, we used commercially-available human blood serum which had been treated for steroid removal (hereinafter, desteroidized human blood serum), and confirmed the measurability of cortisol from the low concentration region rather than from its normal value.

In addition, when using LC/MS/MS, contamination in the MS is an issue when measuring biological specimens. However, use of the IonFocus unit of the LCMS-8060NX enables high-sensitivity analysis while reducing contamination in the MS because only ions are introduced efficiently into the MS (Fig. 1).

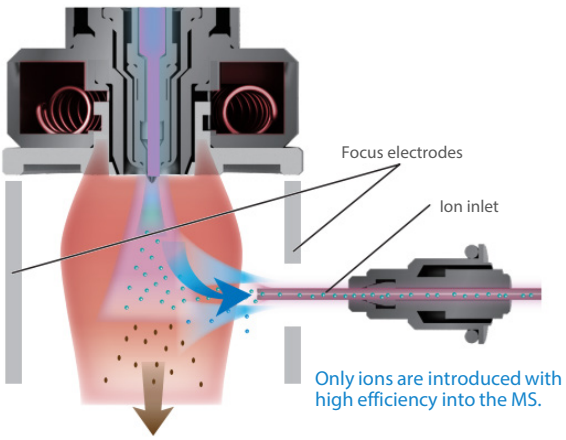

Fig. 1 Concept of IonFocus Unit

■ Sample Preparation

Serial dilution of a reference standard of cortisol was carried out with 50 % methanol (aq), and a calibration curve was prepared using standard samples adjusted to concentrations of 2.5 to 100000 pg/mL.

Spiked samples of the desteroidized blood serum were prepared using an EVOLUTE EXPRESS ABN 30 mg plate. Fig. 2 shows the workflow of sample preparation.

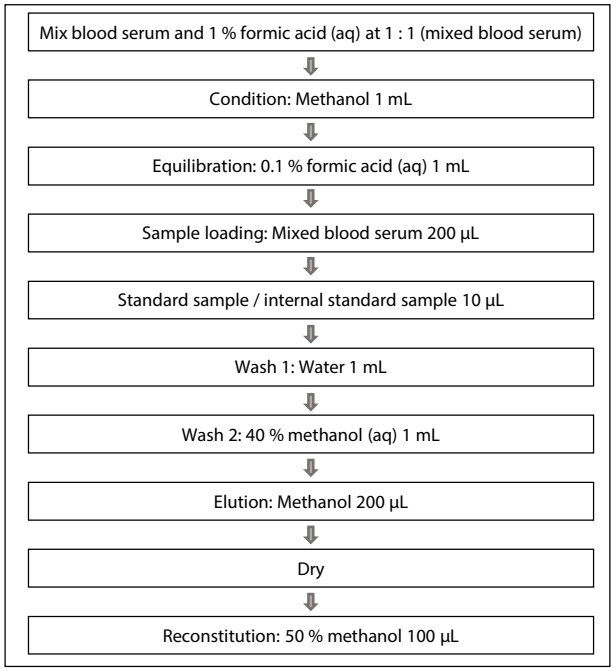

Fig. 2 Workflow of Sample Preparation

■ Analysis Conditions

Table 1 shows the LC conditions. Tables 2 and 3 show the MS conditions and the MRM transitions, respectively.

Table 1 LC Conditions

[HPLC conditions] (Nexera™ X3)	
Column	: Shim-pack Scepter™ C18-120, 50 mm × 2.1 mmI.D., 3 µm*1
Mobile phases	: A) 0.2 mM NH_4F in water B) methanol
Gradient Program	: B 50 % (0 min) → 95 % (4 - 6 min) → 50 % (6.1 -8 min)
Flow rate	: 0.3 mL/min
Injection volume	: 2 µL

*1: P/N 227-31012-03

Table 2 MS Conditions

[MS conditions] (LCMS-8060NX)	
Ionization	: ESI (Positive mode)
Mode	: MRM
Interface voltage	: 0.5 kV
IonFocus voltage	: 3.0 kV
Nebulizing gas flow	: 2.0 L/min
Drying gas flow	: 5.0 L/min
Heating gas flow	: 15.0 L/min
DL temp.	: 150 ˚C
Block heater temp.	: 500 ˚C
Interface temp.	: 400 ˚C
Probe position	: +1.5 mm

Table 3 MRM Transitions

Compound	Precursor *m/z*	Product *m/z*
Cortisol	363.0	121.1
Cortisol-D_4	367.1	121.1

■ Results of Analysis of Standard Sample

Linearity was confirmed by 3 repeated measurements of the standard samples used for the calibration curve spiked with 1 ng/mL of the internal standard solution (50 % methanol). Fig. 3 shows the calibration curve prepared by the internal standard method. Good linearity was obtained, as the coefficient of determination R^2 = 0.999 at all concentrations from 2.5 to 100000 pg/mL, and the accuracy at all calibration points was within 80 to 110 %.

