This Application Note contains important information about this product

AFFINILUTE™ MIP – Nitroimidazoles

Description	Quantity	Part Number
AFFINILUTE MIP Nitroimidazoles 50 mg/3 mL	50	M34-0005-B

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymers- 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, guides 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.

Extraction of Nitroimidazoles from various matrices

The following methods have been determined for Nitroimidazoles that can be optimized for a number of matrices. The nitroimidazoles that we have tested so far include: dimetridazole (DMZ), metronidazole (MNZ), ipronidazole (IPZ), ronidazole (RNZ); and their respective metabolites: DMZOH, MNZOH, and IPZOH.

The first procedure is a general procedure that can be followed if a matrix specific method is not included in this data sheet. This general procedure represents a recommended starting point for further optimization. The general procedure is followed by matrix specific procedures.

Protocol for Extraction of Nitroimidazoles – General Procedure:

Sample Pre-Treatment for solid/tissue samples:

1. Homogenize 2.5 g of sample with I.S.; and add 10 mL DI H_2O.
2. Remove particulates via centrifugation

For liquid samples:

1. Dilute samples 1:1 to 1:5 with DI water or 10 mM ammonium acetate, pH 6

Note: *Deuterated I.S. is recommended for each analyte for accurate quantitation. Use polypropylene or silanized glassware only. Nitromidazoles may adsorb onto standard glassware resulting in loss of recovery.*

The sample should be completely aqueous prior to SPE processing. No organic modifiers should be present in the sample. Additional sample pre-treatment may be required depending on the complexity of the sample. For example, a protein ppt step may be necessary for samples that contain high levels of protein.

Condition column with:
1 mL toluene
1 mL acetonitile
1 mL 10 mM ammonium acetate, pH 6

Note: *Do not allow the phase to dry during conditioning. Recondition if the phase dries out.*

Recommended flow rate during sample load is ≤1 mL/min. If possible use gravity flow during the sample load step.

Load sample:
Apply a maximum of 2 mL sample

Wash #1:
1 mL ultra-pure water

Important: *Apply a strong vacuum through cartridge for at least **5 min**. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or .70 kPa).*

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 #2:
2 x 1 mL hexane

Apply a gentle vacuum (-0.4 bar or –12 in Hg for 5-10 s) between each wash step unless noted otherwise.

Wash #3:
1 mL heptane:toluene 3:1 (v/v)

Important: *Apply a strong vacuum through cartridge for at least **5 min**. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or –70 kPa*

Recommended flow rate during elution is ~0.2 mL/min

Analyte elution:
Elute Nitroimadazoles with 2 x 1 mL acetonitrile: water (60:40. v/v) with 0.5% acetic acid.

Apply a gentle vacuum (-0.4 bar or .12 in Hg for 5-10 s) between each wash step unless noted otherwise.

Evaporate the elution solvent to 20-50 µL at 50 °C under N_2 and reconstitute in 500 µL LC-MS mobile phase, filter prior to analysis if necessary.

Application Note

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This Application Note contains important information about this product

AFFINILUTE™ MIP – Fluoroquinolones

Description	Quantity	Part Number
AFFINILUTE MIP Fluoroquinolones 25 mg/3 mL	50	M69-0002-B

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymers 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, guides 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.

Application Note

Extraction of Fluoroquinolones from various matrices

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_2PO_4, 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_5 – norfloxacin) at 75 ng/g.*

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

Note: *Do not allow the phase to dry. Recondition completely if the phase dries.*

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 of 1 mL sample

Note: *Ensure that the sample is pH 7.4 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 #1:
3 mL ultra pure water

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).*

Wash #2:
1 mL acetonitrile
1 mL 0.5% acetic acid in acetonitrile (v/v)

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

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).*

Wash #3:
1 mL 0.1% ammonia in ultra pure water

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 in Hg, or –70kPa).*

Recommended flow rate during elution is ~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 temperature 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.*

