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Application News

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LC-MS

High Sensitivity Analysis of Testosterone in Human Serum Using LCMS™-8060NX

This report introduces an example of high sensitivity analysis of testosterone in serum using LCMS-8060NX, a triple quadrupole mass spectrometer. Calibration curves prepared using standard samples showed linearity in the concentration range of 0.05 - 1000 pg/mL (R^2 =0.9996), with %RSD of concentration of 2.47% at the lower limit of quantification and accuracy at each calibration point ranged from 94.64 - 111.04%. Furthermore, the accuracy of serum samples spiked with testosterone (1 pg/mL) was 105%, indicating that the method used in the analysis is suitable for the actual samples.

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Introduction

Testosterone (Fig. 1), an androgen produced in the testes and the adrenal glands, is a steroid hormone which contributes to the growth enhancement of bones and muscles and hematopoiesis. The free-testosterone level in blood plays a key clinical role as a biomarker for a variety of diseases, such as Cushing's syndrome and tumors. Testosterone levels in blood have usually been measured using immunoassay, but these levels may be affected by the cross reactivity caused by steroid hormones having similar structures. LC/MS/MS with high selectivity prevents the problems caused by the conventional method and enables more accurate quantitative analysis, and it is expected that it will come into clinical use.

This report introduces an example of high sensitivity quantitative analysis of testosterone in serum using LCMS-8060NX.

Sample Preparation

Standard samples were serially diluted with 50% methanol, prepared at concentrations of 0.05 - 1000 pg/mL and used for preparation of the calibration curve.

For the samples spiked with serum, commercially available steroid-free human serum was spiked with testosterone to make a serum concentration of 1 pg/mL. The standard sample and internal standard were spiked with 100 µL of serum, and then shaken well with a mixture of hexane and ethyl acetate (9 : 1). The solution obtained was centrifuged for 10 minutes, followed by evaporating the organic solvent layer to dryness, and then re-dissolved with 50% methanol.

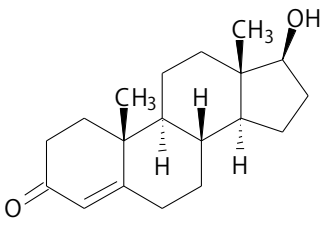

Fig. 1 Structural Formula of Testosterone

Analytical Conditions

The analytical conditions for HPLC and MS are shown in Table 1. The MRM transitions are shown in Table 2.

Table 1 Analytical Conditions

[HPLC conditions] (Nexera™ X3)	
Column	: Shim-pack Scepter™ C18-120, 50 mm × 2.1 mm I.D., 1.9 µm*1
Mobile phases	: A) 0.05 mM NH_4F in water B) methanol
Mode	: Gradient elution
Flow rate	: 0.3 mL/min
Injection volume	: 25 µL
[MS conditions] (LCMS-8060NX)	
Ionization	: ESI (Positive mode)
Mode	: MRM
Interface voltage	: 1 kV
IonFocus voltage	: 3 kV
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 15.0 L/min
Heating gas flow	: 15.0 L/min
DL temp.	: 250 °C
Block heater temp.	: 500 °C
Interface temp.	: 350 °C
Probe position	: +1.5

*1 : P/N 227-31012-03

Table 2 MRM Transition

Compound	Precursor *m/z*	Product *m/z*
Testosterone	289.25	97.15
Testosterone-[13C3]	292.25	100.20

Calibration Curve

The calibration curve prepared using the standard sample (internal standard method, n=3 for each concentration) showed good linearity in a wide dynamic range from 0.05 - 1000 pg/mL with a coefficient of determination (R^2) of 0.9996. The %RSD of concentration at the lower limit of quantification (0.05 pg/mL) was 2.47% with the accuracy at each calibration point ranging from 94.64 - 111.04%. Fig. 2 shows the calibration curve, and Fig. 3 shows the MRM chromatogram of the 0.05 pg/mL standard solution.

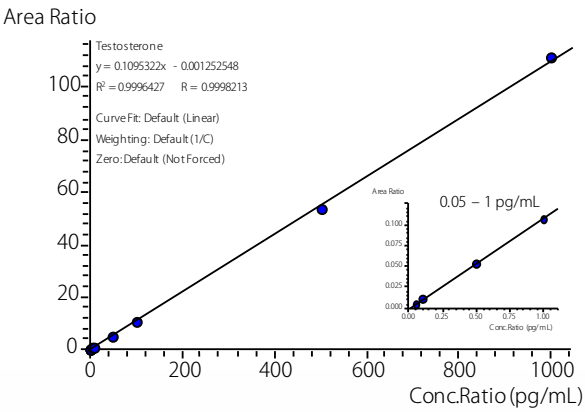

Fig. 2 Calibration Curve (0.05 - 1000 pg/mL)

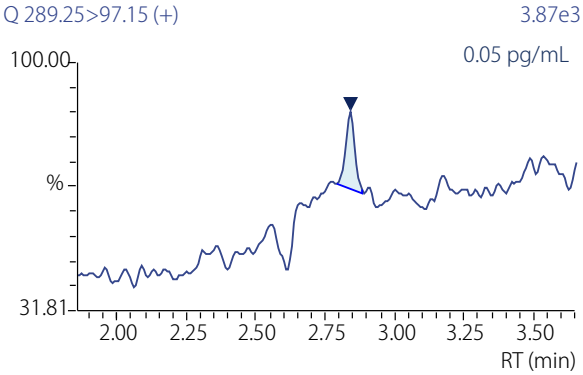

Fig. 3 MRM Chromatogram (neat STD: 0.05 pg/mL)

Sample Spiked with Human Serum

Based on the area obtained by the measurement of the pretreated sample, the testosterone level was calculated using the calibration curve shown in Fig. 2. Good accuracy was obtained and the mean level of testosterone spiked at 1 pg/mL with serum was 1.05 pg/mL (n=3). Fig. 4 shows the MRM chromatogram of the sample spiked with human serum.

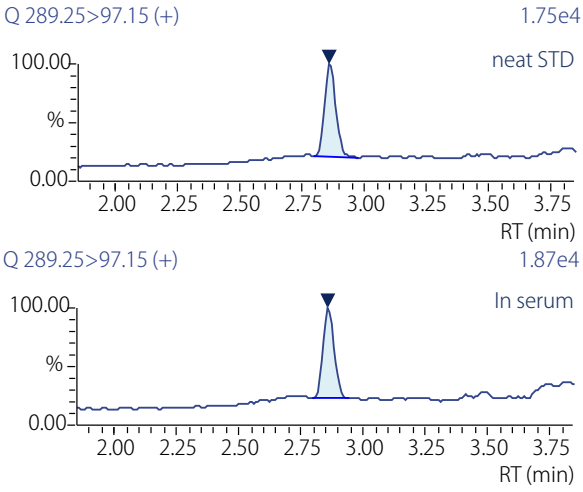

Fig. 4 MRM Chromatogram at 1 pg/mL (Upper: neat STD, Lower: Sample Spiked with Human Serum)

Conclusion

Quantitative analysis of testosterone using LCMS-8060NX demonstrated that the testosterone level can be determined across a wide range of 0.05 - 1000 pg/mL, and that good accuracy is achieved with samples spiked with human serum.

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