Assay Methodology

Urinary tobacco alkaloids (nicotine and its six major metabolites, plus minor tobacco alkaloids anatabine and anabasine) were measured by an isotope dilution high-performance liquid chromatography/tandem mass spectrometric method.1 The LODs for these alkaloids ranged from 0.39 to 10.5 ng/mL, depending on the analyte. The highly-sensitive assays for cotinine and trans-3’-hydroxycotinine used a modified version of isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry as described by Bernert et al.2 The LOD was much lower than the usual assay (0.030 ng/mL) for both analytes in these sensitive assays. TSNAs were measured by isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry using a modified version of the method described by Xia et al.3 The LOD for urinary TSNAs ranged from 0.0006 to 0.0042 ng/mL. The 7 PAH metabolites were quantified by online solid phase extraction coupled with high-performance liquid chromatography-isotope dilution tandem mass spectrometry, as previously described.4 The LODs for PAHs ranged from 0.008 to 0.09 ng/mL. Urinary VOC metabolite concentrations were measured using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry according to a published procedure.5 LODs for VOCs ranged from 0.500 to 15.0 ng/mL. Finally, creatinine was measured by a commercial automated, colorimetric enzymatic (creatinase) method implemented on a Roche/Hitachi Cobas 6000 Analyzer.

In addition to the internal laboratory quality control (QC) measures, we used 30 random samples from among Golestan Cohort participants to make a pooled QC sample. This pool was aliquoted similarly to the study samples, and analyzed randomly at regular intervals throughout the batches. The laboratory team was unaware of the number, and location of these QC samples in the batches. Table 1 shows the coefficients of variation (CVs) of the tests calculated on blind pooled samples from GCS; all but two were below 20% and 19 were below 10% showing excellent assay performance.

**References**

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3. Xia B, Xia Y, Wong J, et al. Quantitative analysis of five tobacco-specific N-nitrosamines in urine by liquid chromatography-atmospheric pressure ionization tandem mass spectrometry. *Biomed Chromatogr* 2014;28(3):375-84. doi: 10.1002/bmc.3031

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5. Alwis KU, Blount BC, Britt AS, et al. Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). *Anal Chim Acta* 2012;750:152-60. doi: 10.1016/j.aca.2012.04.009

|  |  |  |
| --- | --- | --- |
| **Tobacco Use Group** | **Baseline cotinine concordant with self-report** | **Repeat cotinine concordant with self-report** |
| Never tobacco user (n=60) | 58 (96.7%) | 59 (98.3%) |
| Cigarette only (n=35) | 33 (94.3%) | 29 (82.9%) |
| Waterpipe only (n=40) | 37 (92.0%) | - |
| Nass only (n=30) | 30 (100%) | - |
| TOTAL | 158 (95.8%)\* | 88 (92.6%)\*\* |

Supplementary Table 1. Comparison between urinary cotinine concentrations and self-reported tobacco use in the Golestan Cohort samples

\* kappa=0.91+0.08; \*\* kappa=0.84+0.10

Supplementary Table 2. Baseline and demographic characteristics of the study population selected from the Golestan Cohort participants

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Never tobacco-users (n=58)\*** | **Exclusive cigarette smokers (n=33)\*** | **Exclusive waterpipe smokers (n=37)\*** | **Exclusive nass users (n=30)\*** |
| **Age: mean (SD)** | 51.2(8.4) | 50.7(7.6) | 52.6(9.0) | 57.1(9.2) |
| **Sex: male/female** | 28/30 | 33/0 | 4/33 | 25/5 |
| **Ethnicity** |  |  |  |  |
| Turkmen: % | 70.7 | 72.7 | 8.1 | 63.3 |
| Other: % | 29.3 | 27.3 | 91.9 | 36.7 |
| **Residence** |  |  |  |  |
| Urban: % | 19.0 | 42.4 | 24.3 | 10 |
| Rural: % | 81.0 | 57.6 | 75.7 | 90 |
| **education** |  |  |  |  |
| None: % | 63.8 | 39.4 | 86.5 | 86.7 |
| 1-8 years: % | 25.9 | 30.3 | 13.5 | 10 |
| > 8 years: % | 10.3 | 30.3 | 0 | 3.3 |
| **BMI** |  |  |  |  |
| Underweight: % | 1.8 | 0 | 10.8 | 3.3 |
| Normal: % | 31.0 | 42.4 | 27.0 | 46.7 |
| Overweight: % | 36.2 | 39.4 | 43.2 | 43.3 |
| Obese: % | 31.0 | 18.2 | 19.0 | 6.7 |
| **Age when tobacco use started: mean (SD)** | NA | 25.2(7.4) | 40.9(13.5) | 38(15.8) |
| **Daily tobacco use: %** | NA | 97.0 | 78.4 | 100.0 |

