Advances in Biomonitoring Methods for Volatile Organic Compounds

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NHANES

• Ongoing CDC survey designed to collect data on the health and nutritional status of the U.S. population

• Conducted by National Center for Health Statistics

• Complex, multistage, area probability design: samples the U.S. population based on age, sex, race/ethnicity, income

• NHANES surveys: I (71-75), II (76-80), III (88-94), 99-00, 01-02, 03-04, 05-06...

➤ Thorough interview and physical exam, including blood and urine collection

➤ Biomarkers of exposure to environmental chemicals quantified in blood and/or urine
**VOCL Methods to support biomonitoring activities**

**ACTIVE METHODS:**

1) **VOCs in Blood**
   - 44 analytes
   - GC/MS

2) **VOC Metabolites in Urine**
   - 30 analytes in urine
   - UPLC-MS/MS

3) **Aromatic Diamines in Urine**
   - 5 analytes
   - UPLC-MS/MS

**ADDITIONAL METHODS:**

1) **Aliphatic Diamines in Urine**
   - 4 analytes
   - UPLC-MS/MS

2) **Aldehydes in Serum**
   - 19 analytes
   - GC-HRMS
**Volatile Organic Compounds in Blood (VOCB)**

**Quantification of 31 volatile organic compounds in whole blood using solid-phase microextraction and gas chromatography-mass spectrometry.**

Blount BC¹, Kobelski RJ, McElrath DO, Ashley DL, Morrow JC, Chambers DM, Cardinali FL.

**Abstract**

The prevalence of exposure to volatile organic compounds (VOCs) has raised concern about possible health effects resulting from chronic human exposure. To support studies exploring the relation between VOC exposure and health effects, we developed an automated analytical method using solid-phase microextraction (SPME), capillary gas chromatography (GC), and quadrupole mass spectrometry (MS). This method quantifies trace levels (low parts per trillion) of 14 halogenated alkanes, 5 halogenated alkenes, 10 aromatic compounds, and 2 other VOCs in human blood. Detection limits for the SPME-GC-MS method range from 0.005 to 0.12 microg/L, with linear calibration curves spanning three orders of magnitude. The improved throughput of this method will enable us to expand biomonitoring efforts to assess nonoccupational VOC exposure in large epidemiological studies.

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**VOCB method overview**

**Approach**
- Blood and water samples are collected and prepared with a labeled internal standard – all using VOC-free materials and reagents.
- Volatiles are concentrated from the sample headspace with an adsorbent (SPME), which is analyzed by cryogenic trapping gas chromatography-mass spectrometry.

Volatile organic compounds can be measured accurately and precisely in human blood using SPME-GC/MS.
Volatile Organic Compound Metabolites in Urine (VOCm)

Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS).

Aiwis KU1, Blount BC, Britt AS, Patel D, Ashley DL.

Abstract
Volatile organic compounds (VOCs) are ubiquitous in the environment, originating from many different natural and anthropogenic sources, including tobacco smoke. Long-term exposure to certain VOCs may increase the risk for cancer, birth defects, and neurocognitive impairment. Therefore, VOC exposure is an area of significant public health concern. Urinary VOC metabolites are useful biomarkers for assessing VOC exposure because of non-invasiveness of sampling and longer physiological half-lives of urinary metabolites compared with VOCs in blood and breath. We developed a method using reversed-phase ultra high performance liquid chromatography (UPLC) coupled with electrospray ionization tandem mass spectrometry (ESI/MSMS) to simultaneously quantify 28 urinary VOC metabolites as biomarkers of exposure. We describe a method that monitors metabolites of acrolein, acrylamide, acrylonitrile, benzene, 1-bromopropane, 1,3-butadiene, carbon-disulfide, crotonaldehyde, cyanide, N,N-dimethylformamide, ethylbenzene, ethylene oxide, propylene oxide, styrene, tetrachloroethylene, toluene, trichloroethylene, vinyl chloride and xylene. The method is accurate (mean accuracy for spiked matrix ranged from 84 to 104%), sensitive (limit of detection ranged from 0.5 to 20 ng mL(-1)) and precise (the relative standard deviations ranged from 2.5 to 11%). We applied this method to urine samples collected from 1203 non-smokers and 347 smokers and demonstrated that smokers have significantly elevated levels of tobacco-related biomarkers compared to non-smokers. We found significant (p<0.0001) correlations between serum cotinine and most of the tobacco-related biomarkers measured. These findings confirm that this method can effectively quantify urinary VOC metabolites in a population exposed to volatile organics.
VOC metabolites method overview

Metabolism pathways

VOC metabolites in urine
VOC metabolites (VOCm) method overview

**Approach**

- Urine samples are collected and an aliquot (50 µL) is combined with a labeled internal standard

- VOC metabolites are analyzed by reversed phase UPLC-ESI-MS/MS (negative ion)
  - C18 Waters HSS T3
  - 9 minute run time
  - 96-well plate format for high-throughput

30 Volatile organic compound metabolites can be measured accurately and precisely in urine using UPLC-MS/MS
Isotope Dilution UPLC-APCI-MS/MS Method for the Quantitative Measurement of Aromatic Diamines in Human Urine: Biomarkers of Diisocyanate Exposure.

