ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Method for Accurate Quantitation of Volatile Organic Compounds in Urine Using Point of Collection Internal Standard Addition

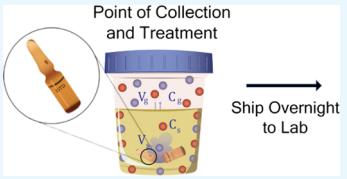
David M. Chambers,* Kasey C. Edwards, Eduardo Sanchez, Christopher M. Reese, Alai T. Fernandez, Benjamin C. Blount, and Víctor R. De Jesús

Cite This: *ACS Omega* 2021, 6, 12684−12690

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: A method to achieve accurate measurement of unmetabolized volatile organic compounds (VOCs) in urine was developed and characterized. The method incorporates a novel preanalytical approach of adding isotopically labeled internal standard (ISTD) analogues directly to the collection container at the point of collection to compensate for analyte loss to the headspace and the collection container surfaces. Using this approach, 45 toxic VOCs ranging in water solubility and boiling point were evaluated and analyzed by headspace solid-phase microextraction/gas chromatography−mass spectrometry. Results show that urine VOCs could be equally lost to the container headspace as to the container surface suggesting similarity of these two regions as partition phases. Surface adsorption loss was found to trend with compound water solubility. In particular, with no headspace, more nonpolar VOCs experienced substantial losses (e.g., 48% for hexane) in a standard 120 mL urine cup at concentrations in the low- and sub-ppb range. The most polar VOCs evaluated (e.g., tetrahydrofuran) showed no significant loss. Other commonly practiced methods for urine sample collection and analysis such as aliquoting, specimen freezing, and use of surrogate ISTD were found to significantly bias results. With this method, we achieved errors ranging from −8.0 to 4.8% of spiked urine specimens. Paired urine and blood specimens from cigarette smokers were compared to assess this method.



INTRODUCTION

Exposure to certain volatile organic compounds (VOCs) has been associated with increased adverse health effects including cancer (e.g., benzene[1]), organ damage (e.g., trichloroethylene and tetrachloroethylene[2]), neurotoxicity, and developmental neurotoxicity (e.g., toluene[3]). Blood levels of harmful VOCs among U.S. nonsmokers[4] are highest for toluene, xylenes, and ethylbenzene (often found in petroleum-based products), 1,4-dichlorobenzene (used in some deodorizers and pesticides), and chloroform (a byproduct of water chlorination). Smokers have elevated blood levels for smoke VOC biomarkers such as benzene, toluene, ethylbenzene, xylenes (BTEX), styrene, 2,5-dimethylfuran, and furan.[6]

For most VOCs, inhalation is generally the primary exposure route because 100% of the blood circulates through the lungs, where more nonpolar (i.e., lipid-soluble) and volatile VOCs are readily absorbed through a thin alveoli membrane. Although VOCs can enter the body by dermal adsorption and ingestion, dermal absorption is generally slowed by the stratum corneum layer and ingestion is limited because only approximately 20% of the blood passes through the gastrointestinal system.[5] VOCs in urine and blood are expected to distribute according to the urine:blood partition constant (KUB) of the VOC,[6] where KUB can be estimated from the ratio of urine:air (KUA) and blood:air (KBA) partition constants for the VOC, assuming that there are no active transport mechanisms. For those VOCs that do not readily undergo decomposition (e.g., hydrolysis) or biotransformation, blood concentrations provide the most direct and sensitive measure of burden experienced by tissues and organs. It is important to note that blood measurements generally reflect tissue load as VOC half-lives are short, less than 1 h. Nevertheless, it is not always practical to collect blood samples, which requires a phlebotomist and specially cleaned collection tubes with low VOC residue levels.[7,8] Given these constraints, urine measurements have been pursued as an alternative to blood measurements.

