

Analysis of Chloramphenicol in Honey by LC/MS

Chloramphenicol is an effective antimicrobial with a broad antibacterial spectrum which shows antibiotic effects against both gram-positive and gram-negative bacteria. However, serious adverse drug reactions have been confirmed with this drug and it is not permitted as a residue in food by the WHO. In the Positive List System in Japan, chloramphenicol is stipulated as a substance that should not be detected in foods, but many reports in the past have described detection in seafood such as shrimp and eel, as well as in other products including honey and royal jelly.

Introduced here is an example of the analysis of chloramphenicol (added sample) in honey. Isocratic conditions (see Fig. 5) are described in the official test method. However, in consideration of the column-cleaning process, gradient conditions were used here. The calibration curve obtained under these analysis conditions was linear between 0.05 and 5.0 µg/L. Honey samples were pretreated using solid-phase extraction in accordance with the test method. Honey samples with 0.2 ng/g of added chloramphenicol were pretreated and then analyzed, with recovery rates of ≥90%.

Figure 1. Structure of chloramphenicol

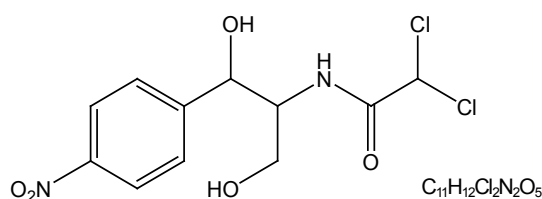


Table 1. Conditions

Column:	TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm
Mobile phase:	A: 10mmol/L ammonium formate, pH3.75 B: acetonitrile
Gradient:	0min (30% B) → 10min (100%B) → 12min (100%B)
Flow rate:	0.2mL/min
Temperature:	40°C
Injection vol.:	5µL
Instrument:	QTRAP [®] (Applied Biosystems)
Ion Source:	ESI
Polarity:	Positive
Precursor ion:	321.0
Product ion:	152.0

Figure 2. MRM chromatograms of chloramphenicol reference standard, honey, and honey with added chloramphenicol

