

This Application Note contains important information about this product

AFFINILUTE™ MIP – β -agonists

Description	Quantity	Part Number
AFFINILUTE MIP β -agonists 25 mg/10 mL	50	M02-0002-G
AFFINILUTE MIP β -agonists 25 mg/3 mL	50	M02-0002-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.

Extraction of β -agonists from biological matrices

The following methods have been developed for the selective extraction of beta-agonists from biological matrices. Methods have been developed for both biological tissues (e.g. bovine muscle) and fluids (e.g. urine). The methods are highly reproducible and offer β -agonist recoveries in the range of 35-90%. Since the methods are amenable to the extraction of a wide range of β -agonists, recoveries may vary for each specific molecule. It is recommended to use the prescribed method as a screening tool to identify which β -agonists are present. Once specific β -agonists are identified, conditioning, wash, and elution steps can be further optimized to offer higher recoveries if required.

Extraction Procedure:

Recommended flow rate is \sim 0.5 mL/min. For analyte elution, a flow rate at \sim 0.2 mL/min. is recommended.

Application Name:	Extraction of β -agonists from bovine muscle and other tissues ¹	Extraction of β -agonists from urine and other biological fluids ²
Analyte:	β -agonists	β -agonists
Sample Matrix:	Validated for bovine muscle but is amenable with other tissues such as rabbit, duck, turkey, liver, and fish.	Urine
General Comments:	The compounds cimaterol, cimbuterol, ractopamine, clenpropolol, clenbuterol, brombuterol, mabuterol, mapenterol and isoxsuprine meet the requirements for quantitative determination. Screening is reliable to below 1 μ g/kg.	Typical recoveries are over 70% for ritrodriene, clenbuterol, formoterol, salmeterol, ractopamine, tulobuterol, brombuterol, and mapenterol; between 40-70% recovery is observed for terbutaline, metaproterenol and cimbuterol. Isoproterenol, salbutamol, fenoterol, and isoxsuprine cannot be determined via HPLC-UV due to interfering peaks.
Sample Pre-treatment:	<ul style="list-style-type: none"> Combine 5 g thawed minced muscle; 50 μL of 0.1 ng/μL of internal standard (deuterated analog in methanol); 5 mL Tris buffer, pH 9.5; and \sim 5 mg protease. Digest samples overnight at 60 °C. After cooling to room temperature, hydrolyze conjugates by adding: 15 μL concentrated acetic acid; 1 mL 2 M acetic buffer, pH 5.2; and 40 μL <i>suc d'Helix Pomatia</i> (Roche Diagnostics). Incubate for 2 hours at 37 °C. Adjust to pH > 12 with 10 M NaOH. Liquid-liquid extract the mixture with 10 mL ethyl acetate. Isolate upper organic layer, and extract mixture again with 5 mL ethyl acetate. Combine the organic layer from both extractions, and evaporate under N₂ at 55 °C. Reconstitute with 4 mL 20% methanol in water. Adjust to pH 1 with concentrated HCl. Remove fats from the sample by adding 1 mL heptane, shake vigorously, centrifuge at 4000 g, and remove/ discard the upper and intermediate layers³. Repeat the fat removal procedure with an additional 1 mL heptane. Neutralize the sample (lower aqueous layer) with 50 μL 10 M NaOH and 2 mL 0.1 M phosphate buffer, pH 6. 	<p>Urine (centrifuged at 3000 x g for 10 min.) diluted 1:1 (v/v) with DI water.</p> <p>50 μL β-glucuronidase – arylsulfatase 4h at room temperature.</p> <p>For β-glucuronidase treatment, please refer to Widstrand, 2004; and Fiori., 2005.</p>