↓

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

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

↓

Recommended flow rate during sample load is ≤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 in Hg, 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 in Hg, or –70kPa).*

Recommended flow rate during elution is ~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 temperature 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 Milk:

Sample Pre-treatment
Dissolve milk in an equal amount of 10 mM ammonium acetate, pH 5. Centrifuge for 5 in. 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 column/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 dries.*

↓

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 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 in Hg, 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 in Hg, or –70kPa).*

Recommended flow rate during elution is ~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 temperature 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:
LC-MS-MS

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
Temperature:	ambient
Flow rate:	0.5 mL/min.

Gradient:

Time (min.)	%A	%B
0.0	95	5
7.0	85	15
7.2	20	80
8.2	95	5
11.0	95	5

Detection:	MS/MS, MRM transitions sarafloxacin (386.1/299.1) norfloxacin (320.2/276.2) enrofloxacin 360.2/245.2) ciprofloxacin (332.4/288.2) d_5-norfloxacin I.S. (325.3/288.1)
Polarity:	Positive
Ion source:	Turbospray
Ion spray voltage:	4500 V
Decluster potential:	sarafloxacin – 46 V, norfloxacin – 41 V Enrofloxacin – 49 V ciprofloxacin – 45 V, d_5- norfloxacin – 46 V
Entrance potential:	sarafloxacin – 5 V, norfloxacin – 3 V enrofloxacin – 4 V ciprofloxacin – 4 V, d_5- norfloxacin – 4 V
Source temp:	500 °C
Collision gas:	5 psi
Curtain:	15 psi
Ion-source gas 1:	50 psi
Ion-source gas 1:	60 psi
Swell time:	200 msec.
Run time:	10 min.
Injection volume:	3 µL

Ordering Information

Description	Quantity	Part Number
AFFINILUTE MIP - Fluoroquinolones		
25 mg/3 mL	50	M69-0002-B

NORTH AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
press (4) at the auto attendant
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
1-pointsupport@biotage.com

EUROPE
Main Office: +46 18 56 5900
Fax: +46 18 59 1922
Order Tel: +46 18 56 57 10
Order Fax: +46 18 56 57 05
order@eu.biotage.com

JAPAN
Tel: +81 422 28 1233
Fax: +81 422 28 1236
jp_order@biotage.com

To locate a distributor please visit our web site at www.biotage.com.

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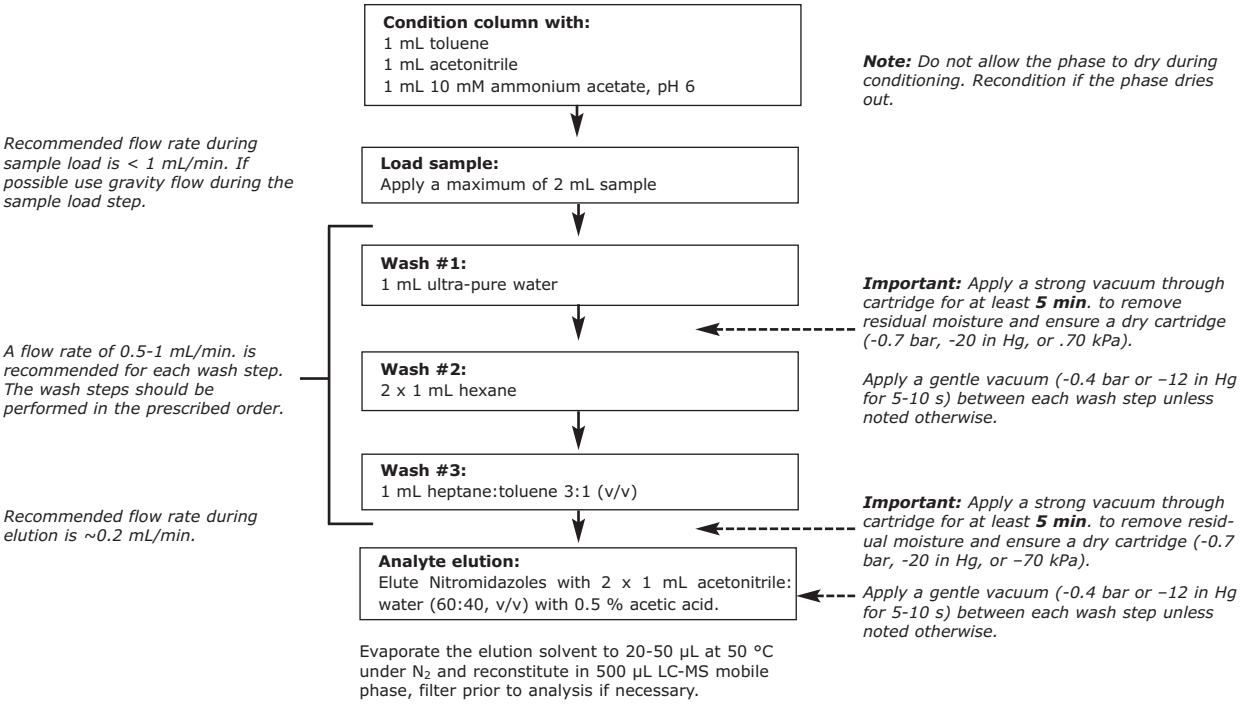
Protocol for Extraction of Nitroimidazoles from Milk & Egg:

Sample Pre-treatment
For **egg powder**, combine 2.5 g of egg powder with 10 mL DI water in a centrifuge tube.
For raw egg, combine 10 g of raw egg with 10 mL DI water in a centrifuge tube.
For **milk**, transfer 10 mL milk to a centrifuge tube.

1. Add I.S. to 10 mL of egg powder, raw egg, or milk sample from above.
2. Shake vigorously for 2 min.; and add 10 mL MeCN. Shake for an additional 2 min.
3. Centrifuge for 15 min. at 4000 x g. Isolate supernatant and combine with 2 g NaCl.
4. Shake manually and centrifuge for 5 min. at 4000 x g. Remove supernatant and evaporate to dryness at 50°C under nitrogen.
5. Reconstitute in 2 mL DI water or 10 mM ammonium acetate, pH 6. Sonicate for 3 min.

Note: *Deuterated I.S. is recommended for each analyte for accurate quantitation. Use polypropylene or silanized glassware only. Nitroimidazoles may adsorb onto standard glassware resulting in loss of recovery.*



Note: *Do not allow the phase to dry during conditioning. Recondition if the phase dries out.*

Recommended flow rate during sample load is < 1 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.

Important: *Apply a strong vacuum through cartridge for at least **5 min**. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or .70 kPa).*

Apply a gentle vacuum (-0.4 bar or –12 in Hg for 5-10 s) between each wash step unless noted otherwise.

Recommended flow rate during elution is ~0.2 mL/min.

Important: *Apply a strong vacuum through cartridge for at least **5 min**. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or –70 kPa).*

Apply a gentle vacuum (-0.4 bar or –12 in Hg for 5-10 s) between each wash step unless noted otherwise.

Evaporate the elution solvent to 20-50 µL at 50 °C under N_2 and reconstitute in 500 µL LC-MS mobile phase, filter prior to analysis if necessary.

