

**Quantitative Analysis of Oxytocin in Rat Plasma
Using LC/MS/MS**

Oxytocin is a peptide hormone that consists of nine amino acids and one S-S bond and is secreted by the posterior pituitary gland. It is used to induce labor pains, as it promotes contraction of the uterine muscles. Oxytocin has attracted attention in recent years as it is thought to be strongly related to interpersonal relationships, particularly bonding between mothers and children, family members, and romantic partners.

This article introduces an example in which oxytocin in rat plasma was analyzed with an LCMS™-8050 triple quadrupole mass spectrometer.

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Sample and Pretreatment

A rat plasma sample was collected after centrifuging blood (rat SLC Wistar derived) treated with heparin to inhibit coagulation. Protein removal treatment was conducted by adding a 10-fold volume (1 mL) of acetonitrile to 100 µL of this plasma. After additional centrifuging, 1 mL of the supernatant was transferred to another tube and centrifugally concentrated under a reduced pressure. Fig.1 shows the pretreatment workflow of the rat plasma sample.

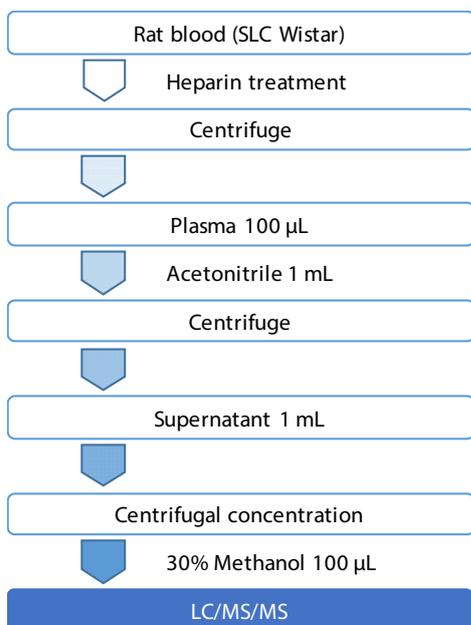


Fig. 1 Pretreatment Workflow of Rat Plasma Sample

Analysis of Standard Substance

The standard substance of oxytocin was properly dissolved and diluted using a 30% methanol solution, and LC/MS/MS measurements were conducted under the analysis conditions shown in Table 1. Fig. 2 shows a typical MRM chromatogram (10 ng/mL) of oxytocin.

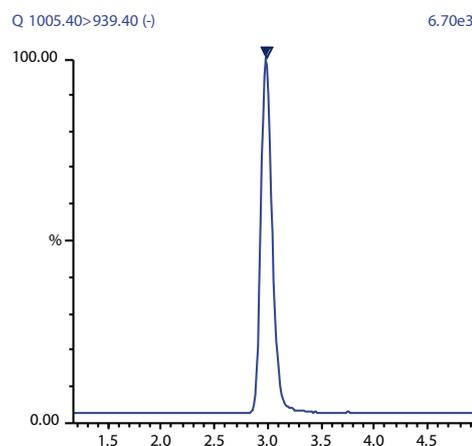


Fig. 2 Typical MRM Chromatogram of Oxytocin (10 ng/mL)

Table 1 Analysis Conditions

[HPLC conditions] (Nexera™ X2)	
Column	: Shim-pack™ GISS-HP (2.1 × 50 mm, 3 µm)
Mobile phases	: A) Water B) Methanol
Time programs	: B conc. 30% (0 min) → 50% (5 min) → 30%(7.01-10 min)
Column temp.	: 40 °C
Flow rate	: 0.3 mL/min
Injection volume	: 2 µL
[MS conditions] (LCMS-8050)	
Ionization	: ESI (Negative mode)
Mode	: MRM
Nebulizing gas flow	: 2.0 L/min
Drying gas flow	: 15.0 L/min
Heating gas flow	: 5.0 L/min
DL temp.	: 150 °C
Block heater temp.	: 500 °C
Interface temp.	: 350 °C
MRM Transition	: 1005.4 > 939.4 (CE : +56 V)

Linearity of Calibration Curve

Fig. 3 shows the calibration curve of oxytocin. When calibration curves were prepared in the concentration range from 0.1 to 500 ng/mL, satisfactory linearity with a contribution rate $r^2=0.999$ or more was obtained. Table 2 shows the measurement results of the oxytocin standard substance solution.