1. Condition/equilibrate cartridge with:	<ul style="list-style-type: none"> • 1 mL methanol • 1 mL DI water • 1 mL 25 mM ammonium or sodium acetate, pH 6.7 	
2. Load sample: Note: recommended flow rate ~0.5 mL/min.	Apply sample to the cartridge.	
3. Wash (interference elution): Note: Apply gentle vacuum between each wash step.	<ul style="list-style-type: none"> • Apply 2 min. of full vacuum to remove residual moisture from the cartridge. • 1 mL acetonitrile • 1 mL 0.5% acetic acid in acetonitrile (selective removal of hydrophobic interferences)⁴ • 1 mL 50 mM ammonium acetate, pH 6.7 (selective removal of electrostatically bonded interferences)⁴ • 1 mL 60% acetonitrile/40% DI Water (selective removal of hydrogen bonded interferences)³ • Apply full vacuum through cartridge for 2 min. to remove residual solvent. 	<ul style="list-style-type: none"> • 1 mL DI water (selective elution/removal of salts and hydrophilic matrix components) • Apply full vacuum through cartridge for 2 min to remove residual moisture from cartridge. • 1 mL 1% acetic acid in acetonitrile (selective removal of hydrophobic interferences)⁴ • 1 mL 50 mM ammonium acetate, pH 6.7 (selective removal of electrostatically bonded interferences)⁴ • 1 mL 60% acetonitrile/40% DI Water (selective removal of hydrogen bonded interferences)³ • Apply full vacuum through cartridge for 2 min. to remove residual solvent.
4 Analyte elution: Note: recommended flow rate ~0.2 mL/min.	Elute β -agonists with 2 x 5 mL 10% acetic acid in methanol. Apply a gentle vacuum between each fraction. Evaporate and reconstitute with LC mobile phase prior to analysis.	Elute β -agonists with 2 x 1 mL 10% acetic acid in methanol. Apply a gentle vacuum between each fraction. Evaporate and reconstitute with LC mobile phase prior to analysis.

Recommended Analytical Technique: HPLC-UV or LC-MS

Column: Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2.7 μ m particle size (53822-U)
 Instrument: Applied Biosystems 3200 Q-TRAP
 Mobile phase: 10 mM ammonium acetate (pH unadjusted) in methanol (A) and MS-grade water (B)
 Flow rate: 0.2 mL/min
 (B) methanol
 Temperature: 35 °C
 Detection: MS/MS
 MRM transitions:

1. Metaproterenol	212.19/152.10
2. Terbutaline	226.21/152.20
3. Formeterol	345.21/121.00
4. Salmeterol	416.33/91.20
5. Salbutamol	240.23/148.30
6. Ritodrine	288.14/121.20

Ion mode: Positive
 Ion source: Turbospray
 Ion spray voltage: 2700 V
 Source temp: 400 °C
 Collision gas: 40 psi
 Injection volume: 30 μ L

Gradient:	Min	A%	B%
	0.0	25	75
	2.0	100	0
	4.0	100	0
	4.1	25	75
	8.0	25	75

1. Procedure based on:

The analysis of beta-agonists in bovine muscle using molecularly imprinted polymers with ion trap LCMS screening, Kootstra PR, CJPF Kuijpers, KL Wubs, D van Doorn, SS Sterk, LA van Ginkel and RW Stephany, 2005, Anal. Chim. Acta, 529:75-81

2. Procedure based on:

Multi-residue liquid chromatography/tandem mass spectrometric analysis of beta-agonists in urine using molecularly imprinted polymers. Van Hoof et al., Rapid Commun. Mass Spectrom. 2005; 19: 2801-2808 Evaluation of MISPE for the multi-residue extraction of beta-agonist from calves urine. Withstrand et al., J Chromatogr B Analyt Technol Biomed Life Sci. 2004, May 5; 804(1):85-91 Evaluation of two different clean-up steps, to minimize ion suppression phenomena in ion trap liquid chromatography-tandem mass spectrometry for the multi-residue analysis of beta agonists in calves urine. Fiori M. et al., Analytica Chimica Acta 529 (2005) 207-210

3. If the intermediate layer is very viscous, only the top uppermost layer is removed, and an additional 4 mL 20% methanol is added prior to further extraction with 1 mL hexane.

4. The prescribed wash procedure has been optimized to maximize sample clean-up prior to analysis. To increase recovery, reduce the acetic acid content of the second 1 mL 1% acetic acid in acetonitrile wash step. Recovery can be further improved by eliminating the last two 50 mM ammonium acetate and 60% acetonitrile wash step.

