LC/MS/MS Analysis of Chloramphenicol from Various Matrices using Strata™-X SPE and a Kinetex® 2.6 µm C18 HPLC/UHPLC Column

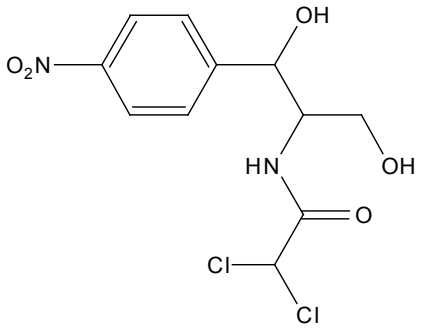
Several food matrices, including shrimp, milk, and honey were analyzed by LC/MS/MS for chloramphenicol (CAP).

This method has been optimized for the following AB SCIEX mass spectrometers: API 3200™, 3200 QTRAP®, API 4000™, 4000 QTRAP, Triple Quad™ 4500, QTRAP 4500, Triple Quad 5500, and QTRAP 5500.

Analytes

Chloramphenicol: $C_{11}H_{12}Cl_2N_2O_5$

CAS: 56-75-7 MW = 311.015 amu



For AB SCIEX mass spectrometer users, this method can be instantly implemented by installing the iMethod™ Test. iMethod Tests are verified across several laboratories and contain everything you will need to start running samples including sample preparation recommendations, consumables, LC running conditions, optimized MRM parameters, reporting templates, and complete method documentation.

Visit **www.phenomenex.com/iMethod** for more information about available iMethod Tests.

Materials and Methods

Extracting Chloramphenicol from Shrimp

Sample Preparation

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

   Note: A small amount of water may need to be added to facilitate homogenization of the tissue.
2. Weigh out 5 g of homogenized shrimp and transfer to a 15 mL polypropylene tube.
3. Add 50 µL of d_5-Chloramphenicol internal standard (I.S.) solution.
4. Mix thoroughly using a vortex mixer to ensure adequate distribution of the I.S. throughout the homogenate.
5. Add 2 mL of water.
6. Mix well using a vortex mixer.
7. Add 5 mL of ethyl acetate.
8. Transfer the tubes to a mechanical shaker and shake rigorously for 30 minutes.
9. Centrifuge at 7000 rpm for 15 minutes.
10. Transfer the supernatant to a new 15 mL polypropylene test tube, reserving the pelleted shrimp homogenate tissue.
11. Add 5 mL of ethyl acetate to the pelleted shrimp homogenate tissue from step 10 and repeat the extraction process.
12. Combine the resulting ethyl acetate supernatant with the extraction previously obtained in step 10.
13. Dry the combined supernatant extracts under nitrogen gas at 55 °C.
14. Reconstitute with 300 µL of methanol, and then dilute to 10 mL with water.

    Note: The sample is ready for solid phase extraction.

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Solid Phase Extraction (SPE)

SPE can be done using a Phenomenex Strata® SPE Vacuum Manifold*

- 12-Position Manifold (Part No. AH0-6023)
- 24-Position Manifold (Part No. AH0-6024)

* Manifold includes: vacuum-tight glass chamber, polypropylene lid with gasket, bleed valve and gauge, stopcock valves, collection racks, polypropylene needles.

Sorbent:	Strata™-X, 60 mg/3 mL
Part No.:	8B-S100-UBJ
Condition:	3 mL Methanol
Equilibrate:	3 mL Water
Load:	10 mL pre-treated sample
Wash:	3 mL Water
Dry:	5 min at 10 in. of Hg
Elute:	3 mL Ethyl acetate
Dry Down:	Dry down @ 55 °C under a stream of nitrogen
Reconstitute:	Reconstitute in 0.5 mL of water/acetonitrile (80:20)

The sample can be filtered through a Phenex™ 0.45 µm filter and then transferred to a Verex™ autosampler vial for LC/MS/MS analysis.

Ethyl acetate should be used as a rinse solution to prevent chloramphenicol contamination (for example, used to rinse needle from blow-down station or interior of the stopcock that is positioned into the manifold).

Comments on the Extraction and SPE of Chloramphenicol

Chloramphenicol has a tendency to exhibit carry-over effects. Extreme caution and good laboratory techniques should be used in order to minimize these effects. In particular, make sure that the SPE manifold and blow-down apparatus are thoroughly cleaned before and after every use (methanol is an appropriate solvent for cleaning these devices). In addition, it is always a good practice to run an extracted solvent blank (processed exactly as the normal samples, but lacking the shrimp tissue) if contamination of any of the solvents is suspected.

