

SupelMIP™ SPE – Chloramphenicol

Product Description:

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related target compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guide the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s). **It is therefore critical for analysts to use the methodology described below when using this phase.** Conventional generic methodologies employed with conventional SPE chemistries (e.g., reversed-phase C18) will yield sub-optimal results when employed with this phase.

The following methods have been developed and optimized for the extraction of chloramphenicol from a variety of sample matrixes including milk, plasma, honey, urine, and shrimp/prawns for subsequent LC-MS/MS analysis. The methods are highly reproducible and offer low limits of detection. Lower limits of detection using the described SupelMIP SPE and LC-MS-MS procedures are as follows:

Chloramphenicol in:	LLOD
Milk	0.1 ng/mL
Plasma	0.02 ng/mL
Urine and Honey	0.02 µg/kg
Shrimp/Prawns	7 ng/kg

Protocol for Extraction of Chloramphenicol from Milk & Plasma:

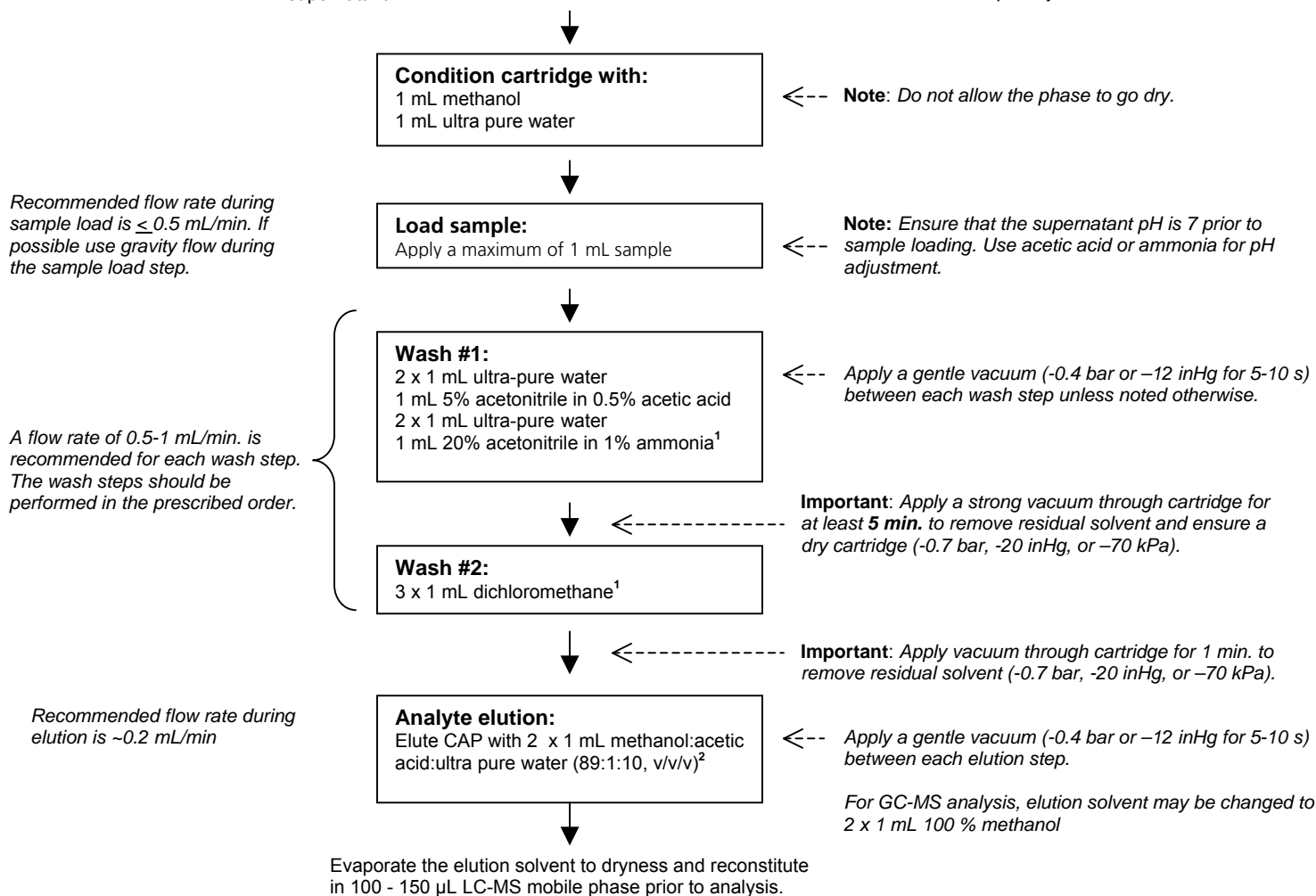
Sample Pre-treatment

No sample pre-treatment is necessary for skim milk. For raw milk, centrifuge milk for 15 minutes at 5000 rpm. Collect the layer between the upper lipid layer and above the protein pellet.

For plasma samples, centrifuge for 10 min. at 3000 rpm and collect supernatant.

Note: Spike either milk or plasma sample at 1 µg/L d_5 - chloramphenicol internal standard.

Adjust the pH of the sample supernatant to pH 7 as necessary. Use acetic acid or ammonia for pH adjustment.



- For enhanced wash steps, replace wash step: 1 mL 20% acetonitrile in 1% ammonia with up to 3 x 1 mL 20% acetonitrile in 1% ammonia OR replace wash step 3 x 1 mL dichloromethane with 3 x 1 mL 2% acetic acid in dichloromethane
- For cleaner extracts replace elution step 2 x 1 mL methanol:acetic acid:ultra pure water (89:1:10, v/v/v) with 2 x 1 mL methanol:dichloromethane (90:10).

Protocol for Extraction of Chloramphenicol from Honey & Urine:

