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Application News

No. C199

Liquid Chromatography Mass Spectrometry

Analysis of Chloramphenicol in Shrimp and Chicken Egg Extracts Using Triple Quadrupole LC/MS/MS

Chloramphenicol is an antibiotic with a broad antimicrobial spectrum and is widely used as a veterinary medicine for the prevention and treatment of livestock diseases.

When the positive list system was introduced, chloramphenicol was set as a component that was not to be detectable in food. During the 2014 review, it could not be denied that it is genotoxic and possibly carcinogenic, so it was reevaluated as a component that should not have a set acceptable daily intake (ADI) which must not be contained in food continuously.

In addition, since it has been confirmed that chloramphenicol glucuronide conjugates are hydrolyzed in vivo, generating chloramphenicol, the test method for chloramphenicol was revised in 2017 (Notification No. 49 of the Ministry of Health, Labour and Welfare, 2017), adding chloramphenicol glucuronide conjugates as a target of measurement. In this study, we present an example analysis of quantified chloramphenicol in shrimp and chicken eggs in accordance with the revised test method.

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Sample Pretreatment

The shrimp was shredded and homogenized, and 10 g was weighed and taken. In addition, the chicken eggs were well mixed and homogenized and 10 g was weighed and taken.

Methanol was added to each sample, and after fine homogenization they were centrifuged twice to remove the supernatant, then made up to the fixed volume of 100 mL with methanol. 4 mL was collected, the solvent was removed, then after hydrolyzation by adding 9 mL of phosphate buffer and 1 mL of β-glucuronidase solution, ethyl acetate was added and the ethyl acetate layer was removed by centrifugation. Two extractions with ethyl acetate were followed by purification using a divinylbenzene-N-vinylpyrrolidone copolymer column to achieve the sample for measurement.

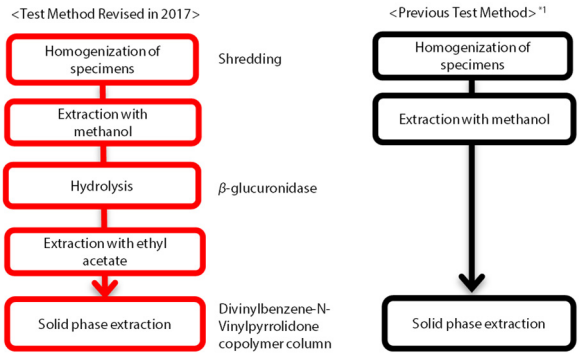


Fig. 1 Pretreatment Operation

*1 Notification No. 499 of the Ministry of Health, Labor and Welfare, 2005
Notification No. 370 of the Ministry of Health and Welfare, 1959

Analysis of Chloramphenicol and Chloramphenicol Glucuronide Conjugate Mixed Standard Solution

The MRM chromatograms obtained by measuring the concentration of chloramphenicol and chloramphenicol glucuronide conjugates in the 1 µg/L mixed standard solution are shown in Fig. 2. The analysis conditions were set such that the retention time for chloramphenicol was 4 min.

Concurrently with the revision of the test method, notice was given of points requiring attention: it is necessary to confirm in advance that interference peaks derived from enzymes do not affect the quantification, and that the chloramphenicol glucuronide conjugates are sufficiently hydrolyzed in pretreatment (Notification 0223-3, February 23, 2017).

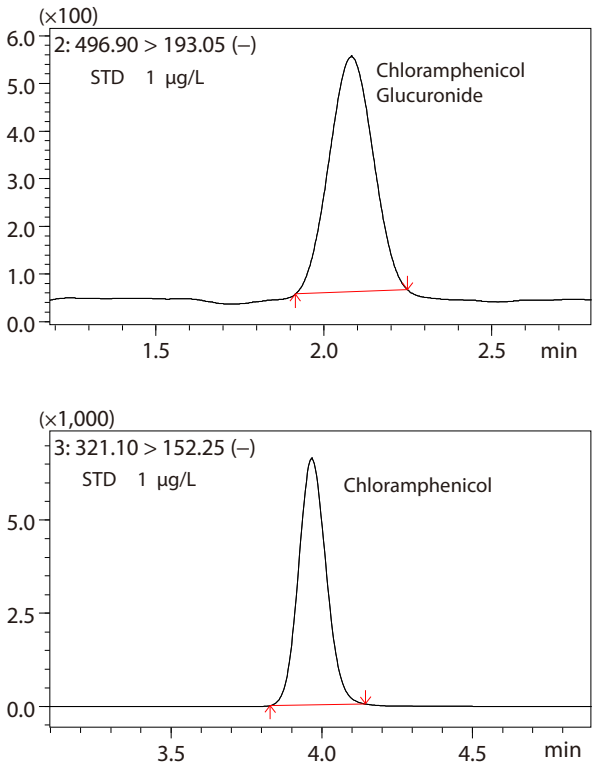


Fig. 2 MRM Chromatograms for Chloramphenicol and Chloramphenicol Glucuronide Conjugates in Mixed Standard Solution

