LC/MS/MS Analysis of Chloramphenicol in Shrimp Using Solid Phase Extraction

Philip J. Koerner, Matthew Trass, Liming Peng, and Jeff Layne
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA
Chloramphenicol (CAP) is a broad spectrum antibiotic that exhibits activity against both gram-positive and gram-negative bacteria. CAP has been commonly used in aquaculture as a disinfectant to prevent diseases, or as a chemotherapeutic agent to control diseases.

CAP has been implicated as a probable causative agent of aplastic anemia and as a possible carcinogen. Therefore, its use in aquaculture and meat-producing animals has been banned in the European Union (EU), Canada, and the United States (USA).

Despite this ban, CAP is still used illegally to treat seafood products because of its broad spectrum activity, ready availability, and low cost.

The present methodology specified in the EU has defined a maximum residue limit (MRL) of CAP at 0.3 ng/g, while China has set an MRL of 0.5 ng/g.

The current official USFDA method uses HPLC coupled with MS/MS detection. We describe here an approach for the analysis of chloramphenicol in shrimp that utilizes liquid-liquid extraction of the shrimp tissue followed by solid phase extraction (SPE) for sample cleanup and concentration, and ultra-fast LC/MS/MS analysis using a Kinetex® core-shell C18 HPLC column.
Chloramphenicol is a hydrophobic compound that is very slightly acidic (due to its amide functionality). Therefore, chloramphenicol is an ideal candidate for SPE using hydrophobic retention followed by reversed phase HPLC.
Kinetex Core-Shell Technology

2.6 µm Core-Shell Particle

Performance equivalent to or better than fully porous sub-2 µm particles

Can be used on conventional HPLC systems and UHPLC/UPLC® systems

0.35 µm Porous Shell

1.9 µm Solid Core

™
Homogenize ~100 g of thawed shrimp using a blender or tissue homogenizer.

Weigh out 5 g of homogenized shrimp and transfer to a 15 mL polypropylene tube.

Add 50 μL of Chloramphenicol-d5 internal standard (ISTD) solution and vortex thoroughly to ensure adequate distribution of the ISTD throughout the homogenate.

Add 2 mL of water and vortex well to mix.

Add 5 mL of ethyl acetate. Transfer the tube to a mechanical shaker and shake vigorously for 30 minutes.

Centrifuge at 7000 rpm for 15 minutes.

Transfer the supernatant to a new 15 mL polypropylene test tube, reserving the tissue pellet.

Add 5 mL of ethyl acetate to the tissue pellet from the previous step and repeat the extraction process. Combine the resulting ethyl acetate supernatant with the previously obtained extract.

Dry the combined supernatant extracts under nitrogen gas at 55 ºC.

Reconstitute with 300 μL of methanol and dilute to 10 mL with water. At this point, the sample is ready for solid phase extraction.
Solid Phase Extraction

The extracted shrimp tissue is further cleaned up and concentrated using SPE

**Cartridge:** Strata™-X 60 mg / 3 mL  
**Part No.:** 8B-S100-UBJ  
**Condition:** 3 mL Methanol (1-2 mL/min)  
**Equilibrate:** 3 mL Water (1-2 mL/min)  
  - **Load:** 10 mL extracted shrimp tissue sample (1 mL/min)  
  - **Wash:** 3 mL of Water  
  - **Dry:** >10" Hg for 5-10 minutes to remove residual water  
  - **Elute:** 3 x 1.0 mL Ethyl acetate (1 mL/min)  
**Dry down:** Nitrogen gas at 55 ºC  
**Reconstitute:** 500 μL of Acetonitrile/Water (20:80)
Solid Phase Extraction

HPLC conditions:
- **Column**: Kinetex 2.6 µm C18 100A
- **Dimensions**: 50 x 2.1 mm ID
- **Part No.**: 00B-4462-AN
- **Mobile Phase**: A: 5 mM Ammonium bicarbonate
  B: Acetonitrile

