This Application Note contains important information about this product

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 fluids[2]

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, propanolol, 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_4Ac), pH 5.
1. Condition/equilibrate cartridge with:	• 1 mL methanol • 1 mL DI water	• 1 mL methanol • 1 mL DI water • 1 mL 25 mM ammonium acetate (NH_4Ac), 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.	• 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 inter action with beta-blockers) • Apply full vacuum through cartridge for 2 min. to remove residual solvent from cartridge.	• 1 mL 50 mM ammonium acetate (NH_4Ac), 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.	

Application Note

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. propranol (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

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