Ordering Information

Description	Quantity	Part Number
AFFINILUTE MIP - β-agonists (class selective)		
25 mg/10 mL	50	M02-0002-G
25 mg/3 mL	50	M02-0002-B

Related Products

Description	Quantity	Part Number
AFFINILUTE MIP - β-Blocker (class selective)		
25 mg/10 mL	50	M18-0002-G
25 mg/3 mL	50	M18-0002-B

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AFFINILUTE™ MIP – β -blockers

Description	Quantity	Part Number
AFFINILUTE MIP β -blockers 25 mg/10 mL	50	M18-0002-G
AFFINILUTE MIP β -blockers 25 mg/3 mL	50	M18-0002-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.

Extraction of Beta-blockers from urine and other biological fluids²

The following methods have been developed for the selective extraction of β -blockers from both biological matrices and water. The methods are highly reproducible and offer β -blocker recoveries of > 80%. The method minimizes matrix effects and offers limits of detection of less than 5 ppt in water and less than 10 ppt for plasma and urine. Since the methods are amenable to the extraction of a wide range of β -blockers, recoveries may vary for each specific molecule. It is recommended to use the prescribed method as a screening tool to identify which β -blockers are present. Once specific β -blockers are identified, conditioning, wash, and elution steps can be further optimized to offer higher recoveries if required.

Extraction Procedure:

A flow rate of ~0.5 mL/min. is recommended. For analyte elution a flow rate of ~0.2 mL/min. is recommended.

Application Name:	Trace level extraction of Beta-blockers from water ¹	Extraction of β -blockers from urine and other biological fluids ²
Analyte:	β -Blockers	β -Blockers
Sample Matrix:	Water	Urine or plasma
General Comments:	Typical recoveries are over 80% for atenolol, betaxolol, carazolol, metoprolol, pindolol, propranolol, sotalol and timolol.	Typical recoveries are over 85% for metoprolol, propranolol, carzalolol and atenolol.
Sample Pre-treatment:	None	Urine or plasma (centrifuged at 3000 x g for 10 min.) diluted 1:1 (v/v) with 25 mM ammonium acetate (NH ₄ Ac), pH 5.
1. Condition/equilibrate cartridge with:	<ul style="list-style-type: none"> • 1 mL methanol • 1 mL DI water 	<ul style="list-style-type: none"> • 1 mL methanol • 1 mL DI water • 1 mL 25 mM ammonium acetate (NH₄Ac), pH 5
2. Load sample: Note: recommended flow rate is 3 mL/min for natural water, and ~0.5 mL/min. for urine/plasma	Apply 100 mL water sample to the cartridge, pH 5-7. To increase head space volume, stack an empty 70 mL reservoir (Cat. No. 120-1009-F) on top of a 3 mL AFFINILUTE MIP SPE cartridge using an SPE tube adapter (120-1100 or 120-1103). For Waste water extractions apply 25 mL of sample.	Apply up to 10 mL diluted urine or 5 mL plasma sample to the cartridge.
3. Wash (interference elution): Note: Apply gentle vacuum between each wash step.	<ul style="list-style-type: none"> • 2 x 1 mL DI water (selective elution/removal of salts and hydrophilic matrix components) • Apply full vacuum through cartridge for 2 min. to remove residual moisture from cartridge. • 1 mL acetonitrile (selective removal of hydrophobic interferences) • Apply full vacuum through cartridge for 10 min. to remove residual solvent from cartridge. • 1 mL dichloromethane (to selectively enhance MIP interaction with beta-blockers) • Apply full vacuum through cartridge for 2 min. to remove residual solvent from cartridge. 	<ul style="list-style-type: none"> • 1 mL 50 mM ammonium acetate (NH₄Ac), pH 6.5 • 1 mL DI water (selective elution/removal of salts and hydrophilic matrix components) • Apply full vacuum through cartridge for 2 min. to remove residual moisture from cartridge. • 1 mL acetonitrile (selective removal of hydrophobic interferences) • 1 mL 60% acetonitrile/40% DI Water (selective removal of hydrogen bonded interferences) • Apply full vacuum through cartridge for 10 min. to remove residual solvent from cartridge.
4 Analyte elution: Note: recommended flow rate ~0.2 mL/min.	For 10 mL cartridges, elute β -blockers with 2 x 1 mL 10% acetic acid in methanol. For 3 mL cartridges, elute β -blockers with 3 x 1 mL 10% acetic acid in methanol. Apply a gentle vacuum between each fraction. Evaporate and reconstitute with LC mobile phase prior to analysis.	