Troubleshooting:

Improve Recovery:

- Do not exceed the recommended load and wash volumes.
- When evaporating the SPE eluent prior to reconstitution and analysis, do not evaporate to dryness. Analyte loss may occur.
- Use polypropylene or silanized glassware throughout the SPE procedure. Nitromidazoles may adsorb onto standard glassware.
- Implement the SPE tube drying steps (e.g., between wash steps and elution steps) as recommended.
- Minimize flow rate during sample load and elution.
- Increase elution from 2 x 1 mL to 3 x 1 mL

Improve Sample Cleanup:

Adjust the elution solvent from "2 x 1 mL acetonitrile:water (60:40, v/v) with 0.5 % acetic acid" to "2 x 1 mL acetonitrile:water (50:50, v/v) with 0.5 % acetic acid"

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:	(A) 0.1% formic acid in LC-MS grade water		
	(B) 0.1% formic acid in acetonitrile		
Gradient:	**Min.**	**A%**	**B%**
	0.0	95	5
	1.0	95	5
	8.0	0	10.0
	12.0	0	10.0
	13.0	95	5
	18.0	95	5
Flow rate:	0.3 mL/min.		
Temperature:	ambient		
Detection:	MS/MS, MRM transitions		
	DMZ (142/96)	IPZOH (186/168)	
	DMZ-d_3(145/99)	IPZOH-d_3 (189/171)	
	DMZOH (158/140)	MNZ (172/128)	
	DMZOH-d_3 (161/143)	MNZOH (188/126)	
	IPZ (170/124)	RNZ (201/140)	
	IPZ-d_3 (189/171)	RNZ-d_3 (204/143)	
Polarity:	Positive		
Ion source:	Turbospray		
Ion spray voltage:	1200 V		
Source temp:	350 °C		
Collision gas:	4 psi		
Curtain gas:	50 psi		
injection volume:	30 µL		

Ordering Information

Description	Quantity	Part Number
AFFINILUTE MIP - Nitroimidazoles		
50 mg/3 mL	50	M34-0005-B

NORTH AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
press (4) at the auto attendant
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
1-pointsupport@biotage.com

EUROPE
Main Office: +46 18 56 5900
Fax: +46 18 59 1922
Order Tel: +46 18 56 57 10
Order Fax: +46 18 56 57 05
order@eu.biotage.com

JAPAN
Tel: +81 422 28 1233
Fax: +81 422 28 1236
jp_order@biotage.com

To locate a distributor please visit our web site at www.biotage.com.

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This Application Note contains important information about this product

AFFINILUTE™ MIP – Fluoroquinolones

Description	Quantity	Part Number
AFFINILUTE MIP Fluoroquinolones 25 mg/3 mL	50	M69-0002-B

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymers 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, guides 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.

Application Note

Extraction of Fluoroquinolones from various matrices

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_2PO_4, 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_5 – norfloxacin) at 75 ng/g.*

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

Note: *Do not allow the phase to dry. Recondition completely if the phase dries.*

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 of 1 mL sample

Note: *Ensure that the sample is pH 7.4 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 #1:
3 mL ultra pure water

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).*

Wash #2:
1 mL acetonitrile
1 mL 0.5% acetic acid in acetonitrile (v/v)

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

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).*

Wash #3:
1 mL 0.1% ammonia in ultra pure water

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 in Hg, or –70kPa).*

Recommended flow rate during elution is ~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 temperature 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.*

↓

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

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

↓

Recommended flow rate during sample load is ≤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 in Hg, or –70kPa).*

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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 in Hg, or –70kPa).*

Recommended flow rate during elution is ~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 temperature 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 Milk:

Sample Pre-treatment
Dissolve milk in an equal amount of 10 mM ammonium acetate, pH 5. Centrifuge for 5 in. 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 column/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 dries.*

↓

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 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 in Hg, 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 in Hg, or –70kPa).*

Recommended flow rate during elution is ~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 temperature 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:
LC-MS-MS

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
Temperature:	ambient
Flow rate:	0.5 mL/min.

