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Application News

No. C202

Liquid Chromatography Mass Spectrometry

Analysis of Chlorpromazine in Milk and Chicken Egg Extracts using Triple Quadrupole LC/MS/MS

Chlorpromazine hydrochloride is used as a tranquilizer (pharmaceutical). At the same time, the use of veterinary medicines that have chlorpromazine as an active constituent is prohibited on animals to be used for food, and those which produce milk, eggs, etc. to be shipped for food. (Ministry of Agriculture, Forestry and Fisheries Ordinance No. 44, 2013)

In addition, in the Positive List system, chlorpromazine is classified as a substance which must not be contained in food, and the LC/MS method has been cited as the method for testing for it in the "Standards for Food, Food Additives, etc." (Ministry of Health and Welfare Notification No. 370, 1959).

However, this test method cannot be applied to all livestock and seafood, and it is being reviewed because it may not be possible to obtain good analysis results depending on the food.

In March 2019, the Pharmaceutical Affairs and Food Sanitation Council (food sanitation subcommittee, agricultural chemicals and veterinary medicines group) reported a consultation document (Ministry of Health, Labour and Welfare Notification 0220-4) on a new chlorpromazine test method whose development has been completed.

In this article, we present an example analysis of chlorpromazine in milk and chicken eggs in accordance with the test method described in the consultation document.

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Sample Pretreatment

In accordance with the draft report on the test method, 10 g of milk or chicken egg was weighed out, subjected to extraction twice using acetone, then made up to the fixed volume of 100 mL.

A volume of 10 mL was collected, ultrapure water and formic acid were added, and solid phase extraction was performed using a sulfonate-modified methacrylate copolymer mini-column.

After concentrating the eluate to about 1 mL at 40 °C, it was accurately made up to the fixed volume of 5 mL with a mixture of 0.1% formic acid solution and 0.1% formic acid acetonitrile (3:2), which was used as the sample for measurement.

Although the sample coverage has broadened, there are fewer treatment processes than those with the conventional test method, making the pretreatment easier.

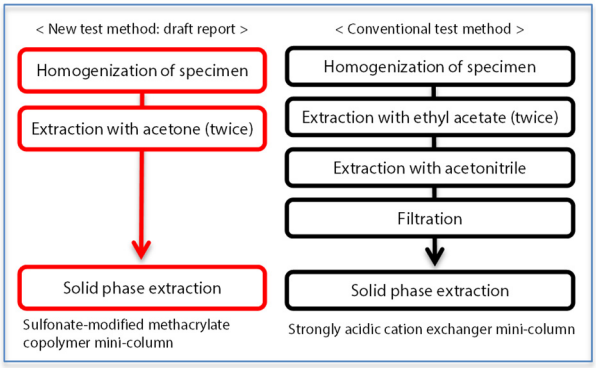

Fig. 1 Pretreatment Operations

Linearity of MRM Chromatograms and Calibration Curves of Chlorpromazine Standard Solution

The chlorpromazine standard solution (10 ng/L) was analyzed and the resulting MRM chromatogram is shown in Fig. 2. The lower limit of detection for the test method being reported is taken to be 20 ng/L when an injection volume is 5 µL, but if the LCMS™-8050 is used, it is possible to measure from 10 ng/L as a quantitative lower limit concentration even if the injection volume is reduced to 2 µL.

Fig. 3 shows the calibration curve from 10 to 1,000 ng/L; good linearity was obtained with a coefficient of determination of R^2>0.9998. The analysis conditions for this are shown in Table 1.