Recommended Analytical Technique: LC-MS

Standard Conditions:

Column: Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particle size (53822-U)
Instrument: Waters Micromass ZQ
Mobile phase A: 10 mM ammonium acetate (pH unadjusted) in 10% acetonitrile
Mobile phase B: Acetonitrile
Flow rate: 1 mL/min., split to MS
Temperature: 35 °C
Detection: MS, ESI(+) in selected ion recording (SIR)
Injection volume: 10 µL
Gradient:

Min	A%	B%
0.0	100	0
1.0	100	0
15.0	0	100
16.0	100	0

Peak ID:

1. atenolol (M+H)+ :	267.16
2. pindolol (M+H)+ :	249.15
3. timolol (M+H)+ :	317.15
4. metoprolol (M+H)+ :	268.18
5. propranolol (M+H)+ :	260.15
6. betaxolol (M+H)+ :	308.21

Trace Conditions:

Column: Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particle size (53822-U)
Instrument: Applied Biosystems 3200 Q-TRAP MRM
Mobile phase: 10 mM ammonium acetate (pH unadjusted) in 10% acetonitrile:acetonitrile (74:26)
Flow rate: 0.2 mL/min.
Temperature: 35 °C
Detection: MS/MS
Ion mode: Positive
Ion source: Turbospray
Ionspray voltage: 3400 V
Source temp.: 375 °C
Collision gas: 45 psi
Injection: 5 µL
MRM transitions:

1. atenolol (267.27/145.20)
2. pindolol (249.15/116.20)
3. timolol (317.23/261.20)
4. metoprolol (268.29/126.10)
5. propranolol (260.12/116.20)
6. betaxolol (309.00/116.20)

1. Procedure based on worked conducted by Prof. Damia Barcelo et al at the Department of Environmental Chemistry, IIQAB-CSIC, Barcelona, Spain.

Ordering Information

Description	Quantity	Part Number
AFFINILUTE MIP - β-blocker (class selective)		
25 mg/10 mL (LRC)	50	M18-0002-G
25 mg/3 mL	50	M18-0002-B

Related Products

Description	Quantity	Part Number
AFFINILUTE MIP - β-agonists (class selective)		
25 mg/10 mL (LRC)	50	M02-0002-G
25 mg/3 mL	50	M02-0002-B

NORTH AMERICA

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AFFINILUTE™ MIP – NSAIDs

Description	Quantity	Part Number
AFFINILUTE MIP NSAIDs 25 mg/3 mL	50	M72-0002-B
AFFINILUTE MIP NSAIDs 25 mg/10 mL	50	M72-0002-G

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 NSAIDs from various matrices

The following methods have been determined for **non-steroidal anti-inflammatory drugs (NSAIDs)** that can be optimized for a number of matrices.

The following NSAIDs have been successfully extracted: **Naproxen, Clofibric Acid, Diclofenac, and Ibuprofen.**

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

Important Note: The below procedure(s) may require further optimization depending on the NSAID of interest.