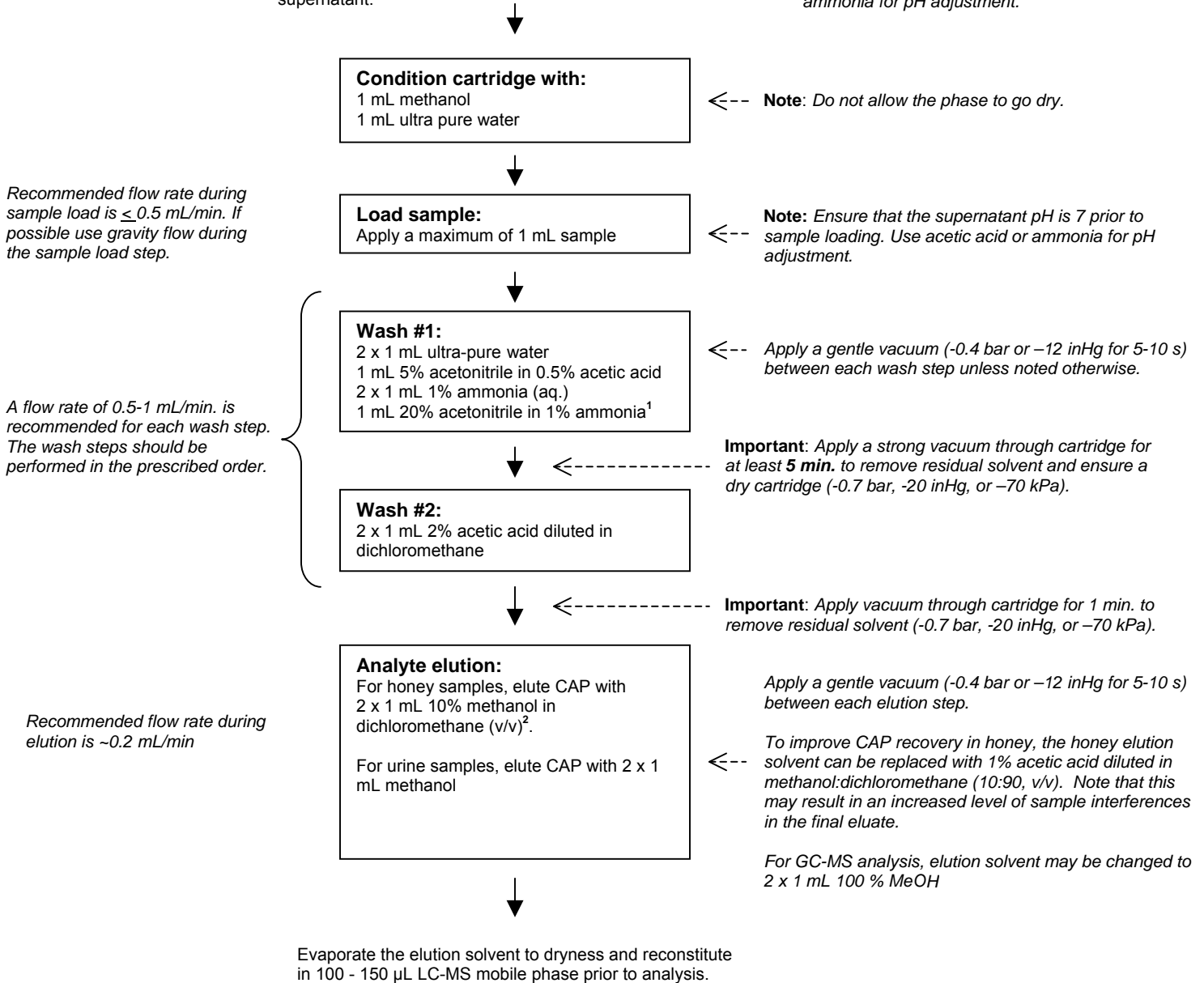
Sample Pre-treatment

Dissolve 1 g of honey into 1 mL DI water. Solubility can be improved by heating the sample to 45 °C.

For urine samples, adjust to pH 7. For particulate laden urine samples, centrifuge at 3000 g for 10 minutes and collect supernatant.

Note: Spike either honey or urine sample at 1 µg/L d₅-chloramphenicol internal standard.

Adjust the pH of the sample supernatant to pH 7 as necessary. Use acetic acid or ammonia for pH adjustment.



- For enhanced wash steps, replace wash step: 1 mL 20% acetonitrile in 1% ammonia with up to 3 x 1 mL 20% acetonitrile in 1% ammonia
- To improve CAP recovery in honey, the honey elution solvent can be replaced with 1% acetic acid diluted in methanol:dichloromethane (10:90, v/v). Note that this may result in an increased level of sample interferences in the final eluate.

Protocol for Extraction of Chloramphenicol from Shrimp/Prawns:

Sample Pre-treatment

Homogenize 5 g peeled shrimp (raw or boiled) and add 20 mL ethyl acetate. Vortex for 2 min.. Centrifuge for 5 min. at 2000 rpm or filter through a 150 µm porosity filter. Evaporate supernatant to dryness and reconstitute residue in 10 mL ultra pure water. Filter reconstituted sample as necessary

Note: Spike shrimp sample with d_5 -chloramphenicol internal standard at the level of 200 ng/kg prior to EtOAC homogenization.

Adjust the pH of the reconstituted sample supernatant to pH 7 as necessary using acetic acid or ammonia.

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Condition/equilibrate cartridge with:
1 mL methanol
1 mL ultra pure water

←-- **Note:** Do not allow the phase to go dry. Recondition if the phase goes dry.

Recommended flow rate during sample load is ≤ 0.5 mL/min. If possible use gravity flow during the sample load step.

↓
Load sample:
Apply a maximum volume of 2 mL reconstituted sample

←-- **Note:** Ensure that the supernatant pH is 7 prior to sample loading. Use acetic acid or ammonia for pH adjustment.