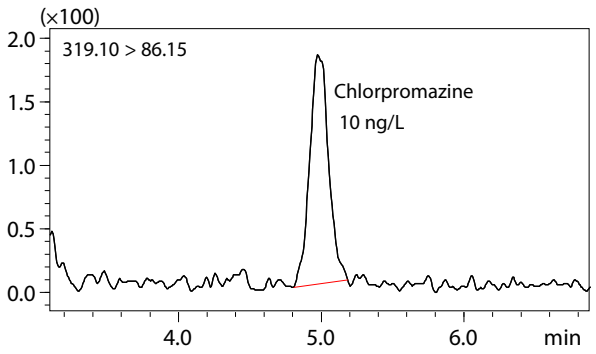

Fig. 2 MRM Chromatogram of Chlorpromazine Standard Solution

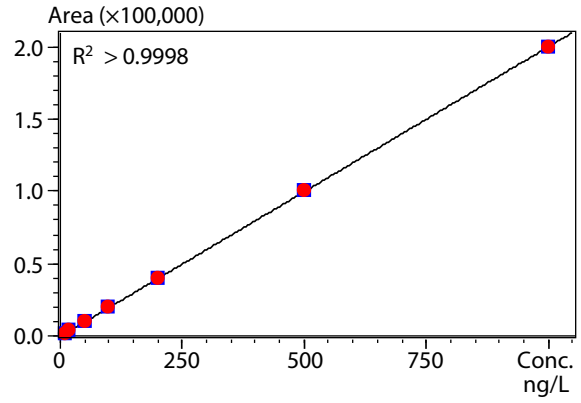

Fig. 3 Calibration Curves

Table 1 Measurement Conditions

Column	:	Shim-pack™ HR-ODS (150 mmL. × 2.1 mm i.d., 3 µm, Shimadzu Corp.)
Mobile phases	:	0.1% formic acid water / 0.1% formic acid acetonitrile = 72 / 28 (v/v)
Flow rate	:	0.20 mL/min
Column temperature	:	40 °C
Injection volume	:	2 µL
Probe voltage	:	+1.0 kV (ESI-positive)
DL temperature	:	250 °C
Block heater temperature	:	350 °C
Interface temperature	:	300 °C
Nebulizing gas flow	:	2 L/min
Drying gas flow	:	5 L/min
Heating gas flow	:	15 L/min
MRM transition	:	*m/z* 319.10 > 86.15 (quantifier ion) 321.10 > 58.10 (qualifier ion)

Milk and Egg Analysis

A blank, including pretreatment, was analyzed to ensure that no analytes were detected. (See Fig. 4)

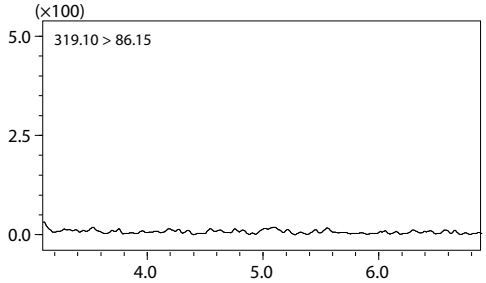

Fig. 4 Blank MRM Chromatogram

Store-bought milk and chicken eggs produced in Japan were pretreated, and the MRM chromatograms obtained for each by analyzing their extracts are shown in the upper figures in Fig. 5 and Fig. 6. With milk, minor peaks were detected, but they were generally calculated to be less than 1/5th of the quantitative lower limit, and were not detected with chicken eggs.

In addition, the chlorpromazine standard solution was added to milk and chicken eggs to achieve the equivalent of 0.0001 mg/kg, then pretreated test solutions were prepared by following the procedure shown in Fig. 1.

The MRM chromatograms obtained by analyzing them are shown in the lower figures in Fig. 5 and Fig. 6 respectively. The concentration of the test solution equivalent to 0.0001 mg/kg in the sample is 20 ng/L. As shown in Table 2 and Table 3, the recovery factors (trueness) were very good, at 103% for milk extract and 102% for chicken egg extract. Using the LCMS-8050 in this way makes it possible to accurately measure chlorpromazine.

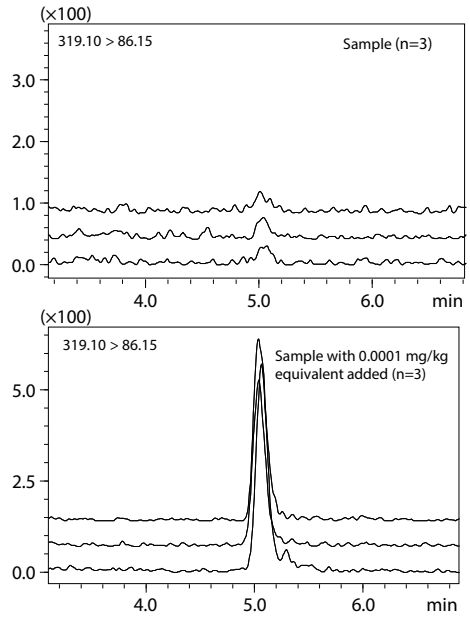

Fig. 5 MRM Chromatogram (n=3, milk)

Table 2 Spike and Recovery Test Results (n=3, milk)

	Average concentration	Average recovery	Area %RSD
Spiked sample	20.69 ng/L	103%	5.71

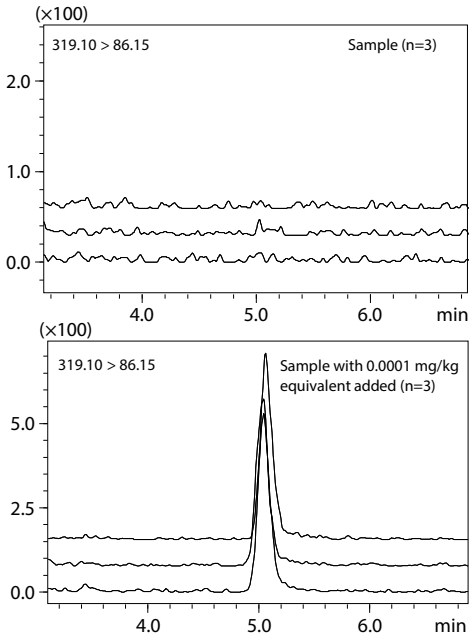

Fig. 6 MRM Chromatogram (n=3, chicken egg)

Table 3 Spike and Recovery Test Results (n=3, chicken eggs)

	Average concentration	Average recovery	Area %RSD
Spiked sample	20.50 ng/L	102%	2.45

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