APPLICATIONS

Extraction Techniques for Chloramphenicol in other Foodstuffs

Sample Preparation (Honey)

Based on a method by Nieland et al.[1]

1. Dissolve 5 g of honey in 20 mL of water.
2. Add 100 µL of internal standard (D_5-CAP 1 µg/mL).
3. Mix using a vortex mixer until uniform suspension.
4. Extract with 20 mL ethyl acetate in an ultrasonic bath for 5 minutes.
5. Centrifuge for 10 minutes at 4000 rpm.
6. Transfer the upper phase (ethyl acetate) into a glass test tube.
7. Evaporate until dry under a stream of nitrogen and gentle heating (55 °C).
8. Dissolve the residue in 1 mL of water.
9. Filter using a Phenex™ 0.45 µm filter and then transfer to a Verex™ autosampler vial for LC/MS/MS analysis.

Sample Preparation (Royal Jelly)

1. Measure 2 g of royal jelly.
2. Add 100 µL of internal standard (D_5-CAP 1 µg/mL).
3. Add 20 g of Sepra™ Bulk Florisil® (Part No. 04G-4411).
4. Homogenize with a glass rod.
5. Load homogenized mix onto a Strata® FL-PR (Florisil) 5 g/20 mL Tube (Part No. 8B-S013-LEG)
6. Flush vial, glass rod and column with 250 mL of ethyl acetate/cyclohexane (50:50).
7. Evaporate to dryness under a stream of nitrogen and gentle heating (55 °C).
8. Dissolve the residue in 1 mL of water.
9. Filter using a Phenex 0.45 µm filter and then transfer to a Verex autosampler vial for LC/MS/MS analysis.

Sample Preparation (Fish and Seafood)

Based on a method by Effkemann et al.[2]

1. Measure and homogenize 5 g of fish or seafood tissue.
2. Add 100 µL of internal standard (D_5-CAP 1 µg/mL).
3. Add 10 mL of ethyl acetate.
4. Homogenize for 1 minute using an ULTRA-TURRAX®.
5. Centrifuge for 10 minutes at 3000 rpm.
6. Transfer the upper phase (ethyl acetate) into a glass test tube.
7. Evaporate until dry under a stream of nitrogen and gentle heating (55 °C).
8. Dissolve the residue in 1 mL of water.
9. Add 1 mL of hexane and mix.
10. Centrifuge for 10 minutes at 3000 rpm.
11. Remove the lower phase (water) and then filter it through a Phenex 0.45 µm filter and then transfer to a Verex autosampler vial for LC/MS/MS analysis.

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Sample Preparation (Milk)
Based on a method by Hormazabal et al.[3]

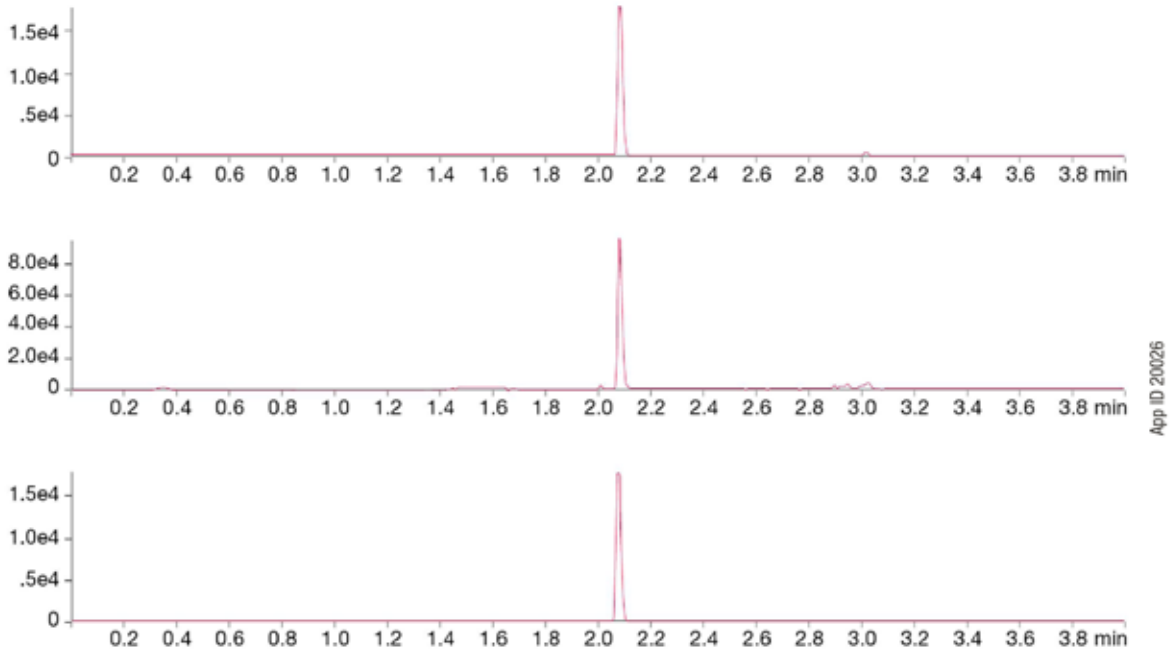
1. Measure 2 mL of milk sample.
2. Add 100 µL of internal standard (D_5-CAP 1 µg/mL).
3. Extract with 5 mL of acetonitrile.
4. Centrifuge for 10 minutes at 4000 rpm.
5. Wash supernatant with 5 mL of chloroform.
6. Centrifuge for 10 minutes at 4000 rpm.
7. Discharge upper phase (water).
8. Dry lower phase under a stream of nitrogen.
9. Dissolve residue in 100 µL of methanol.
10. Add 3 mL of water and 0.5 mL disodium hydrogen phosphate (0.5 M, pH 6.0).
11. Condition a Strata® SDB-L 200 mg/6 mL SPE cartridge (Part No. 8B-S014-FCH) with 2 mL methanol followed by 2 mL water.
12. Load sample onto SPE cartridge.
13. Wash twice with 2 mL of water and 0.3 mL of water/methanol (50:50).
14. Elute twice with 0.3 mL of methanol.
15. Evaporate until dry under a stream of nitrogen and gentle heating (55 °C).
16. Reconstitute in 1 mL of water.
17. Filter using a Phenex™ 0.45 µm filter and then transfer to a Verex™ autosampler vial for LC/MS/MS analysis.

