Analysis of the Tobacco-specific Nitrosamine 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol in Urine by SPE on a Molecularly Imprinted Polymer (MIP) Column and LC MS/MS

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OVERVIEW

- Purpose
  - -Introduce and optimize new SPE column
  - -Faster and more effective sample cleanup
- Method
  - -Using MIP column in SPE
  - -LC/MS/MS PE Sciex 4000
- Results
  - -Optimization of pH, ionic strength, elution in SPE
  - -Comparison of β-glucuronidases
  - -Precision, accuracy, carryover and sample stability
  - -10 smokers and 5 exposed nonsmokers

INTRODUCTION

- Tobacco-specific nitrosamines such as NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, is significant components of tobacco and tobacco smoke.
- NNK and its metabolite, NNAL (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol), are potent lung carcinogens.
- NNAL and its glucuronide (NNAL-gluc) are present in the urine of smokers and nonsmokers (Figure 1).
- Urinary NNAL levels are quite low (parts per trillion level in smokers, and down to parts per quadrillion level in minimally exposed nonsmokers).

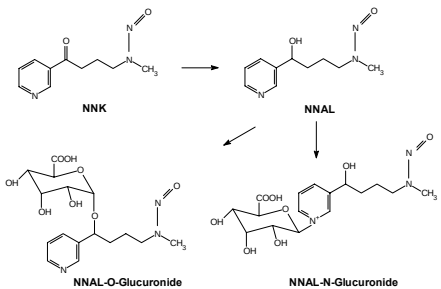


Figure 1. Metabolism of NNK and NNAL

Time (min)	Mobile Phase A	Mobile Phase B	System Controller
0.01			Start
0.02	100%	0%	
1.61	0%	100%	
1.81	100%	0%	
3.50			Stop

Table 1. Mobile phase gradient table in HPLC seperation

METHODS

SPE using MIP column

- Column conditioning with 1 ml CH_2Cl_2, 1 ml methanol and 1 ml water
- Sample loaded at 0.5 ml/min and dried for 5 min under N_2
- MIP column washed with 1 ml water, 1 ml toluene and 1 ml toluene:CH_2Cl_2(9:1)
- Sample collected by 3 x 1 ml CH_2Cl_2

Free NNAL and total NNAL

- Free NNAL: 20 µl [1', 2', 3', 4', 5', 6'-$^{13}C_6$]NNAL (ISTD, 100 ng/ml) was spiked into 5 ml urine, followed by the addition of 2 ml PO4 buffer (0.5 M, pH 6.4). The mixture was filtered.
- Total NNAL (free NNAL + NNAL-Gluc): 20 µl [$^{13}C_6$]NNAL (ISTD, 100 ng/ml) was spiked into 5 ml urine, followed by the addition of 10 ml PO4 buffer (0.5 M, pH 6.4) and 0.5 ml 20,000 unit/ml β-glucuronidase solution. The solution was incubated at 37 0C for 48 hours and filtered before SPE.

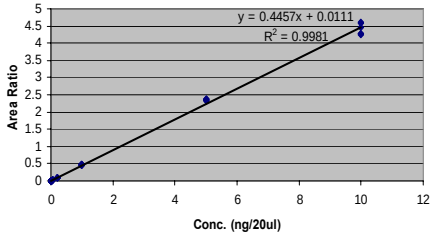
LC/MS/MS

- A Shimadzu SCL-10A system controller, two Shimadzu LC-10AD liquid chromatographs, a Shimadzu DGU-14A degasser, an Agilent 1100 autosampler, an Agilent 1100 column oven and a PE Sciex API 4000 triple quadrupole mass spectrometer.
- XTerra® MS C18 narrow-bore column(3.5 µm, 2.1x100mm), maintained at 40 ^{0}C, flow rate 0.600 ml/min. Gradient of mobile phase A (8mM ammonium acetate, pH 5) and B (100% acetonitrile) as in Table 1.
- Turbo-ionspray ionization source at 500 ^{0}C with a spray voltage of 5500 volts, MRM transitions: 210.2/93.2 (for confirmation), 210.2/180.1 (for quantitation), and 216.1/186.1 (ISTD).

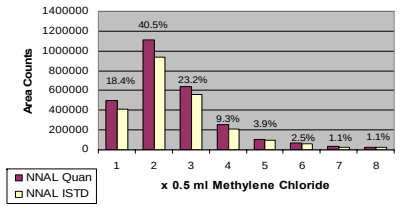
RESULTS

- Maximum recovery by MIP extraction is pH 6.4
- SPE elution is achieved by 3 x 1 ml CH_2Cl_2
- Type IX-A from *E. coli.* β-glucuronidase was found to be more efficient than type H-1 from *E. Coli.*
- NNAL level in exposed nonsmokers is much lower than in smokers.

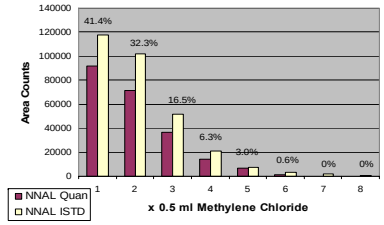
NNAL STDs Calibration Curve



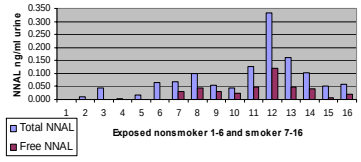
NNAL Elution Fractions from MIP Columns (500 pg/ml)



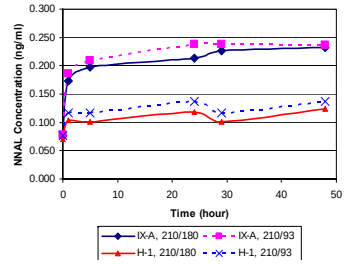
NNAL Elution Fractions from MIP Columns (50 pg/ml)



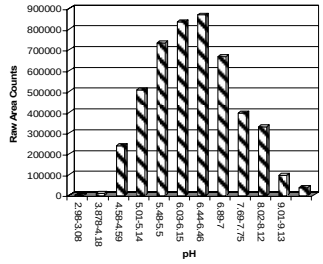
NNAL levels in smokers and nonsmokers' urine

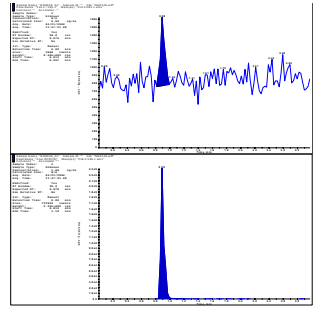


β-Glucuronidases Study



pH Evaluation of MIP





Chromatogram of 2.5 pg NNAL(top) and 1 ng $^{13}C_6$]NNAL(bottom)

Conclusions

- **Using MIP in SPE was a success for NNAL measurement.**
- **Faster and more efficient SPE by using MIP allows higher sample throughput.**