Gradient:

Time (min.)	%A	%B
0.0	95	5
7.0	85	15
7.2	20	80
8.2	95	5
11.0	95	5

Detection:	MS/MS, MRM transitions sarafloxacin (386.1/299.1) norfloxacin (320.2/276.2) enrofloxacin 360.2/245.2) ciprofloxacin (332.4/288.2) d_5-norfloxacin I.S. (325.3/288.1)
Polarity:	Positive
Ion source:	Turbospray
Ion spray voltage:	4500 V
Decluster potential:	sarafloxacin – 46 V, norfloxacin – 41 V Enrofloxacin – 49 V ciprofloxacin – 45 V, d_5- norfloxacin – 46 V
Entrance potential:	sarafloxacin – 5 V, norfloxacin – 3 V enrofloxacin – 4 V ciprofloxacin – 4 V, d_5- norfloxacin – 4 V
Source temp:	500 °C
Collision gas:	5 psi
Curtain:	15 psi
Ion-source gas 1:	50 psi
Ion-source gas 1:	60 psi
Swell time:	200 msec.
Run time:	10 min.
Injection volume:	3 µL

Ordering Information

Description	Quantity	Part Number
AFFINILUTE MIP - Fluoroquinolones		
25 mg/3 mL	50	M69-0002-B

NORTH AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
press (4) at the auto attendant
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
1-pointsupport@biotage.com

EUROPE
Main Office: +46 18 56 5900
Fax: +46 18 59 1922
Order Tel: +46 18 56 57 10
Order Fax: +46 18 56 57 05
order@eu.biotage.com

JAPAN
Tel: +81 422 28 1233
Fax: +81 422 28 1236
jp_order@biotage.com

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www.biotage.com

This Application Note contains important information about this product

AFFINILUTE™ MIP – Chloramphenicol

Description	Quantity	Part Number
AFFINILUTE MIP Chloramphenicol 25 mg/10 mL	50	M10-0002-G
AFFINILUTE MIP Chloramphenicol 25 mg/3 mL	50	M10-0002-B

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymers 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, guides 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.

Application Note

Extraction of Chloramphenicol from various matrices

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 AFFINILUTE MIP 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 and 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.

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Condition column with:
1 mL methanol
1 mL ultra pure water

Note: *Do not allow the phase to dry. Recondition if the phase dries.*

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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.

Load sample:
Apply a maximum of 1 mL sample

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

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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 5% acetonitrile in 0.5% acetic acid
2 x 1 mL ultra-pure water
1 mL 20% acetonitrile in 1% ammonia[1]

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

Important: *Apply a strong vacuum through cartridge for at least **5 min**. to remove residual solvent and ensure a dry cartridge (-0.7 bar, -20 inHg, or –70 kPa).*

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Wash #2:
3 x 1 mL dichloromethane[2]

Important: *Apply vacuum through cartridge for **1 min**. to remove residual solvent (-0.7 bar, -20 inHg, or –70 kPa).*

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Recommended flow rate during elution is ~0.2 mL/min

Analyte elution:
Elute CAP with 2 x 1 mL methanol:acetic acid:ultra pure water (89:1:10, v/v/v)[3]

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 % methanol.

