# Extraction of Tobacco-Specific Nitrosamines (TSNAs) from Urine Using ISOLUTE® SLE+ Prior to UPLC/MS/MS Analysis

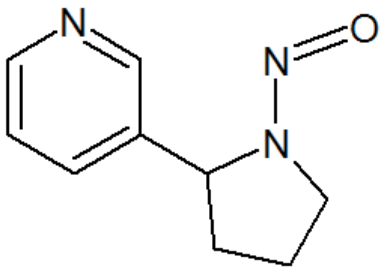


**Figure 1.** Structure of N-nitrosonornicotine (NNN).

## Introduction

TSNAs, or tobacco-specific nitrosamines, are carcinogens found in tobacco products, including e-cigarettes and smokeless tobacco. NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol), and NNN (n-nitrosonornicotine) are the most commonly analyzed TSNAs. NNK has been seen to cause lung cancer in laboratory animals, NNAL is a metabolite of NNK, and NNN has been seen to cause esophageal cancer in laboratory animals. This proof of concept study focusses on NNN (n-nitrosonornicotine).

TSNAs can be difficult to accurately detect, as, like nicotine, there can be false positives for secondhand and third-hand contamination. Secondhand contamination, or secondhand smoke, causes non-smokers to test positive for TSNAs if the detection limits for the method are low. Third-hand contamination can be from test tubes, extraction media, pipette tips, or any number of items used throughout the sample extraction and analysis processes.

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

## Analytes

This proof of concept study focusses on NNN (n-nitrosonornicotine), but the supported liquid extraction methodology described is a also suitable for NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)

## Sample Preparation Procedure

Most donor urine samples will test positive for various TSNAs, especially at lower limits of quantitation/detection. Because of this, water or a synthetic urine (i.e. Urisub) should be used for all controls/calibrators.

### Format

ISOLUTE® SLE+ 1 mL sample volume columns (tabless), p/n 820-00140-CG.

### Sample Loading

Load 1 mL of the pre-treated (IS spiked) urine sample onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

### Analyte Extraction

Apply 1.5 mL of dichloromethane and allow to flow under gravity for 5 minutes. Apply a further aliquot of dichloromethane (1.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining extraction solvent (5–10 seconds).

### Post Elution and Reconstitution

Dry the extract in a stream of air or nitrogen using a TurboVap® at 40 °C, 1.5 L/min, for 20 minutes.

Reconstitute in Mobile Phase A/ Mobile Phase B (90:10, v/v, 100 µL).



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## HPLC Parameters

### Instrument
Shimadzu Nexera X2

### Column
Restek Raptor Biphenyl (50 x 3 mm, 2.1 µm)

### Mobile Phase A (MOB A)
0.1% formic acid in water

### Mobile Phase B (MOB B)
Acetonitrile

### Flow Rate
0.45 mL/min

### Column Temperature
50°C

## MS/MS Parameters

### Instrument
SCIEX 5500 Triple Quadrupole Mass Spectrometer

### Curtain Gas
30 °C

### CAD
8

### Ionspray
1500 V

### Temperature
400 °C

### GS1
50

### GS2
50

### Entrance Potential
10

**Table 1.** LC Gradient for Restek Raptor Biphenyl 50 x 3 mm, 2.1 µm column.

| Time | % MOB B |
|---|---|
| 0.01 | 5 |
| 0.5 | 15 |
| 4.75 | 60 |
| 5.0 | 95 |
| 5.8 | 95 |
| 6.0 | 5 |

**Table 2.** Transitions for SCIEX 5500 Triple Quadrupole Mass Spectrometer.

| Compound | Q1 | Q3 | DP | CE | CXP |
|---|---|---|---|---|---|
| NNN-$D_4$ 1 | 182.045 | 124.1 | 181 | 25 | 8 |
| NNN- $D_4$ 2 | 182.045 | 122.1 | 181 | 41 | 8 |
| NNN- $D_4$ 3 | 182.045 | 109.1 | 181 | 25 | 8 |
| NNN 1 | 178.009 | 120.1 | 156 | 25 | 8 |
| NNN 2 | 178.009 | 119.1 | 156 | 41 | 8 |
| NNN 3 | 178.009 | 105.1 | 156 | 25 | 8 |




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## Results

When using this method, chromatography for the TSNAs and their internal standards is acceptable. If determination of higher TSNA concentrations are desired, detuning the compounds may be necessary. Column overload could be seen at concentrations of 10 and 100 ng/mL when using this method. Limits of quantitation/detection (LOQ/LODs) of 10 pg/mL can be achieved when using ISOLUTE® SLE+ for sample clean up.

## Discussion

Due to the potential for contamination of control urine samples, spiked water or synthetic urine should be run to determine LOQ/LODs along with water or synthetic urine that has not been spiked. This will help determine at what concentration the negative samples may contain TSNAs. Running several donor urine samples from both smokers and non-smokers could help to determine reasonable cutoff concentrations. This method can be difficult to develop due to secondhand and third-hand TSNA contamination.

## Conclusion

Using an ISOLUTE SLE+ sample preparation method allows for a fast extraction method with sufficient sample clean up resulting in cleaner extracts.

## Ordering Information

| Part Number | Description | Quantity |
|---|---|---|
| **820-0140-CG** | ISOLUTE SLE+ 1 mL Supported Liquid Extraction Column Tabless | 30 |
| **PPM-48** | Biotage PRESSURE+ 48 Positive Pressure Manifold for Columns. | 1 |
| **SD-9600-DHS-EU** | Biotage® SPE Dry 96 Sample Evaporator 220/240V | 1 |
| **SD-9600-DHS-NA** | Biotage® SPE Dry 96 Sample Evaporator 100/120V | 1 |
| **C103198** | TurboVap® LV Without Racks 100/120V | 1 |
| **C103199** | TurboVap® LV Without Racks 220/240V | 1 |

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