This Application Note contains important information about this product

AFFINILUTE™ MIP – Amphetamines

Description	Quantity	Part Number
AFFINILUTE MIP Amphetamines 25 mg/3 mL	50	M28-0002-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 Amphetamines from Urine

The following method(s) have been developed for the class-selective extraction of Amphetamine, Methamphetamine, Phentermine, MDA, MDMA, MDEA from human urine. The method is highly reproducible and offers an average recovery greater than 80%. Lower limits of detection and quantification from 1 mL urine using the described AFFINILUTE and LC-MS-MS procedures are as follows:

	LOD (ng/mL)	LOQ (ng/mL)
Methamphetamine	0.0020	0.0066
Amphetamine	0.0022	0.0073
MDA	0.0129	0.0430
MDMA	0.0009	0.0030
MDEA	0.0008	0.0025
Phentermine	0.0044	0.0150

Extraction Procedure:

A flow ≤ 0.5 mL/min. is recommended during sample load, 0.5-1 mL/min. during wash steps, and ~0.2 mL/min. for elution. If possible, use gravity flow during the sample load step. A gentle vacuum (-0.4 bar or –12 inHg for 5-10 s) should be applied between each wash step and between the elution fractions unless described otherwise.

Analyte:	Amphetamine, Methamphetamine, Phentermine, MDA, MDMA, MDEA
Sample Matrix:	Human urine
General Comments:	The method is optimized for the class-selective extraction of trace levels of multiple amphetamines in human urine. Using the described procedure, ion-suppression is reduced thereby allowing lower limits of quantification relative to traditional SPE procedures.
Sample Pre-treatment:	Dilute up to 5 mL urine with 10 mM NH_4Ac pH 8.0 (1:1, v/v). Adjust to pH 7.5-8.5 with NH_3 or CH_3COOH. For particulate laden samples, centrifuge at 3000 g for 10 minutes and isolate supernatant for SPE preparation. Apply deuterated internal standard as necessary.
1. Condition/equilibrate cartridge with:	• 1 mL methanol • 1 mL 10 mM NH_4Ac buffer pH 8.0
2. Load sample: Note: recommended flow rate is 3 mL/min for natural water, and ~0.5 mL/min. for urine/plasma	Apply 1 mL diluted urine sample. Note that up to 10 mL diluted urine sample can be applied. Note: Flow rate at ≤0.5 mL/min. is recommended during sample load. If possible use gravity flow during the sample load step.
3. Wash (interference elution): The wash steps should be performed in the prescribed order. A flow rate of 0.5-1 mL/min. is recommended for each wash step.	• 2 x 1 mL DI water (elution of salt and matrix components). **Important:** Do not let the cartridge dry after the water wash steps • 1 mL 60% acetonitrile in DI water (elution of hydrophobic matrix components) **Important:** Apply vacuum through cartridge for 5-10 min. to remove residual moisture from cartridge (-1 bar, -20 in Hg, or –70kPa). • 1 mL 1% acetic acid in acetonitrile Apply a gentle vacuum (-0.4 bar or –12 inHg) for ~30 sec. to the cartridge before elution.
4. Analyte elution: Note: recommended flow rate ~0.2 mL/min.	Elute amphetamines with 2 x 1 mL 1% formic acid in methanol. Apply a gentle vacuum (-0.4 bar or –12 inHg) for ~30 sec. to the cartridge between each elution fraction. Evaporate the elution solvent to dryness and reconstitute in 100 µL mobile phase (90% A and 10% B) prior to analysis. For GC-MS analysis, reconstitute and derivatize according to the selected method.

Application Note

Note that the original LC-MS-MS method developed and validated using TFA as a mobile phase pH modifier/ion-pairing agent was based on a method described by Fuh et al (1). Under these run conditions, excellent peak shape/efficiency and minimal ion-suppression was observed allowing for maximum sensitivity during chromatographic analysis.

However, for analysts concerned with using TFA as a mobile phase ion-pairing agent, an alternative method was developed using ammonium acetate buffer. Please note that when using the ammonium acetate method, instrumental LOQs for the amphetamine compounds will be inferior relative to the TFA method, and a run time of at least 17 minutes will be required to achieve proper re-equilibration of HPLC column.

Column:	Ascentis® C18, 15 cm x 2.1 mm I.D., 5 µm particles (581304-U)
Instrument:	Sciex API 3200
Mobile phase A:	0.05% TFA in DI water **OR** 13 mM ammonium acetate, pH 7
Mobile phase B:	0.05% TFA in acetonitrile **OR** 13 mM ammonium acetate in acetonitrile (when using ammonium acetate buffer as mobile phase A)
Temperature:	22 °C
Flow rate:	0.2 mL/min. split

Gradient:

Min	A%	B%
0.0	90	10
7.0	70	30
10.0	70	30
11.0	10	90
11.2	90	10
15.0	90	10

Detection: **MS/MS, MRM transitions:**

Compound	Rt (min.)	Q1/Q3	DP	EP	CEP	CE	CXP
Amphetamine	7.80	136/119	20	7.5	12	13	2
		136/91				30	2
Methamphetamine	8.33	150/119	25	5	12	14	2
		150/91				29	2
Methampetamine D8 (IS)	8.33	158/124	30	5	12	14	2
		158/93				25	2
Phentermine	8.66	150/133	25	5	12	13	2
		150/91				29	2
MDA	8.04	180/163	25	5	12	14	2
		180/105				33	2
MDMA	8.48	194/163	27	5	15	18	2
		194/105				33	2
MDMA D_5 IS	8.48	199/165	25	4	10	17	4
		199/136				29	4
MDEA	9.18	208/163	27	5	15	18	2
		208/105				35	2

Polarity:	Positive
Ion source:	Turbospray
Ion spray voltage:	5500 V
Source temp:	600 °C
Collision gas:	6 psi
Curtain:	10 psi
Ion source gas 1	60 psi
Ion source gas 2	60 psi
Dwell time:	100 msec.
Run time:	15 min.
injection Volume	20 µL

Ordering Information

Description	Quantity	Part Number
AFFINILUTE MIP - Amphetamines (class selective)		
25 mg/3 mL	50	M28-0002-B

1.Determination of amphetamine and methamphetamine in urine by solid phase extraction and ion-pair liquid chromatography electrospray–tandem mass spectrometry, Fuh M, Wu T, Lin T, Talanta 68 (2006) 987–99.

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AFFINILUTE™ MIP – TSNAs

Description	Quantity	Part Number
AFFINILUTE MIP TSNAs 50 mg/10 mL	50	M21-0005-G
AFFINILUTE MIP TSNAs 50 mg/3 mL	50	M21-0005-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 TSNAs from urine

The following methods have been developed for the selective extraction of tobacco specific nitrosamines (NNN, NNK, NAT, and NAB) from human urine. The method is highly reproducible and offers an average recovery of 80% (95% for NNK and NAT; and 70% for NNN and NAB). Detection limits of 4 pg/mL are readily achieved for urine samples using LC-MS-MS analysis.

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.

Analyte:	NNN, NNK, NAT and NAB
Sample Matrix:	Human urine
General Comments:	The method is optimized for the extraction of tobacco specific nitrosamines from urine. Using the exhaustive wash steps detailed in this method, TSNA detection levels of 4 pg/mL urine are achievable via LC-MS/MS analysis.
Sample Pre-treatment:	Adjust sample pH to 5.5 with acetic acid. Add 1 ng/mL NNK d3 internal standard. For urine glucoronidase treatment, please refer to Stepanov, 2005.
1. Condition/equilibrate cartridge with:	• 1 mL methanol • 1 mL DI water Do not allow the cartridge to dry during conditioning
2. Load sample: Note: recommended flow rate ~0.5 mL/min.	Apply 1 mL sample to the cartridge.
3. Wash (interference elution): Note: Apply gentle vacuum between each wash step.	• 1 mL 10 mM ammonium acetate, pH 5.5 • Apply full vacuum through cartridge for 10 min. to remove residual moisture from cartridge. • 1 mL heptane (selective removal of hydrophobic interferences) • Apply full vacuum through cartridge for 5 min. to remove residual solvent.
4 Analyte elution: Note: recommended flow rate ~0.2 mL/min.	Elute TSNAs with 2 x 1 mL 10% methanol in dichloromethane. Apply a gentle vacuum between each fraction. Evaporate and reconstitute with 100 µL LC mobile phase prior to analysis.

Application Note

Recommended Analytical Technique:
LC-MS-MS or LC-MS

Column:	Ascentis® C18, 5 cm x 3 mm I.D., 3 µm particles (581307-U)		
Instrument:	Sciex API 3200		
Mobile phase A:	10 mM ammonium formate, pH 6.1		
Mobile phase B:	acetonitrile		
Temperature:	25 °C		
Flow rate:	0.5 mL/min.		
Gradient:	**Time (min.)**	**%A**	**%B**
	0.0	90	10
	1.0	90	10
	4.0	60	40
	5.0	30	70
	6.0	30	70
	6.1	90	10
	9.0	90	10

Detection:	MS/MS, MRM transitions NNK (208.10/122.00) NNN (178.20/148.10) NAB (192.20/162.20) NAT (190.10/160.20) I.S. (211.30/122.10)
Polarity:	Positive
Ion source:	Turbospray
Ion spray voltage:	5500 V
Decluster potential:	25 V for NNK, 22 V for NNN, 30 V for NAB, 20 V for NAT, 25 V for NNK-*d3*,
Entrance potential:	5.0 V
Source temp:	500 °C
Collision gas:	5 psi
Collision cell exit potential:	5.0 V
Curtain:	30 psi
Dwell time:	100 msec.
Run time:	9 min.
Injection volume:	10 µL

Tobacco-Specific Nitrosamines and Their Pryridine-N-glucuronides in Urine of Smokers and Smokeless Tobacco Users, Stepanov I, Hecht S, Cancer Epidemiology Biomarkers and Prevention Vol. 14, 885-891, April 2005