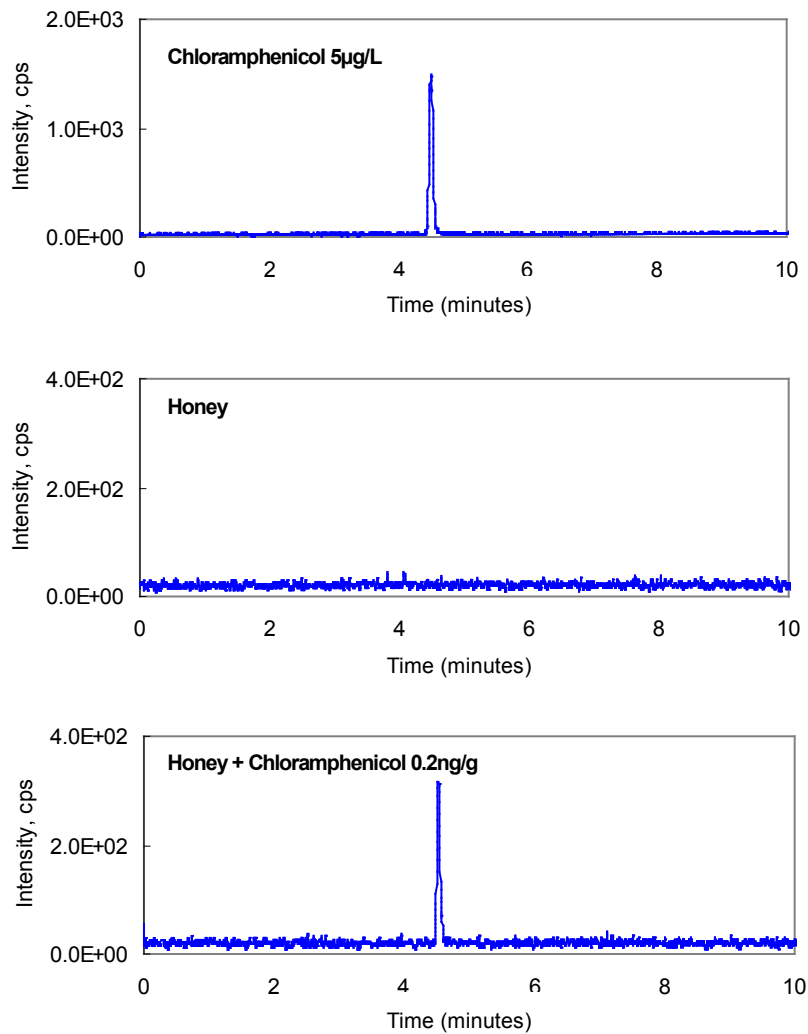


Figure 3. Honey pretreatment procedure

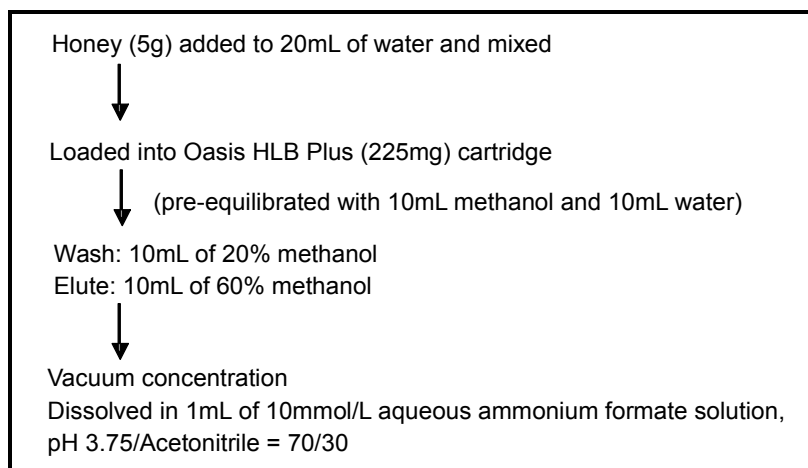


Figure 4. Chloramphenicol calibration curve

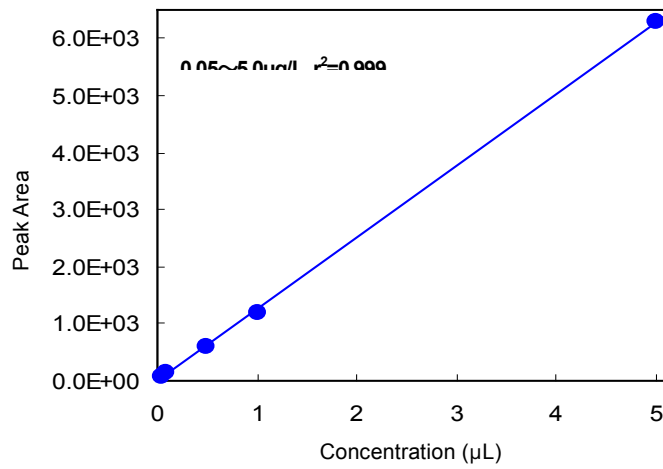


Figure 5. MRM chromatogram of chloramphenicol reference standard performed in accordance with official analysis conditions (isocratic conditions)

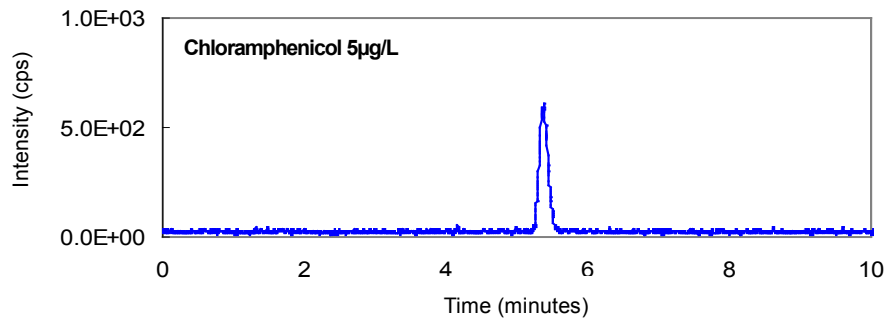


Table 2. Conditions

Column:	TSKgel ODS-100V, 3µm, 2.0mm ID × 15cm
Mobile phase:	10mmol/L ammonium formate, pH 3.75/acetonitrile = 70/30
Flow rate:	0.2mL/min
Temperature:	40°C
Injection vol.:	25µL