

## SupelMIP™ SPE – Fluoroquinolones

### 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.

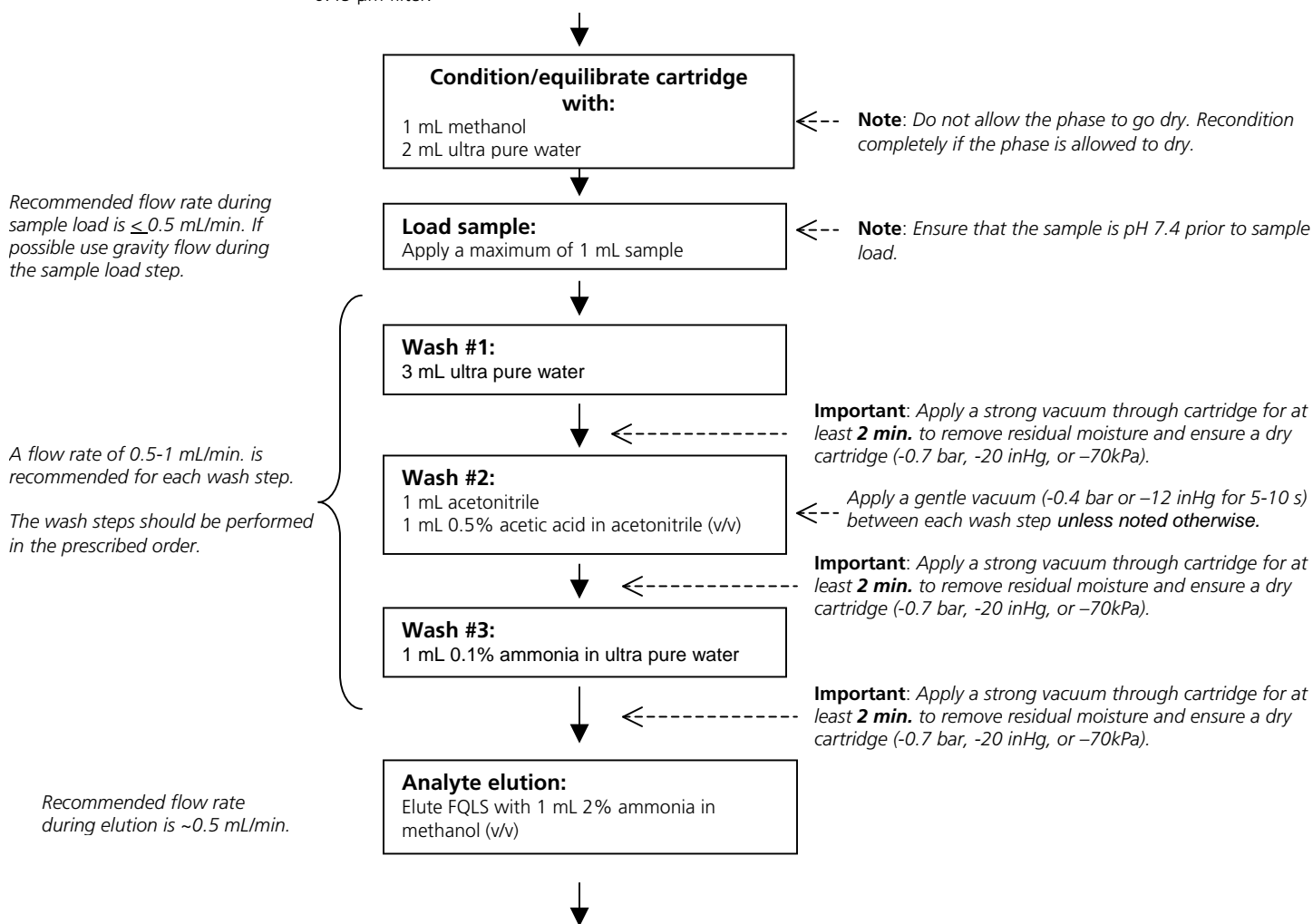
The following methods have been developed and optimized for the extraction of fluoroquinolones (FQL) from a variety of sample matrixes including bovine kidney, honey, and milk. Example FQLs include sarafloxacin, norfloxacin, enrofloxacin, ciprofloxacin, lomefloxacin, and ofloxacin.

### Protocol for Extraction of Fluoroquinolones from Bovine Kidney:

#### Sample Pre-treatment

Homogenize 2 g kidney in 30 mL 50 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.4. Centrifuge for 10 min. at 5000 rpm. Filter the supernatant using a 0.45 µm filter.