Bhandari D¹, Ruhl J¹, Murphy A¹, McGahee E¹, Chambers D¹, Blount BC¹.

Abstract
Urinary diamines are biomarkers of diisocyanate exposure. Diisocyanates are considered as skin and respiratory sensitizers and are the most frequently reported cause of occupational asthma. Herein we report on the development and validation of an ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the measurement of five aromatic diamines, 4,4′-methyleneedianiline (MDA), 2,4-toluenediamine (4TDA), 2,6-toluenediamine (6TDA), 1,5-naphthalenediamine (NDA), and p-phenylenediamine (PPDA) in human urine. The method incorporates sample preparation steps, which include a 4 h acid hydrolysis followed by high-throughput solid-phase extraction prior to chromatographic separation. Chromatographic separation was achieved using a C18 reversed phase column with gradient elution of basic mobile phases (pH 9.2). The duty cycle of the method was less than 5 min, including both the column equilibration and autosampler movement. Analytical detection was performed using positive ion atmospheric pressure chemical ionization tandem mass spectrometry (APCI-MS/MS) in scheduled multiple reaction monitoring (sMRM) mode. Excellent linearity was observed over standard calibration curve concentration ranges of 3 orders of magnitude with method detection limit ranging from 10 to 100 pg/mL. The interday and intraday reproducibility and accuracy were within ±15%. This method is fast, accurate, and reproducible and is suitable for assessment of exposure to the most common aromatic diisocyanates within targeted groups as well as larger population studies such as the National Health and Nutrition Examination Survey (NHANES).
Urinary aromatic diamines method overview

**Approach**

- Urinary diamines are biomarkers of diisocyanate exposure (polyurethane-based products)
- Urine samples are collected and combined with a labeled internal standard, then hydrolyzed under acidic conditions
- Post-hydrolysis SPE (Strata XC) is performed, and diamines are analyzed by reversed phase UPLC-APCI-MS/MS (positive ion)
  - Mac-Mod ACE Excel2 SuperC18
  - 5 minute run time
  - 96-well plate format for high-throughput

250 µL aliquot + 50 µL internal standard + 100 µL 6N HCl + 600 µL H₂O

Five aromatic diamines can be measured accurately and precisely in urine using UPLC-MS/MS.
Current Method Development Activities

- **Improved urinary metabolite analysis:**
  - t,t-Muconic acid, N-acetyl-S-(phenyl)-L-cysteine (benzene)
  - 2-Aminothiazoline-4-carboxylic acid (hydrogen cyanide)

- **Urinary aromatic and aliphatic diamines – combined method:**
  - Diisocyanate exposure biomarkers

- **New urinary metabolites for:**
  - N-Methylpyrroloidone (paint and coating removal products)
  - Furfural (found in aerosols of sweet-flavored e-liquids)
  - 5-Hydroxymethylfurfural (found in foods and aerosols of sweet-flavored e-liquids)

- **Terpenes in blood/serum:**
  - Pinenes, limonene
  - Others
VOCL supports national and international biomonitoring studies

In FY17, VOCL reported >660K analyte results:
- NHANES: >10,000 specimens (VOCB, VOCm, ARO, ALDS)
- PATH: >11,000 specimens in FY17 (VOCm)

Occupational and environmental exposure studies
- VOCL supported studies into:
  - CS$_2$ exposure
  - Nail salon workers
  - Gulf coast residents
  - Waterpipe second-hand smoke exposure

International collaboration
- Lund University (Sweden)
  - Examined exposures to diisocyanates
Summary

- Biomonitoring provides useful information about exposure to VOCs and other harmful air pollutants

- Novel analytical approaches developed to better characterize human exposure to VOCs

- VOCL develops robust analytical methods to support biomonitoring activities worldwide
  - Potential multidisciplinary collaboration
  - Exchange of methodological information and materials
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Biomonitoring provides useful information about exposure to VOCs and other harmful air pollutants

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