Most urine analysis methods described in the literature incorporate a number of common procedures that include transfer of the urine from the specimen container using a syringe, freezing and thawing the specimen, and the analysis of milliliter quantities of specimen. To improve headspace analysis, low-milligram amounts of NaCl are added to decrease VOC solubility and acids or bases are added for compound neutralization. Ideally, an internal standard is added to correct for sample loss, but not typically.[9−12] Increasingly, these analyses are performed by equilibrium headspace solid-phase microextraction (SPME)[13]/gas chromatography−mass spectrometry (GC−MS[9−11] and nonseparative mass spectrome-

Received: February 19, 2021
Accepted: April 22, 2021
Published: May 4, 2021

https://doi.org/10.1021/acsomega.1c00854
ACS Omega 2021, 6, 12684−12690

try[14] although some dynamic headspace[15−17] methods continue to be developed and employed. In general, these methods are better suited for analysis of highly polar VOCs (alcohols and carbonyls) that have low volatility and less tendency to adsorb to container and syringe surfaces during collection and sample preparation. Consequently, unmetabolized VOC urine analysis has been mostly limited to assessing occupational exposure levels and has not progressed toward being used for estimation of body burden.[18,19]

Despite method demonstrations over the decades, interest in routine unmetabolized VOC urine biomonitoring has been subdued in our laboratory because of limitations associated with maintaining sample integrity throughout specimen collection and analysis. Method limitations are especially considerable for more nonpolar compounds (e.g., BTEX and alkanes) that are prone to volatilization loss, and more notably, surface adoption loss, especially in a polar aqueous matrix such as urine. For analysis of more nonpolar VOCs, use of these current methods results in sample losses greater than the standard 15% tolerance benchmark for many target analytes.[20] As such, nonpolar VOCs can be substantially lost when the urine specimens are frozen, if not hermetically contained, as most of the VOCs are not miscible in water and can have water solubilities that are in the low mg/L range (e.g., hexane).[21] Finally, for methods that rely on SPME, the compound collection efficiency depends not only on the headspace concentration but also on the SPME fiber sorbent collection specificity, which varies with the concentration of other competing analytes.[22]

To compensate for these limitations, we developed and evaluated a novel preanalytical method of adding isotopically labeled analogues to a standard urine specimen cup at the point of specimen collection or soon after collection that can be used remotely in the field. To accomplish this, an ampule containing 1333 μL of isotopically labeled internal standard (ISTD) is cracked open and dropped into the sample cup to compensate for sample collection, handling, and storage losses and quantitation biases. For this work, we selected 45 VOCs that have been identified as toxic.[4] We identified and characterized biases resulting from urine specimen collection and preparation, specimen freezing and thawing, and use of a surrogate ISTD. Urine levels measured with this method were compared to blood levels among cigarette smokers.

EXPERIMENTAL SECTION

The content in this section describes the supplies, equipment, and procedures that are consistent throughout this work. In the results section, experimental details used to characterize specific analytical figures of merit and procedures specific to experiments that deviate from this experimental section are included.

Standards. Stock standard mixtures in methanol (O2Si Smart Solutions, Charleston, SC) contained 45 toxic VOCs at eight different levels (0 through 7) whose concentrations were confirmed using a similar standard set prepared by Absolute Standards (Hamden, CT). These ampulized, stock standards were diluted by aliquoting 40 μL into 25 mL of low VOC water (O2Si). The final concentrations ranged from low ng/L to low μg/L but varied for each individual analyte depending on the limit of detection (LOD). The stock ISTD mixture consisted of 45 isotopically labeled analogues corresponding to each VOC in methanol and was prepared in a similar manner as for the native standards in which 100 μL was diluted in 25 mL of methanol. Methanol used was purge-and-trap grade (Honeywell-Burdick and Jackson, Muskegon, MI). The ISTD working solution was proportionally aliquoted into the standard and samples to achieve a dilution factor of 75. ISTD concentrations in the standards and samples ranged from 0.029 μg/L (trichloroethylene) to 16 μg/L (nitrobenzene). Positive displacement pipettes were used to transfer all liquids in the microliter range. Stock standards and ISTD ampules were stored in a −70 °C freezer.

Urine and Blood Specimen Collection. Pooled urine specimens were collected anonymously at the CDC under Internal Review Board-approved protocol ID 3994.0. Blood and urine specimens used for method validation were collected by Tennessee Blood Services (Memphis, TN). The participants provided written consent, and the specimen collection protocol (ID 2012 10,385) was approved by the National Institute of Environmental Health Sciences. Participants targeted for this study were cigarette smokers. Urine and coinciding blood specimens were collected from a total of 17 participants. Sterile 120 mL orange-top urine specimen cups were purchased from Parter Medical Products, Inc. (Carson, CA) and preweighed before use. Donors were instructed to fill their urine sampling cup as much as possible to ideally achieve 120 mL. Urine specimens that were under 40 mL were not used (N = 3). Urine specimens were kept refrigerated until shipped.