AB SCIEX™ API 4000™

Ion source conditions:
- **Ionization**: ESI
- **Polarity**: Negative
- **Scan Type**: MRM
- **Gas 1 (GS1)**: 45
- **Gas 2 (GS2)**: 50
- **DP**: -50
- **Collision Gas (CAD)**: 5
- **Temperature (TEM)**: 450
- **IS**: -4500

MRM conditions:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Q1</th>
<th>Q3</th>
<th>CE</th>
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<tbody>
<tr>
<td>Chloramphenicol (quant)</td>
<td>321.2</td>
<td>152.0</td>
<td>-24</td>
</tr>
<tr>
<td>Chloramphenicol (qual)</td>
<td>321.2</td>
<td>257.2</td>
<td>-16</td>
</tr>
<tr>
<td>Chloramphenicol-d5</td>
<td>326.0</td>
<td>157.0</td>
<td>-26</td>
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**Gradient**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>B (%)</th>
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<tbody>
<tr>
<td>0.00</td>
<td>5</td>
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<tr>
<td>2.00</td>
<td>5</td>
</tr>
<tr>
<td>2.01</td>
<td>95</td>
</tr>
<tr>
<td>4.50</td>
<td>95</td>
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**Flow Rate**: 0.4 mL/min
**Column Temperature**: Ambient
**Injection**: 25 µL
**Detection**: API 4000™ MS/MS
Results

Figure 1. Chloramphenicol analysis 5 ng/mL standard

![Chloramphenicol analysis 5 ng/mL standard](image1)

**Conditions:** As noted

**Analytes:**
1. Chloramphenicol 321.2/152.0, 321.2/257.2
2. Chloramphenicol-d5 326.1/157.0

Figure 2. Extracted sample 0.01 ng/g of shrimp

![Extracted sample 0.01 ng/g of shrimp](image2)

**Conditions:** As noted

**Analytes:**
1. Chloramphenicol 321.2/152.0, 321.2/257.2
2. Chloramphenicol-d5 326.1/157.0
3. Impurity
Figure 3. Chloramphenicol standard curve
0.1 ng/mL - 200 ng/mL in mobile phase

$R^2 = 0.9999$
Figure 4. Extracted sample calibration curve with QC samples (1.0 ng/g and 7.5 ng/g) ranging from 0.01 - 20 ng/g of shrimp

\[ R^2 = 0.9997 \]
**Figure 1** shows a representative extracted chloramphenicol sample at a concentration of 0.05 ng/g of shrimp. The chromatogram is very clean due to the selectivity of the solid phase extraction sorbent, which retains chloramphenicol but does not retain any polar impurities.

In addition to a clean baseline, **Figure 1** demonstrates a very narrow analyte peak width. The narrow peak width means that the compound can be detected at very low levels which would not be possible using a conventionally porous HPLC column. In fact, based on the signal-to-noise ratio (115.9) at the lowest level (0.01 ng/g) the limit of quantitation is ≤0.001 ng/g of shrimp (**Figure 2**).

**Figure 4** shows an extracted calibration curve from 0.01 - 20 ng/g of shrimp. The curve is linear with an $R^2$ value of 0.9997. In order to validate the method, QC samples were extracted in replicate (n=6) at two different concentration levels (1.0 ng/g and 7.5 ng/g). The QC samples confirmed the accuracy and reproducibility of the method with RSD values less than 5%.
Conclusions

SPE with Strata-X in conjunction with the outlined sample preparation method is an effective way of concentrating and cleaning up shrimp samples for chloramphenicol analysis.

Analysis of chloramphenicol in shrimp is a very topical method that is being tested more and more frequently. Therefore, a sample cleanup method is important as it reduces the amount of contamination that the costly LC/MS/MS system is subjected to.

Chromatographically, the Kinetex C18 reversed phase column gives a very narrow chloramphenicol peak, resulting in an easily integrated peak with very low detection limits.

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