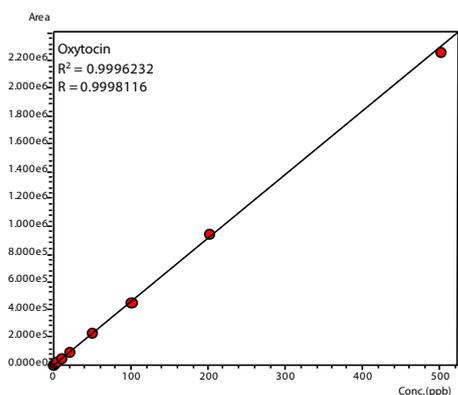


Fig. 3 Calibration Curve of Oxytocin (0.1 to 500 ng/mL)

Table 2 Measurement Results of Oxytocin Standard Substance Solution

Compound	Cal. Range (ng/mL)	Contribution rate r^2	%RSD (0.1 ng/mL, n=3)
Oxytocin	0.1-500	0.999	8.27

Measurement of Plasma Sample

The pretreated rat plasma sample was measured, and quantitation was carried out using the calibration curve obtained from the measurements of the standard substance solution. Oxytocin was also detected from the unspiked rat plasma blank sample. Fig. 4 and Table 3 show the MRM chromatogram and the quantitation results of the rat plasma blank sample, respectively.

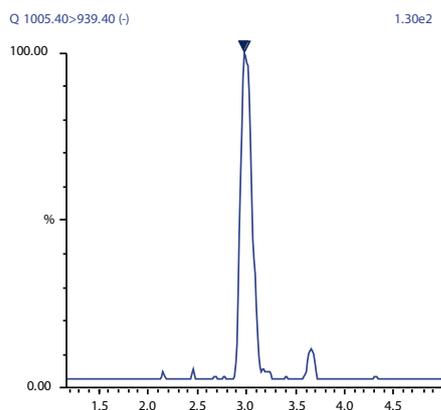


Fig. 4 MRM Chromatogram of Rat Plasma Sample

Table 3 Quantitation Results of Rat Plasma Sample

Sample Name	Conc. (ng/mL)
Rat Plasma Sample	0.23

Plasma Sample Spike and Recovery Test

A spike and recovery test was conducted by spiking a rat plasma sample with the standard substance to an oxytocin concentration of 2 ng/mL. As a result, a satisfactory recovery rate was obtained. Fig. 5 and Fig. 6 show the MRM chromatograms of the oxytocin standard substance (2 ng/mL) and the rat plasma sample (2 ng/mL spiked), respectively. Table 4 shows the results of the spike and recovery test of the rat plasma sample.

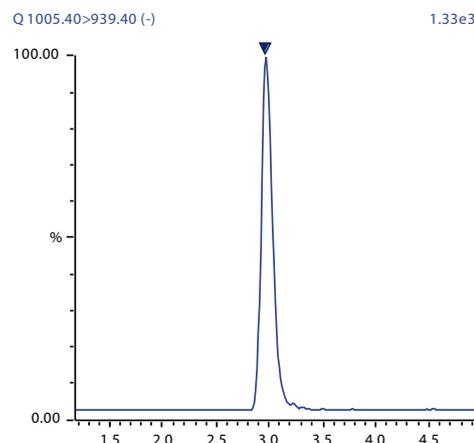


Fig. 5 MRM Chromatogram of Oxytocin Standard Substance (2 ng/mL)

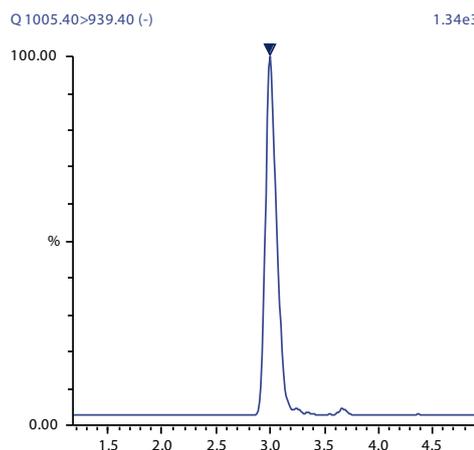


Fig. 6 MRM Chromatogram of Rat Plasma Sample (2 ng/mL Spiked)

Table 4 Results of Spike and Recovery Test of Rat Plasma Sample

Oxytocin	2 ng/mL spiked
Recovery rate	91.08%

<Acknowledgement>

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