Sample Preparation (Milk Powder)
Based on a method by Hormazabal et al.[3]

1. Measure 5 g of milk powder.
2. Add 100 µL of internal standard (D_5-CAP 1 µg/mL).
3. Dilute with 25 mL of water.
4. Homogenize for one minute using an ULTRA-TURRAX®.
5. Continue with step 3 of Sample Preparation (Milk).

HPLC Methodology

Figure 1. Extracted ion chromatograms for the lowest level of chloramphenicol extracted from spiked shrimp tissue (0.05 µg CAP/Kg of shrimp tissue)



The signal-to-noise ratio for the quantifier ion (321.2/152) was 531.8:1. The signal-to-noise ratio for the qualifier ion (321.2/257) was 59.9:1.

LC/MS/MS Conditions

Column: Kinetex® 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: Water
B: Acetonitrile

Gradient:

Time (min)	B (%)
0.00	5
2.01	95
4.00	95

Flow Rate: 0.4 mL/min
Injection Volume: 25 µL
Temperature: 25 °C
Detection: MS/MS, ESI negative (ESI-)
Sample: 1. Chloramphenicol
2. Chloramphenicol-d_5

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MS/MS Detection
Optimized for API 4000™ and 4000 QTRAP® LC/MS/MS systems
TurboV™ source
Negative polarity

Source/Gas Parameters	
CUR:	10 psi
IS:	-4500 V
TEM:	450 °C
GS1:	45 psi
GS2:	50 psi
Ihe:	On
CAD:	5

Compound MRM Parameters and Retention Times	
Dwell Time:	150 ms for analytes and internal standards
MRM Pause Time:	5 ms
Q1 Resolution:	UNIT
Q3 Resolution:	UNIT

References

1. B. Nieland et al.: '*Detection of a range of antibiotics in honey by Liquid Liquid Extraction followed by LC/MS/MS*' poster presented at ASMS Conference on Mass Spectrometry **(2004)** Nashville, Tennessee, USA
2. S. Effkemann: *'Bestimmung von Chloramphenicol in Fischen mittels RP-HPLC mit massenspektrometrischer Detektion'* Prüfvorschrift LC-MS002 LAVES **(2005)** Cuxhaven, Germany
3. V. Hormazabal, M. Yndestad: J. *Liq. Chrom. & Rel. Technol.* 24 **(2001)** 2477

Retention times were collected using the Agilent® 1200 LC system.