**Note:** Spike kidney sample with internal standard (e.g., d<sub>3</sub>- norfloxacin) at 75 ng/g.



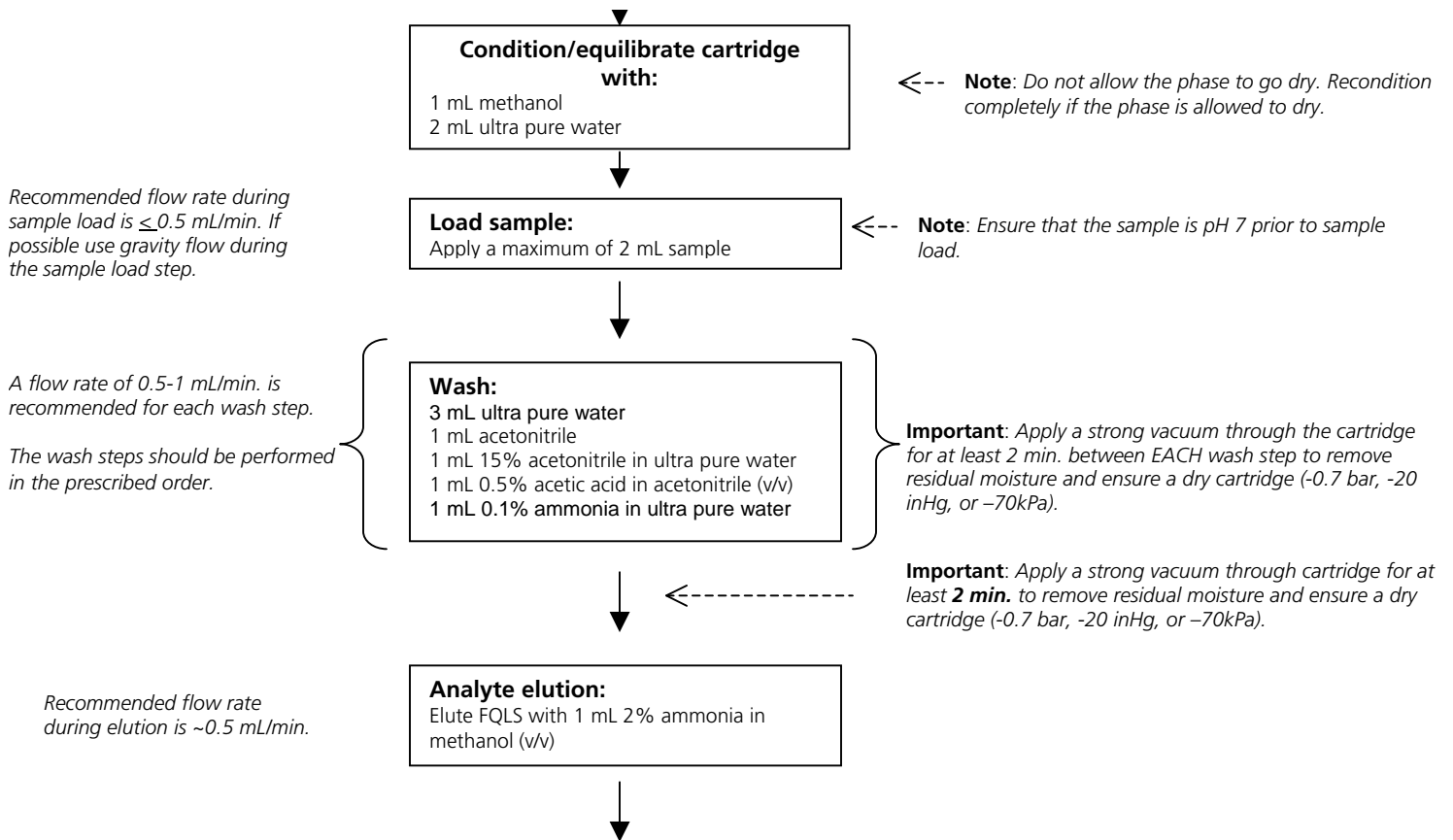
Evaporate the elution solvent to dryness at a maximum temp. of 35 °C under gentle nitrogen. Reconstitute in 150 µL 50% acetonitrile in 0.1% formic acid prior to analysis.

## Protocol for Extraction of Fluoroquinolones from Honey:

### Sample Pre-treatment

Dissolve honey in an equal amount of 10 mM ammonium acetate, pH 7. The sample could be heated to 45 °C to improve solubility. Adjust pH to 7 as necessary with ammonium hydroxide and acetic acid. Centrifuge for 5 min. at 3000 rpm.