\*Numbers exclude individuals who had self-reported tobacco status discordant with measured cotinine concentrations

Supplementary Table 3. Correlations among TNE7 and nitrosamines by study groups

1. **Cigarette**

**smokers**



1. **Waterpipe**

**smokers**



1. **Nass**

**users**



\* p<0.01

Supplementary Table 4. Correlations among TNE2 and different PAH metabolites by study groups

1. **Cigarette**

**smokers**



1. **Waterpipe**

**smokers**



1. **Nass**

**users**



1. **Never tobacco**

**users**



\* p<0.01

Supplementary Table 5. Correlations among TNE2 and different VOC metabolites by study groups

1. **Never tobacco**

**users**



1. **Cigarette**

**smokers**



\* p<0.01

1. **Waterpipe**

**smokers**



1. **Nass**

**users**



\* p<0.01

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Factor loadings** | | |
|  | | **Factor 1 (Tobacco factor)** | **Factor 2 (PAH factor)** | **Factor 3 (VOC factor)** |
| COTT |  | 0.95 | 0.16 | 0.01 |
| HCTT |  | 0.89 | 0.14 | -0.02 |
| COXT |  | 0.94 | 0.17 | 0.08 |
| NCTT |  | 0.92 | 0.22 | 0.04 |
| NICT |  | 0.92 | 0.06 | 0.10 |
| NOXT |  | 0.90 | -0.04 | 0.16 |
| NNCT |  | 0.96 | 0.06 | 0.08 |
| ANBT |  | 0.94 | -0.12 | -0.16 |
| ANTT |  | 0.92 | -0.18 | -0.03 |
| NABT |  | 0.67 | 0.45 | 0.13 |
| NATT |  | 0.80 | 0.27 | 0.28 |
| NNAL |  | 0.42 | 0.42 | 0.15 |
| NNNT |  | 0.85 | 0.09 | 0.15 |
| 1-nap |  | 0.40 | 0.56 | 0.03 |
| 2-nap |  | 0.05 | 0.81 | 0.46 |
| 1-phe |  | 0.10 | 0.85 | -0.01 |
| ∑2,3phe |  | 0.06 | 0.80 | 0.02 |
| 2-flu |  | 0.09 | 0.86 | 0.31 |
| 3-flu |  | 0.20 | 0.86 | 0.33 |
| 1-pyr |  | -0.01 | 0.87 | 0.04 |
| 2MHA |  | 0.19 | 0.55 | 0.20 |
| 34MH |  | 0.17 | 0.55 | 0.32 |
| AAMA |  | 0.11 | 0.56 | 0.43 |
| GAMA |  | 0.21 | 0.60 | 0.14 |
| CYHA |  | 0.07 | 0.63 | 0.61 |
| CYMA |  | -0.09 | 0.67 | 0.62 |
| CEMA |  | 0.00 | 0.06 | 0.81 |
| HPMA |  | -0.02 | 0.14 | 0.86 |
| BMA |  | -0.07 | 0.03 | 0.20 |
| MADA |  | 0.28 | 0.60 | 0.33 |
| PHGA |  | 0.02 | 0.23 | 0.15 |
| PMA |  | 0.11 | 0.34 | 0.28 |
| HPM2 |  | -0.07 | 0.34 | 0.65 |
| AMCA |  | 0.07 | 0.66 | 0.52 |
| DHBM |  | -0.02 | -0.06 | 0.62 |
| MHB3 |  | 0.19 | 0.26 | 0.88 |
| HPMM |  | 0.17 | 0.19 | 0.90 |
| IPM3 |  | 0.16 | 0.39 | 0.76 |
| TTCA |  | 0.12 | 0.33 | -0.06 |
| **Proportion of variance** | | **26%** | **23%** | **17%** |

Supplementary Table 6. Factor loadings and variance proportions in the rotated principal-component factor analysis