Fig. 4 shows the MRM chromatogram of the 2.5 pg/mL standard solution.

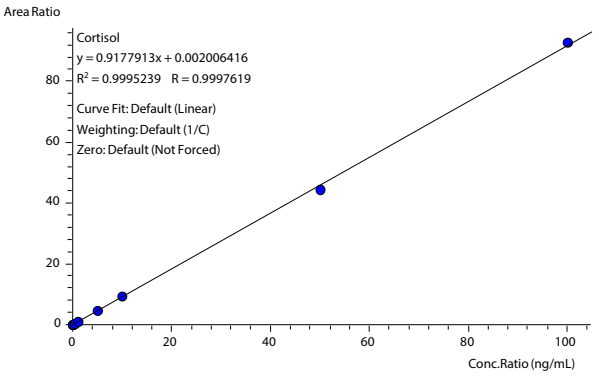

Fig. 3 Calibration Curve

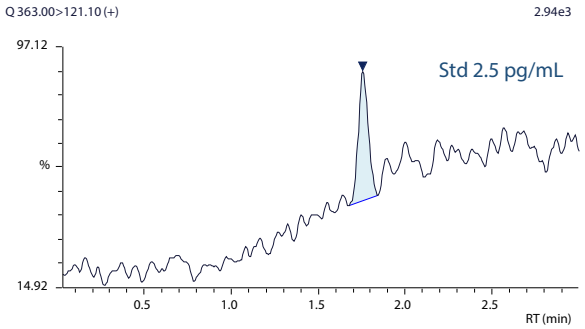

Fig. 4 MRM Chromatogram (Neat STD: 2.5 pg/mL)

■ Results of Analysis of Spiked Human Blood Serum Sample

When the samples prepared in this experiment were measured, cortisol was detected from the commercial desteroidized human blood serum. In comparison with the spiked concentration of 100 pg/mL of the serum, the value obtained by subtracting the amount detected from the desteroidized human blood serum was 92 pg/mL, which was a satisfactory result. Fig. 5 shows the MRM chromatogram of the spiked human blood serum sample.

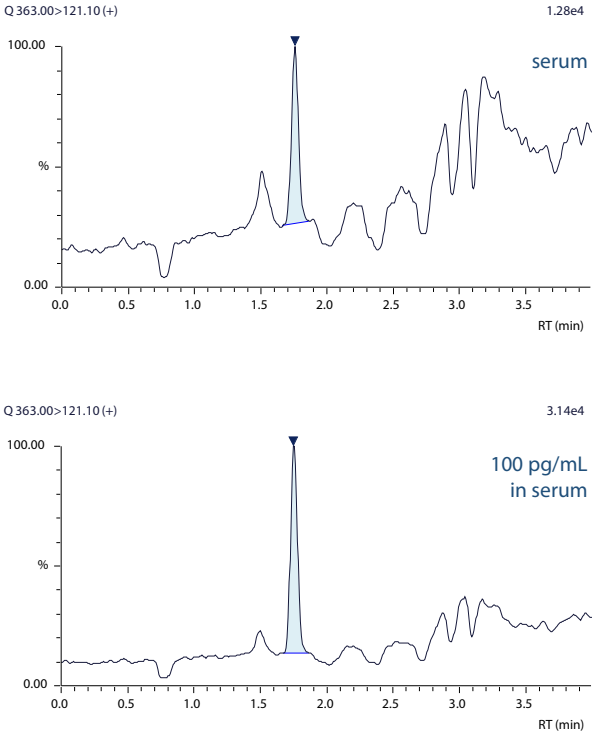

Fig. 5 MRM Chromatograms
(Top: Desteroidized Human Blood Serum,
Bottom: Spiked Human Blood Serum)

■ Conclusion

A quantitative analysis of cortisol was conducted using an LCMS-8060NX. As a result, a quantitative analysis with high sensitivity over a wide concentration range of 2.5 to 100000 pg/mL was possible, and good results were also confirmed with spiked human blood serum. Although cortisol was also detected in the desteroidized human blood serum, this experiment demonstrated the possibility of detecting cortisol in blood serum to the 2.5 pg/mL level.

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