A High-Throughput and Sensitive Method for Quantitation and Identification of Chloramphenicol in Foods of Animal Origin Using UHPLC-MS/MS

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Introduction
Chloramphenicol (CAP) is a broad-spectrum antibiotic and potential carcinogen that is banned by WHO and also banned in the United States, Canada, European and most Asian countries in food products. However, due to its broad activity and low cost, the illegal use still remains. In this study, a fast, high-throughput and sensitive UHPLC-MS/MS method for quantification and identification of CAP was developed and validated to determine sub-µg/kg levels of CAP in infant formula, honey and seafood (shrimp, fish meat, oyster and mussel) samples. The sample preparation consists of dispersing the sample in water followed by extraction with acetonitrile, partitioning the aqueous and organic layers by the addition of sodium chloride, exchanging a portion of the acetonitrile layer into water and then analysis by UHPLC-MS/MS using negative electrospray ionization mode. The isotopically labeled internal standard CAP-d₄ is added to compensate for matrix effects. Method criteria for data acceptance and an approach for handling complex samples with severe ion suppression were established.

Analytical Method
UHPLC System Conditions
UHPLC: Agilent® Fast LC 1290
Column: Phenomenex® Synergi Fusion-RP, 50 x 2 mm, 2.5µ particle size
Column oven temperature: 30°C
Injection volume: 5 µL
Flow rate: 0.5 mL/min
Mobile Phase A: 10 mM Ammonium acetate in water
Mobile Phase B: Acetonitrile

Mass Spectrometer Conditions
Mass spectrometer: Agilent® Triple Quadrupole MS/MS 6490-A
Mode: Negative ion

ESI Parameters
Gas temperature (TEM): 270°C
Gas flow: 17 L/min
 Nebulizer: 30 psi
Sheath gas temperature: 400°C
Sheath gas flow: 12 L/min
Capillary: -4000 V
Nozzle voltage: 0 V
Cell accelerating voltage: 4 V

M RM Method
Analyte Precursor Ion Product Ion Dwell Collision Energy (V)
CAP (Quantification) 321.0 152.0 100 20
CAP (Quantification) 321.0 257.0 100 8
CAP (Quantification 1) 321.0 194.1 100 8
CAP (Quantification 2) 321.0 175.9 100 16
CAP-d₄ Internal Standard 328.0 156.8 100 20

Sample Preparation
1. Weigh 5 g of homogenized sample
2. Add 0.05 mL of CAP-d₄ internal standard solution
3. Add 0.10 mL of CAP standard solution for spiked samples
4. Add 10 mL H₂O and 10 mL ACN, mix by vortexing
5. Add 2 g sodium chloride, shake to mix the sample
6. Centrifuge at 2000 g for 5 minutes
7. Transfer 1 mL of the ACN layer (top layer) of the extract into an HPLC autosampler vial with volume mark
8. Pipet 0.8 mL water into the autosampler vial
9. Evaporate the sample extract to ≤1 mL
10. Adjust the volume to 1.0 mL with ACN
11. Filter with 0.2 µm PTFE syringe filter
12. Analyze by UHPLC-MS/MS

Validation Process
1. Calibration curve: CAP working standards at 0.05, 0.1, 0.2, 0.6, 2, 10 and 10 µg/kg containing CAP-d₄ internal standard at 0.5 ng/mL
2. Matrices tested: infant formula, honey, oyster, mussel, shrimp and fish meal
3. AB Sciex® 4000 was used along with Agilent® 6490 to test the method performance and it was shown to be equivalent for infant formula, honey, oyster and on both instruments. Fish meal, mussel and shrimp were validated only on the Agilent® 6490.
4. Quality control samples: spiked matrices at 0.15, 0.30 and 0.60 µg/kg on AB Sciex® 4000 and at one level of 0.2 µg/kg on Agilent® 6490.
5. Number of batch analyses tested for intra- and inter-reproducibility: two batches (or one batch) with 3 replicates at each QC spike level in two days with two analysts (or one day with one analyst) on AB Sciex® 4000 and one batch with five replicates of QC spikes on Agilent® 6490.
6. Limit of quantification (LOQ): 0.15 ng/mL
7. Reporting limit: 0.30 ng/mL, which is based on the EU Minimum Required Performance Limit (MRPL)

Validation Results

Matrix Effect
CAP recovery in fish meal did not meet the acceptable recovery criteria due to the matrix effect. The ion suppression (measured as a ratio of the internal standard peak area in the sample vs. the average area count of the standards used to generate the calibration curve) was significant (below 25%). To solve this problem, a larger (10-fold) dilution was applied. Acceptable recovery and RSD were obtained at the spiked level of 2.0 µg/kg, and no significant ion suppression was observed anymore. The LOQ and the reporting limit for the matrices with significant ion suppression were raised to 1.5 µg/kg and 3.0 µg/kg, respectively.

Data Acceptance Criteria in Routine Analysis
1. Spike recovery: within 30% of theoretical (70-130%)
2. Calibration curve: a correlation coefficient ≥0.99
3. The retention time difference between the analyte and the isotopically labeled internal standard: within 5%
4. The area recovery of the internal standard: ≥25% when compared to the average area count of the standards used to generate the calibration curve
5. Background: no CAP present in the reagent blank at a level greater than 30% of LOQ (0.15 µg/kg)

Conclusion
The validated method showed acceptable accuracy (98.3-117%) and precision (1.0-6.8%). No significant matrix effects were observed, except for fish meal, in which case a further extract dilution was used. Our method allows for rapid, high-throughput and sensitive UHPLC-MS/MS identification and quantification of the banned antibiotic chloramphenicol in a variety of matrices of animal origin, meeting the EU MRPL of 0.3 µg/kg and offering a lower LOQ of 0.15 µg/kg.

Reference