API 3200™ and 3200 QTRAP® system parameters

Analyte	Q1	Q3	DP	EP	CE	CXP	Agilent RT (min)	Shimadzu® RT (min)
Chloramphenicol 1	321.0	151.9	-30	-4	-24	-4	2.09	2.08
Chloramphenicol 2	321.2	257.2	-30	-4	-14	-6	2.09	2.08
D_5-Chloramphenicol	326.1	157.0	-30	-4	-26	-5	2.08	2.07

API 4000™, 4000 QTRAP, Triple Quad™ 4500, and QTRAP 4500 system parameters

Analyte	Q1	Q3	DP	EP	CE	CXP	Agilent RT (min)	Shimadzu® RT (min)
Chloramphenicol 1	321.0	152.0	-50	-10	-24	-4	2.09	2.08
Chloramphenicol 2	321.2	257.2	-50	-10	-16	-6	2.09	2.08
D_5-Chloramphenicol	326.1	157.0	-50	-10	-26	-5	2.08	2.07

Triple Quad 5500 and QTRAP 5500 system parameters

Analyte	Q1	Q3	DP	EP	CE	CXP	Agilent RT (min)	Shimadzu® RT (min)
Chloramphenicol 1	321.0	151.9	-50	-4	-24	-4	2.09	2.08
Chloramphenicol 2	321.0	257.0	-50	-4	-14	-6	2.09	2.08
D_5-Chloramphenicol	326.1	157.0	-50	-4	-26	-5	2.08	2.07

Results Table for the Standards

Analyte	Expected Concentration (ng/mL)	Number of Values	Mean	Standard Deviation	% CV	% Accuracy
CAP	0.500	2	0.485	0.027	5.6	97.0
CAP	5.000	4	5.878	0.113	1.9	117.6
CAP	25.000	4	23.075	0.814	3.5	92.3
CAP	50.000	4	43.096	0.944	2.2	86.2
CAP	200.000	4	215.431	5.793	2.7	107.7

APPLICATIONS

Ordering Information

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Complete iMethod™ Kit*

Description	Part No.
iMethod Test for Chloramphenicol	KH0-8985

* Kit includes Kinetex® 2.6 µm C18 HPLC/UHPLC column, SecurityGuard™ ULTRA cartridges and holder, in-line filter, Phenex™ Syringe Filters, Verex™ Vial kit, Sure-Lok™ UHPLC Nut and tightening tool, Sure-Lok Fingertight Nut and wrenches, and Strata™-X SPE cartridges.

Strata-X SPE

Sorbent Mass	Part No.	Unit
Tube		
30 mg	8B-S100-TAK	1 mL (100/box)
30 mg	8B-S100-TBJ	3 mL (50/box)
60 mg	8B-S100-UBJ	3 mL (50/box)
100 mg	8B-S100-EBJ	3 mL (50/box)
100 mg	8B-S100-ECH	6 mL (30/box)
200 mg	8B-S100-FBJ	3 mL (50/box)
200 mg	8B-S100-FCH	6 mL (30/box)
500 mg	8B-S100-HBJ	3 mL (50/box)
500 mg	8B-S100-HCH	6 mL (30/box)
Giga™ Tube		
500 mg	8B-S100-HDG	12 mL (20/box)
1 g	8B-S100-JDG	12 mL (20/box)
1g	8B-S100-JEG	20 mL (20/box)
2 g	8B-S100-KEG	20 mL (20/box)
5 g	8B-S100-LFF	60 mL (16/box)

Additional sizes available. Contact your Phenomenex Sample Preparation Specialist for additional information.

Strata® SDB-L SPE

Sorbent Mass	Part No.	Unit/Box
Tube		
100 mg	8B-S014-EAK	1 mL (100/Box)
200 mg	8B-S014-FBJ	3 mL (50/Box)
200 mg	8B-S014-FCH	6 mL (30/Box)
500 mg	8B-S014-HBJ	3 mL (50/Box)
500 mg	8B-S014-HBJ	6 mL (30/Box)
1g	8B-S014-JCH	6 mL (30/Box)
Giga Tube		
10 g	8B-S014-MFF	60 mL (16/Box)

Strata Florisil® SPE

(pesticide residue grade)

Sorbent Mass	Part No.	Unit/Box
Tube		
500 mg	8B-S013-HBJ	3 mL (50/Box)
500 mg	8B-S013-HCH	6 mL (30/Box)
1g	8B-S013-JCH	6 mL (30/Box)
Giga Tube		
1 g	8B-S013-JEG	20 mL (20/Box)
2 g	8B-S013-KDG	12 mL (20/Box)
5 g	8B-S013-LEG	20 mL (20/Box)
10 g	8B-S013-MFF	60 mL (16/Box)

Kinetex Core-Shell HPLC/UHPLC Columns

1.7 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard™ ULTRA Cartridges‡ 3/pk
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782 for 2.1 mm ID

2.6 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard™ ULTRA Cartridges‡ 3/pk
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)

Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard™ ULTRA Cartridges‡ 3/pk
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775 for 3.0 mm ID

2.6 µm Analytical Columns (mm)

Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard™ ULTRA Cartridges‡ 3/pk
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768 for 4.6 mm ID

‡SecurityGuard ULTRA cartridges require holder, Part No. AJ0-9000

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