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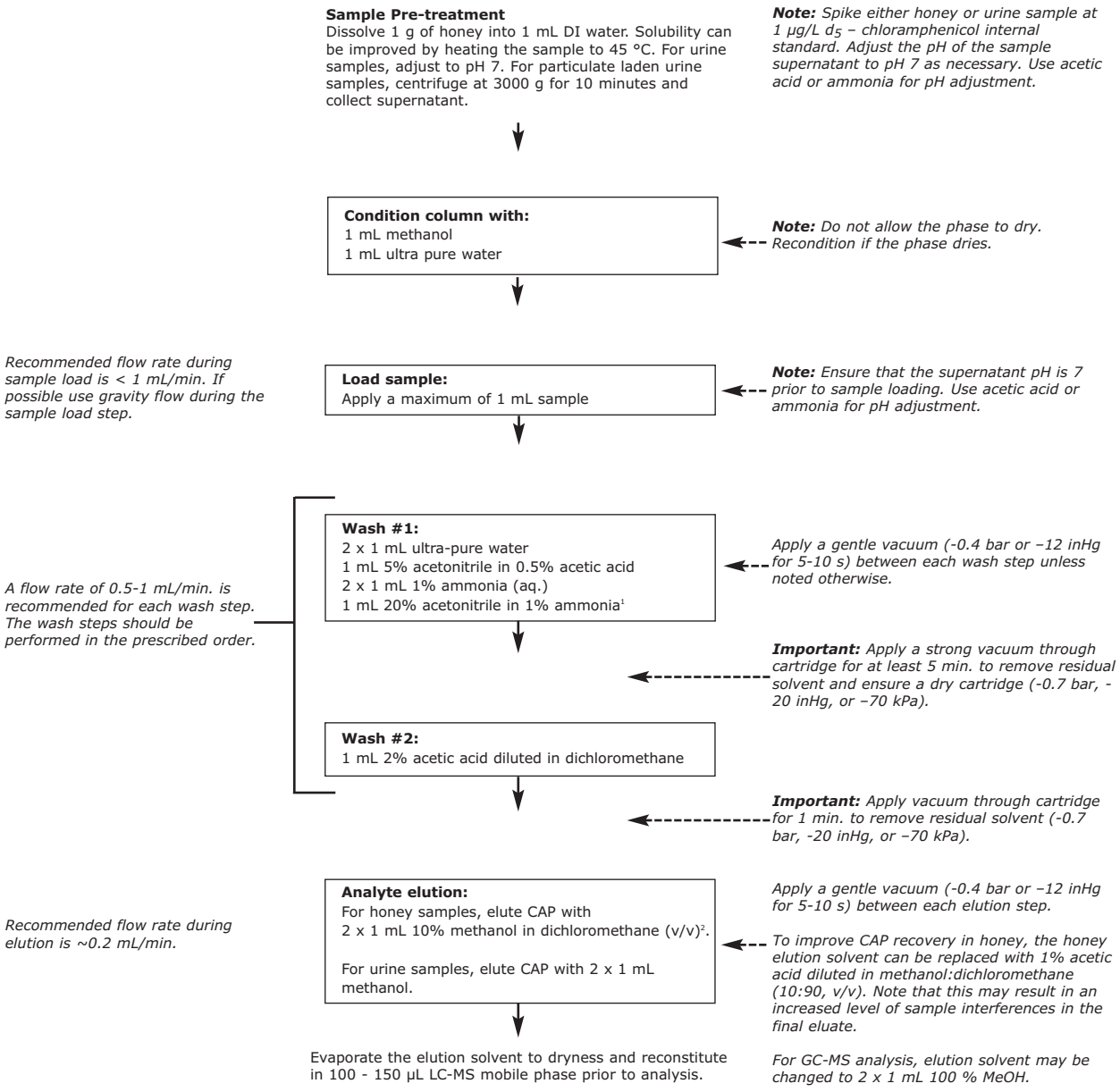
Evaporate the elution solvent to dryness and reconstitute in 100 - 150 µL LC-MS mobile phase prior to analysis.

1. 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**
2. For enhanced wash steps replace wash step **3 x 1 mL dichloromethane with 3 x 1 mL 2% acetic acid in dichloromethane**
3. 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:



1. 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**
2. 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 column with:
1 mL methanol
1 mL ultra pure water

Note: *Do not allow the phase to dry. Recondition if the phase dries.*

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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.

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.*

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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.

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

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.

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Important: *Apply a strong vacuum through cartridge for **2 min**. to remove residual solvent (-0.7 bar, -20 inHg, or –70 kPa).*

Recommended flow rate during elution is ~0.2 mL/min

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

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

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

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.
Temperature:	ambient
Detection:	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 temperature:	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.
injection volume:	20 µL

Ordering Information

Description	Quantity	Part Number
AFFINILUTE - Chloramphenicol		
25 mg/10 mL (LRC)	50	M10-0002-G
25 mg/3 mL	50	M10-0002-B

NORTH AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
press (4) at the auto attendant
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To locate a distributor please visit our web site at www.biotage.com.

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