**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 NaH₂PO₄, pH 7.4. Centrifuge for 10 min. at 5000 rpm. Filter the supernatant using a 0.45 µm filter.

**Condition/equilibrate cartridge with:**

1 mL methanol
2 mL ultra pure water

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**Load sample:**

Apply a maximum of 1 mL sample

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**Wash #1:**

3 mL ultra pure water

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**Wash #2:**

1 mL acetonitrile
1 mL 0.5% acetic acid in acetonitrile (v/v)

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**Wash #3:**

1 mL 0.1% ammonia in ultra pure water

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**Analyte elution:**

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

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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.

**Note:** Spike kidney sample with internal standard (e.g., d₅ – norfloxacin) at 75 ng/g.
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.

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

**Load sample:**
Apply a maximum of 2 mL sample

**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

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

**Analyte elution:**
Elute FQLS with 1 mL 2% ammonia in methanol (v/v)

**Important:** Apply a strong vacuum through 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).

Recommended flow rate during elution is ~0.5 mL/min.

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.

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**Note:** Spike honey sample with internal standard (e.g., d5-norfloxacin) at 2 ng/g.

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.

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

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

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

**Load sample:**
Apply a maximum of 2 mL sample

**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

**Analyte elution:**
Elute FQLS with 1 mL 2% ammonia in methanol (v/v)

**Note:** Spike milk sample with internal standard (e.g., d₈-norfloxacin) at 2 ng/g.

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

**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.**

**Recommended flow rate during elution is ~0.5 mL/min**

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

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

**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).

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. Filter through a 0.45 µm filter if necessary.
Recommended Analytical Technique:

- **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>
<thead>
<tr>
<th>Time (min.)</th>
<th>%A</th>
<th>%B</th>
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<tbody>
<tr>
<td>0.0</td>
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</tr>
<tr>
<td>7.0</td>
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</tr>
<tr>
<td>7.2</td>
<td>20</td>
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<td>5</td>
</tr>
<tr>
<td>11.0</td>
<td>95</td>
<td>5</td>
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</table>
- **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
- **Source Temp**: 500 °C
- **Collision Gas**: 5 psi
- **Curtain**: 15 psi
- **Ion-Source Gas 1**: 50 psi
- **Ion-Source Gas 2**: 60 psi
- **Dwell Time**: 200 msec.
- **Run Time**: 10 min.
- **Inj.**: 3 µL

Product Information:

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<tr>
<th>Description</th>
<th>Pkg. Qty.</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td><strong>SupelMIP SPE - Full Beta-receptors (beta-blockers &amp; beta-agonists)</strong></td>
<td>50</td>
<td>53223-U</td>
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<tr>
<td>25 mg/10 mL (LRC)</td>
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<td>25 mg/3 mL</td>
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<td><strong>SupelMIP SPE - Beta-blocker (class selective)</strong></td>
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*SupelMIP SPE developed by MIP Technologies AB*

*SupelMIP is a trademark of Sigma-Aldrich Biotechnology LP*

Updated 02/11/09