Table 1 Analysis Conditions

Column	: Shim-pack™ HR-ODS (150 mmL. × 2.1 mm i.d., 3 µm, Shimadzu Corp.)
Mobile phases	: 10 mmol/L ammonium acetate water / Acetonitrile = 70 / 30 (v/v)
Flow rate	: 0.35 mL/min
Column temperature	: 40 °C
Injection volume	: 5 µL
Probe voltage	: −1.0 kV (ESI-Negative)
DL temperature	: 300 °C
Block heater temperature	: 500 °C
Interface temperature	: 400 °C
Nebulizing gas flow	: 3 L/min
Drying gas flow	: 10 L/min
Heating gas flow	: 10 L/min
MRM transition	: Chloramphenicol *m/z* 321.10 > 152.25 (Quantifier ion) 321.10 > 257.05 (Qualifier ion)
	: Chloramphenicol glucuronide *m/z* 496.90 > 193.05 (Quantifier ion) 496.90 > 113.00 (Qualifier ion)

Linearity of Calibration Curve

A 5-point calibration curve was created in the concentration range of 0.1 to 2 µg/L for chloramphenicol and 0.5 to 10 µg/L for chloramphenicol glucuronide conjugates.

With the LC/MS method, the lower limit of detection of chloramphenicol in livestock produce is 0.005 mg/kg, and as shown in Fig. 3, good linearity was obtained from 0.1 µg/L as the quantitative lower limit.

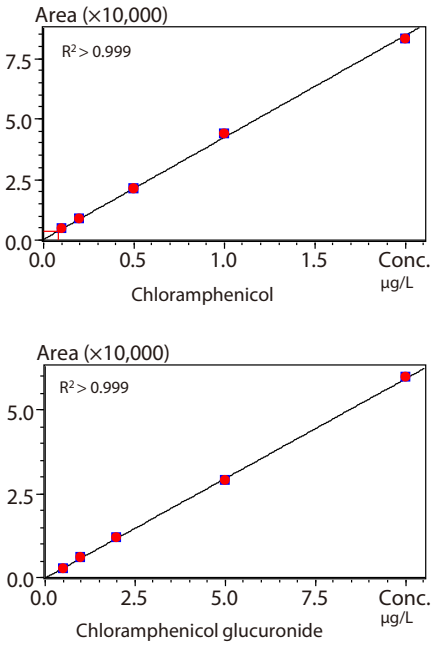
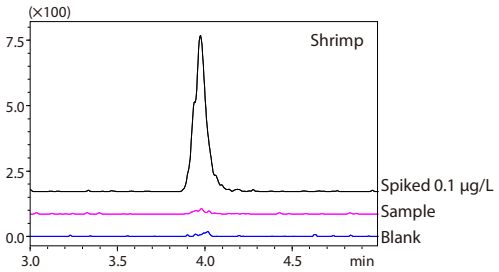


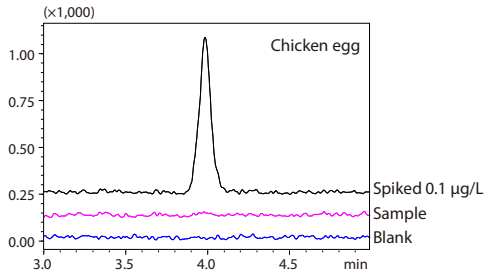
Fig. 3 Calibration Curves

Analysis of Shrimp and Chicken Eggs



Chloramphenicol	0.1 µg/L added
Recovery factor	87.9%
%RSD	7.6%

Fig. 4 Results of Spike and Recovery Test (n=3, Shrimp)



Chloramphenicol	0.1 µg/L added
Recovery factor	95.5%
%RSD	13.5%

Fig. 5 Results of Spike and Recovery Test (n=3, Chicken Egg)

The results of the measurements were that chloramphenicol was not detected in either commercially available shrimp (Indian black tiger) or chicken eggs (domestically produced in Japan). Therefore, only chloramphenicol standard solution was added to both blank samples to achieve a concentration of 0.1 µg/L, measurements were performed and it was confirmed that a recovery rate of 85% or greater can be obtained.

Further, the result of adding only chloramphenicol glucuronide conjugates to the blank solvent and measuring after the same pretreatment was that the chloramphenicol glucuronide conjugates were not detectable, while chloramphenicol was detected, which confirmed that the pretreatment implemented in this study achieved sufficient hydrolyzation.

Using the LCMS™-8050 allows accurate measurement from a concentration of 0.1 µg/L.

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