Protocol for Extraction of NSAIDs – General Procedure:

Sample Pre-Treatment for solid/tissue samples:

1. Homogenize 5.0 g of sample with internal standard and add 10 mL 10 mM ammonium formate, pH 3
2. Remove particulate via centrifugation or using glass fibre filter (0.7 µm)

For liquid samples:

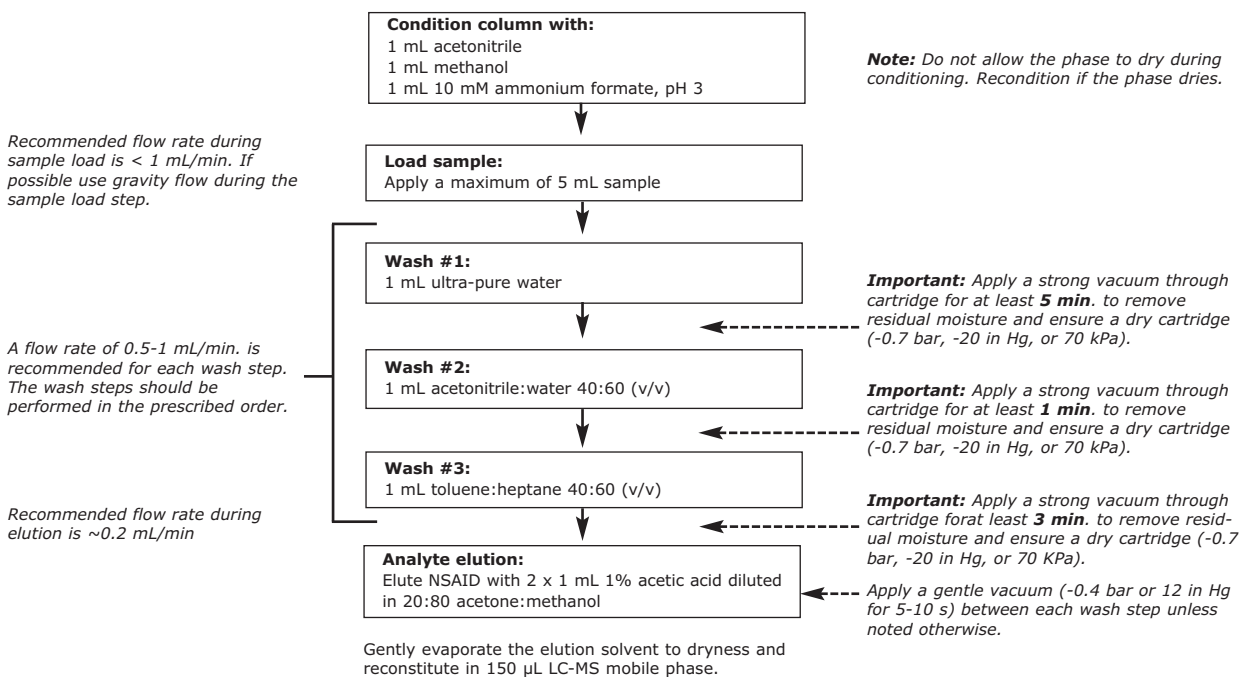
1. Adjust to pH 3 by 10 mM ammonium formate/formic acid

Note: Deuterated internal standard is recommended for each analyte for accurate quantification.

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.

