

**This Data Sheet Contains Important Information About This Product.**

## SupelMIP™ SPE – NNAL

### Product Description:

Molecular 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, 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.

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.**

Application Name:	Extraction of free & total NNAL for LC-MS-MS Analysis <sup>1</sup>
Analyte:	NNAL
Sample Matrix:	Urine
General Comments:	Both free and total NNAL (free NNAL + NNAL-glucoride) can be extracted using this method.
SupelMIP SPE – NNAL:	25 mg/10 mL (LRC) (Cat. No. 53206-U) or 25 mg/3 mL (LRC) (Cat. No. 53203-U)
Sample Pre-treatment:	<i>Free NNAL:</i> Add internal standard NNAL <i>d3</i> to each sample at the level of 200 pg/mL. Centrifuge 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. <i>Total NNAL:</i> Dilute 5 mL urine sample with 10 mL 50 mM ammonium dihydrogen phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$ ), pH 6.4. Add 20 $\mu\text{L}$ of 100 ng/mL [ $^{13}\text{C}_6$ ] NNAL ISTD to diluted sample. Add 0.5 mL 20,000 unit/mL $\beta$ -glucuronidase solution (Type IX-A from <i>E. coli</i> ) and incubate at 37 °C for 24 to 48 hours. Filter diluted sample with 0.45 $\mu\text{m}$ filter.
1. Condition/equilibrate cartridge with:	<ul style="list-style-type: none"> <li>◆ 1 mL dichloromethane</li> <li>◆ 1 mL methanol</li> <li>◆ 1 mL DI water</li> <li>◆ Do not allow to dry prior to sample load</li> </ul>
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 at 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.	<ul style="list-style-type: none"> <li>◆ 2 x 1 mL DI water (selective elution/removal of salts and hydrophilic matrix components)</li> <li>◆ Apply full vacuum through cartridge for 10 min. to remove residual moisture from cartridge.</li> <li>◆ 1 mL toluene</li> <li>◆ 1 mL toluene: DCM (9:1, v/v)</li> <li>◆ 1 mL toluene: DCM (4:1, v/v)</li> <li>◆ Apply full vacuum through cartridge for 2 min. to remove residual solvent.</li> </ul>
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 mL, 10 mM ammonium formate, pH 6.1) prior to analysis.

<b>Recommended Analytical Technique: LC-MS-MS</b>	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.									
	temp.:	25 °C									
	det.:	API3200 MS/MS									
	injection volume:	10 µL									
	MRM transitions:	<b>Analyte</b>	<b>Q1</b>	<b>Q3</b>	<b>Time (ms)</b>	<b>DP</b>	<b>EP</b>	<b>CEP</b>	<b>CE</b>	<b>CXP</b>	
		NNAL Quantification	210.00	180.20	200	27	5	10	14	5	
		NNAL-d3 Quantification	213.00	183.20	200	27	5	10	14	5	
	NNAL Identification	210.00	93.20	200	27	5	10	27	5		
	NNAL-d3 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:	<b>Min.</b>	<b>A%</b>	<b>B%</b>							
		0.00	90	10							
		1.50	70	30							
		2.50	70	30							
		2.60	90	10							
		4.50	90	10							

## Product Information:

Description	Pkg. Qty.	Cat. No.
<b>SupelMIP SPE - Clenbuterol</b>		
25 mg/10 mL (LRC)	50	<b>53201-U</b>
<b>SupelMIP SPE - Beta-agonists (class selective)</b>		
25 mg/10 mL (LRC)	50	<b>53202-U</b>
25 mg/3 mL	50	<b>53225-U</b>
<b>SupelMIP SPE – NNAL</b>		
25 mg/10 mL (LRC)	50	<b>53206-U</b>
25 mg/3 mL	50	<b>53203-U</b>
<b>SupelMIP SPE - Riboflavin (Vitamin B2)</b>		
25 mg/10 mL (LRC)	50	<b>53207-U</b>
<b>SupelMIP SPE - Triazine 10</b>		
25 mg/10 mL (LRC)	50	<b>53208-U</b>
<b>SupelMIP SPE - Chloramphenicol</b>		
25 mg/10 mL (LRC)	50	<b>53210-U</b>
25 mg/3 mL	50	<b>53209-U</b>
<b>SupelMIP SPE - Beta-blocker (class selective)</b>		
25 mg/10 mL (LRC)	50	<b>53218-U</b>
25 mg/3 mL	50	<b>53213-U</b>
<b>SupelMIP SPE - TSNA's (NNK, NNN, NAB, NAT)</b>		
50 mg/10 mL (LRC)	50	<b>53221-U</b>
50 mg/3 mL	50	<b>53222-U</b>
<b>SupelMIP SPE - Full Beta-receptors (beta-blockers &amp; beta-agonists)</b>		
25 mg/10 mL (LRC)	50	<b>53223-U</b>
25 mg/3 mL	50	<b>53224-U</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

*SupelMIP SPE developed by MIP Technologies AB*  
SupelMIP is a trademark of Sigma-Aldrich Co.

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