A flow rate of 0.5-1 mL/min. is recommended for each wash step. The wash steps should be performed in the prescribed order.

↓
Wash #1:
2 x 1 mL ultra pure water
1 mL acetonitrile:0.5% acetic acid (5:95, v/v, aq.)
2 x 1 mL 1% ammonia (v/v, aq.)
1 mL acetonitrile:1% ammonia (20:80, v/v, aq.)

←-- Apply a gentle vacuum (-0.4 bar or -12 inHg for 5-10 s) between each wash step.

↓
Wash #2:
3 x 1 mL dichloromethane

←-- Apply a gentle vacuum (-0.4 bar or -12 inHg for 5-10 s) between each wash step.

↓
Analyte elution:
Elute CAP with 2 x 1 mL methanol:dichloromethane (10:90, v/v)

Recommended flow rate during elution is ~0.2 mL/min

←-- **Important:** Apply a strong vacuum through cartridge for at least 10 min. to remove residual solvent (-0.7 bar, -20 inHg, or -70 kPa).

←-- Apply a gentle vacuum (-0.4 bar or -12 inHg for 5-10 s) between each elution step.

For GC-MS analysis, elution solvent may be changed to 2 x 1 mL 100 % MeOH

↓
Evaporate the elution solvent to dryness and reconstitute in 100 - 150 µL LC-MS mobile phase prior to analysis.

Recommended Analytical Method:

Recommended Analytical Technique: LC-MS-MS or LC-MS	column: Ascentis C18, 10 cm x 2.1 mm I.D., 3 µm particle size (581301-U) instrument: Sciex API 3200 mobile phase: 10 mM ammonium acetate (pH 6.7):acetonitrile (70:30) flow rate: 0.2 mL/min. temp.: ambient det.: MS/MS, MRM transitions Quantification (321.00/152.00) Identification (321.00/257.00) I.S. (326.00/157.00) polarity: Negative ion source: Turbospray ion spray voltage: -2000 V decluster potential: -35 V source temp: 500 °C collision gas: 4 psi ion source gas 1: 70 psi ion source gas 2: 40 psi curtain gas: 10 psi dwell time: 150 msec run time: 5 min. inj.: 20 µL
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Product Information:

Description	Pkg. Qty.	Cat. No.
SupelMIP SPE - Full Beta-receptors (beta-blockers & beta-agonists)		
25 mg/10 mL (LRC)	50	53223-U
25 mg/3 mL	50	53224-U
SupelMIP SPE - Beta-blocker (class selective)		
25 mg/10 mL (LRC)	50	53218-U
25 mg/3 mL	50	53213-U
SupelMIP SPE - Beta-agonists (class selective)		
25 mg/10 mL (LRC)	50	53202-U
25 mg/3 mL	50	53225-U
SupelMIP SPE - Clenbuterol		
25 mg/10 mL (LRC)	50	53201-U
SupelMIP SPE - TSNAs (NNK, NNN, NAB, NAT)		
50 mg/10 mL (LRC)	50	53221-U
50 mg/3 mL	50	53222-U
SupelMIP SPE – NNAL		
25 mg/10 mL (LRC)	50	53206-U
25 mg/3 mL	50	53203-U
SupelMIP SPE - Chloramphenicol		
25 mg/10 mL (LRC)	50	53210-U
25 mg/3 mL	50	53209-U
SupelMIP SPE – Fluoroquinolones		
25 mg/3 mL	50	53269-U
SupelMIP SPE – Amphetamines (class selective)		
25 mg/3 mL	50	53228-U
SupelMIP SPE - Riboflavin (Vitamin B2)		
25 mg/10 mL (LRC)	50	53207-U
SupelMIP SPE - Triazine 10		
25 mg/10 mL (LRC)	50	53208-U

SupelMIP SPE developed by MIP Technologies AB
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