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



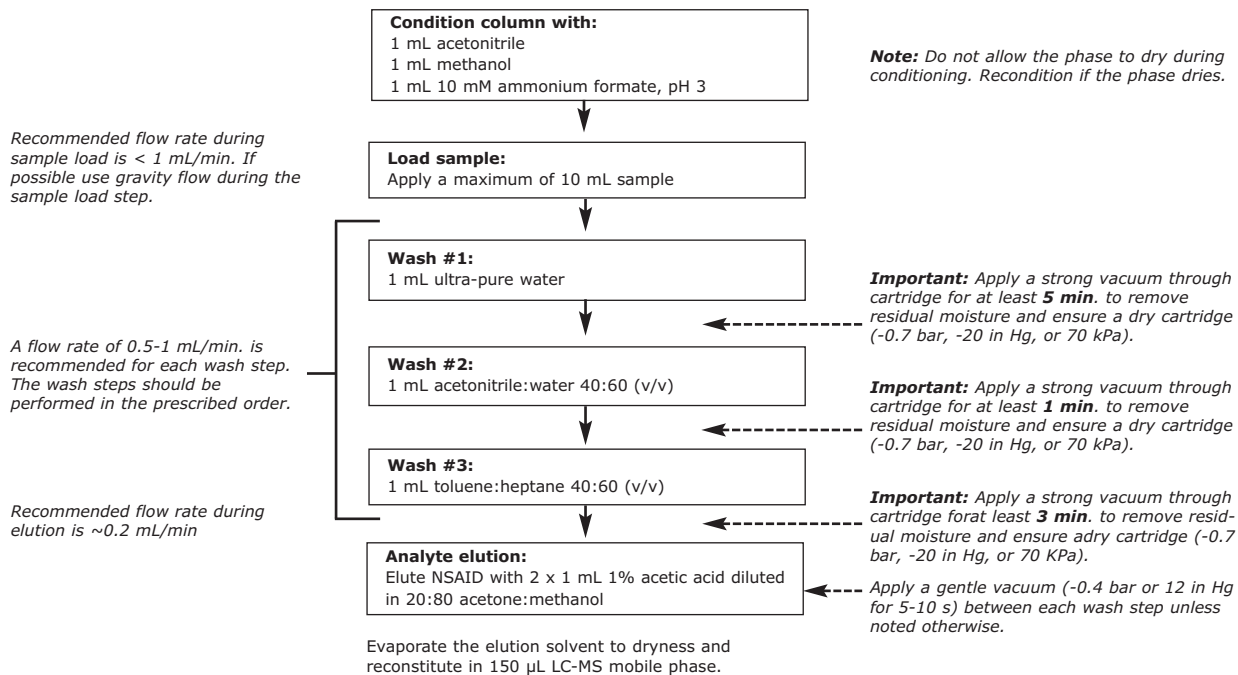
Protocol for Extraction of NSAIDs in Waste Water*

Sample Pre-treatment

1. Store waste water sample at 4°C under dark conditions prior to analysis.
2. Add internal Standard (final concentration 400 ng/mL)
3. Filter the sample as necessary using a 0.7 µm glass fiber filter
4. Adjust the sample to pH 3 using 10 mM ammonium formate buffer.

Note: Deuterated internal standard is recommended for each analyte for accurate quantification.

The sample should be completely aqueous prior to SPE processing. No organic modifiers should be present in the sample.



* Selective determination of acidic pharmaceuticals in wastewater using molecularly imprinted solid-phase extraction. S.Zorita¹, B.Boyd², S.Jönsson², E.Yilmaz², C.Svensson², L.Mathiasson², S.Bergström². 1. Division of Analytical Chemistry, Lund University Lund, Sweden. 2. MIP Technologies AB, Lund, Sweden. Analytica chimica acta 626 (2008) 147-154

Troubleshooting:

Improve Recovery:

- Do not exceed the recommended load and wash volumes.
- Minimize flow rate during sample load and elution.
- Ensure that the sample is pH ≤ 3 prior to sample load.
- The drying steps described in the wash section are critical for maintaining optimal recovery and selectivity.
- Do not exceed the acetonitrile level (40:60) described in wash step #2. Higher concentrations of acetonitrile may result in premature analyte elution.
- Increase elution from 2 x 1 mL to 3 x 1 mL

**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: LC-MS/MS
Mobile phase: (A) 5 mM ammonium formate, pH 6
(B) methanol
Gradient:

	Min.	A%	B%
	0.0	65	35
	13.0	10	90
	13.1	65	35
	17.0	65	35

Flow rate: 0.2 mL/min.
Temperature: Ambient
Detection: MS/MS, MRM transitions
Clofibrac acid 213/127 & 213/85
Naproxen 229/185 & 229/170
Diclofenac 294/250 & 294/214
Ibuprofen 205/161
Ibuprofen-d₃ 208/164
Polarity: Negative
Ion source: Turbospray
Ion spray voltage: 4000 V
Source temp: 500 °C
Nebulizer gas: 60 psi
Curtain gas: 10 psi
Injection volume: 30 µL

Ordering Information

Description	Quantity	Part Number
AFFINILUTE MIP NSAIDs 25 mg/3 mL	50	M72-0002-B
AFFINILUTE MIP NSAIDs 25 mg/10 mL	50	M72-0002-G

NORTH AMERICA

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