Ordering Information

Description	Quantity	Part Number
AFFINILUTE - TSNAs (NNK, NNN, NAB, NAT)		
50 mg/10 mL (LRC)	50	M21-0005-G
50 mg/3 mL	50	M21-0005-B

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AFFINILUTE™ MIP – NNAL

Description	Quantity	Part Number
AFFINILUTE MIP NNAL 25 mg/10 mL	50	M06-0002-G
AFFINILUTE MIP NNAL 25 mg/3 mL	50	M06-0002-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 free & total NNAL for LC-MS-MS Analysis[1]

The following methods have been developed for the selective extraction of the tobacco specific nitrosamine, NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) from aqueous sample matrices such as biological fluids (e.g., urine). The method is highly reproducible and offers NNAL recoveries in the range of 90% and a limit of detection below 5 ppt. The described methods have been adapted for the extraction of free and total NNAL.

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.

Analyte:	NNAL
Sample Matrix:	Urine
General Comments:	Both free and total NNAL (free NNAL + NNAL-glucoonride) can be extracted using this method.
Sample Pre-treatment:	*Free NNAL:* Add internal standard NNAL d_3 (200 pg/mL) to each sample. Certrifuge for 10 min. at 5000 rpm. Adjust supernatant to pH 6-7 with acetic acid. To improve flow, samples can be diluted 1:1 with 50 mM ammonium dihydrogen phosphate buffer, pH 6.4. Total NNAL: Dilute 5 mL urine sample with 10 mL 50 mM ammonium dihydrogen phosphate ($NH_4H_2PO_4$ *$2H_2O$), pH 6.4. Add 20 µL of 100 ng/mL [$^{13}C_6$] NNAL ISTD to diluted sample. Add 0.5 mL 20,000 unit/mL β-glucuronidase solution (Type IX-A from E. coli.) and incubate at 37 ºC for 24 to 48 hours. Filter diluted sample with 0.45 µm filter.
1. Condition/equilibrate cartridge with:	• 1 mL dichloromethane • 1 mL methanol • 1 mL DI water • Do not allow to dry prior to sample load
2. Load sample: Note: recommended flow rate ~0.5 mL/min.	Apply diluted sample (see sample pre-treatment) to the cartridge. A maximum sample volume of 5 mL (undiluted urine) should be applied. Recovery may be reduced with larger volumes.
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 10 min. to remove residual moisture from cartridge. • 1 mL toluene • 1 mL toluene: DCM (9:1, v/v) • 1 mL toluene: DCM (4:1, v/v) • Apply full vacuum through cartridge for 2 min. to remove residual solvent.
4 Analyte elution: Note: recommended flow rate ~0.2 mL/min.	Elute NNAL with 2 x 1 mL 10% methanol in DCM. Apply a gentle vacuum between each DCM fraction. Evaporate and reconstitute with LC mobile phase (150 µL, 10 mM ammonium formate, pH 6.1) prior to analysis.

Application Note

Recommended Analytical Technique:
LC-MS-MS

Column: Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particle size (581307-U)
Instrument: API3200 MS/MS
mobile phase: 10 mM ammonium formate (pH 6.1) (A) and acetonitrile (B)
flow rate: 0.5 mL/min.
temperature: 25 °C
Detection: API3200 MS/MS
Injection volume: 10 µL

MRM transitions:

Analyte	Q1	Q3	Time (ms)	DP	EP	CEP	CE	CXP
NNAL Quantification	210.00	180.20	200	27	5	10	14	5
NNAL-d_3 Quantification	213.00	183.20	200	27	5	10	14	5
NNAL Identification	210.00	93.20	200	27	5	10	27	5
NNAL-d_3 Identification	213.00	93.20	200	27	5	10	27	5

Approximate Retention Time:
NNAL 2.1 min.
NNAL-d3 2.0 min.

Ion mode: Positive
Ion source: Turbospray, ESI
Ion spray voltage: 4000 V
Source temp.: 400 °C

Gradient:

Min.	A%	B%
0.00	90	10
1.50	70	30
2.50	70	30
2.60	90	10
4.50	90	10

Ordering Information

Description	Quantity	Part Number
AFFINILUTE – NNAL		
25 mg/10 mL (LRC)	50	M06-0002-G
25 mg/3 mL	50	M06-0002-B

Related Products

Description	Quantity	Part Number
AFFINILUTE - TSNAs (NNK, NNN, NAB, NAT)		
50 mg/10 mL (LRC)	50	M21-0005-G
50 mg/3 mL	50	M21-0005-B

1. This method is based on the work published by the Center for Disease Control and Prevention: Analysis of the Tobacco-Specific Nitrosamine 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol in Urine by Extraction on a Molecularly Imprinted Polymer Column and Liquid Chromatography/Atmospheric Pressure Ionization Tandem Mass Spectrometry, Xia Y, McGuffey JE, Bhattacharyya S, Sellergren B, Yilmaz E, Wang L, and Bernert JT, Anal. Chem. 77 (2005) 7639-7645.

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