**Note:** Spike honey sample with internal standard (e.g.,  $d_5$ - norfloxacin) at 2 ng/g.



Evaporate the elution solvent to dryness at a maximum temp. of 35 °C under gentle nitrogen. Reconstitute in 150  $\mu$ L 50% acetonitrile in 0.1% formic acid prior to analysis.

## Protocol for Extraction of Fluoroquinolones from Milk:

### Sample Pre-treatment

Dissolve milk in an equal amount of 10 mM ammonium acetate, pH 5. Centrifuge for 5 min. at 5000 rpm. Adjust supernatant to pH 7 as necessary with ammonium hydroxide and acetic acid.

**Note:** Spike milk sample with internal standard (e.g.,  $d_5$ - norfloxacin) at 2 ng/g.

### Condition/equilibrate cartridge with:

1 mL methanol  
2 mL ultra pure water

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

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

### Load sample:

Apply a maximum of 2 mL sample

←-- **Note:** Ensure that the sample is pH 7 prior to sample load.

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:

3 mL ultra pure water  
1 mL acetonitrile  
1 mL 15% acetonitrile in ultra pure water  
1 mL 0.5% acetic acid in acetonitrile (v/v)  
1 mL 0.1% ammonia in ultra pure water

**Important:** Apply a strong vacuum through the cartridge for at least 2 min. between EACH wash step to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 inHg, or -70kPa).

←-- **Important:** Apply a strong vacuum through cartridge for at least 2 min. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 inHg, or -70kPa).

Recommended flow rate during elution is  $\sim 0.5$  mL/min

### Analyte elution:

Elute FQLS with 1 mL 2% ammonia in methanol (v/v)

Evaporate the elution solvent to dryness at a maximum temp. of 35 °C under gentle nitrogen. Reconstitute in 150  $\mu$ L 50% acetonitrile in 0.1% formic acid prior to analysis. Filter through a 0.45  $\mu$ m filter if necessary.

<b>Recommended Analytical Technique:</b>	column:	Ascentis C18, 5 cm x 3 mm I.D., 3 µm particles (581307-U) w/ guard column																		
	instrument:	LC-MS/MS Triple Quadrupole																		
	mobile phase A:	0.1% formic acid																		
	mobile phase B:	acetonitrile																		
	temp.:	ambient																		
	flow rate:	0.5 mL/min.																		
	gradient:	<table border="1"> <thead> <tr> <th>Time (min.)</th> <th>%A</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>95</td> <td>5</td> </tr> <tr> <td>7.0</td> <td>85</td> <td>15</td> </tr> <tr> <td>7.2</td> <td>20</td> <td>80</td> </tr> <tr> <td>8.2</td> <td>95</td> <td>5</td> </tr> <tr> <td>11.0</td> <td>95</td> <td>5</td> </tr> </tbody> </table>	Time (min.)	%A	%B	0.0	95	5	7.0	85	15	7.2	20	80	8.2	95	5	11.0	95	5
	Time (min.)	%A	%B																	
	0.0	95	5																	
	7.0	85	15																	
	7.2	20	80																	
	8.2	95	5																	
	11.0	95	5																	
	det.:	MS/MS, MRM transitions sarafloxacin (386.1/299.1) norfloxacin (320.2/276.2) enrofloxacin 360.2/245.2) ciprofloxacin (332.4/288.2) d5-norfloxacin I.S. (325.3/288.1)																		
	polarity:	Positive																		
ion source:	Turbospray																			
ion spray voltage:	4500 V																			
decluster potential:	sara floxacin – 46 V norfloxacin – 41 V enrofloxacin – 49 V ciprofloxacin – 45 V d5- norfloxacin – 46 V																			
entrance potential:	sara floxacin – 5 V norfloxacin – 3 V enrofloxacin – 4 V ciprofloxacin – 4 V d5- norfloxacin – 4 V																			
source temp:	500 °C																			
collision gas:	5 psi																			
curtain:	15 psi																			
lon-source gas 1:	50 psi																			
lon-source gas 2:	60 psi																			
dwel time:	200 msec.																			
run time:	10 min.																			
inj.:	3 µL																			

## Product Information:

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

SupelMIP SPE developed by MIP Technologies AB  
SupelMIP is a trademark of Sigma-Aldrich Biotechnology LP

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**SUPELCO**  
Bellefonte, PA