Blood collection tubes (aka vacutainers) were 7 mL glass blood tubes with gray butyl rubber stoppers and a potassium oxalate/sodium fluoride anticoagulant (Covidien, Dublin, Ireland). Blood specimens were collected within 20 min of the urine specimens, except for one sample. Blood collection tubes used were precleaned to remove VOC residues using a method previously described.[7] Once a whole blood specimen was collected, it was mixed for at least 5 min to ensure that the anticoagulant was completely dissolved. After the blood specimen was mixed, it was immediately placed into a refrigerator. Urine and blood specimens were shipped cold, but not frozen, overnight at the end of each collection day.

Blood and Urine Sample Preparation. Upon arrival, samples from urine cups and blood tubes were immediately prepared and run on the same instrument. Because Tennessee Blood Services did not deposit the ISTD ampules into the urine specimens at the donation facility, the specimens were immediately given the ISTD treatment when received at our laboratory. Preweighed urine specimen cups were weighed to determine the specimen quantity. The ISTD was added by breaking open a 2 mL flame-sealed ampule containing a 1.33 mL aliquot (O2Si) and depositing the ampule and its contents into the specimen cup, recapping, and vigorously mixing. The ISTD concentration was the same concentration as the working solution described above, which assumed a urine specimen volume of 100 mL. The volume of these specimens ranged from 52 to 96 mL, requiring that a dilution factor be used in proportion to the collection volume for quantitation. Blood was collected and analyzed in a similar manner as described previously[23,24] and run with the urine samples. The VOC smoke biomarkers compared include BTEX, styrene, 2,5-dimethylfuran, and furan.

For analysis of the blood and urine specimens, 3 mL was directly aliquoted into a 10 mL headspace vial with clean 5 mL gas-tight syringes. Headspace 5 mL syringes used for specimen aliquoting were fitted with stainless steel 18-gauge Luer lock needles. The headspace vials were 10 mL crimp top with a

https://doi.org/10.1021/acsomega.1c00854
ACS Omega 2021, 6, 12684−12690

beveled edge and round bottom. Ultralow bleed 3 mm-thick PTFE/silicone 20 mm crimp seal headspace vial caps were purchased from The Lab Depot, Inc. (Dawsonville, GA). For the urine specimens, the cup cap was removed to withdraw 3 mL and for the blood specimens, 3 mL was withdrawn by piercing the syringe needle through the blood tube stopper. The gas-tight syringes, headspace vials, and headspace vial caps used for sample preparation were kept in vacuum ovens at 50 °C before use to outgas any VOC residue.[25]

Instrumentation and Method Parameters. The method used for sample analysis was similar to the one previously described.[23,24] Headspace vial samples were processed with a PAL3 DHR/RSI-RSI automated sampler (LEAP Technologies, Carrboro, NC) and the process involved holding the sample vials at 15 °C in a cooled tray and then transferring a vial to a heated agitator (40 °C and 500 rpm agitation speed) to be sampled with a 75 μm Carboxen/PDMS SPME fiber (MilliporeSigma, St. Louis, MO) for 15 min. After sampling, the SPME fiber was injected into the inlet of an Agilent Technologies (Santa Clara, CA) 7890A GC/5975C MS instrument for analysis of VOCs collected on the fiber. The GC inlet liners were 2 mm ID. The GC inlet nonstick septa (Agilent Technologies, Santa Clara, CA) were 11 mm in diameter and bleed- and temperature-optimized. The GC column was a DB-VRX column (40 m × 0.18 mm × 1 μm, Agilent Technologies). Research-grade helium (99.9999%) from Airgas South (Atlanta, GA) was used as the carrier gas. The GC was fitted with a cryotrap (Scientific Instrument Services, Ringoes, NJ) to narrow peak broadening of low boiling point (BP) VOCs. The cryotrap was held at −100 °C for 1 min and then rapidly heated to 210 °C for the duration of the GC run. The GC inlet was set to the pulsed splitless (50 psi for 1.5 min) mode, 250 °C, and a constant flow of 1.0 mL/min. After 1.5 min, the inlet flow was split to vent with a 30 mL/min purge flow. The GC's oven temperature ramp was set as follows: 0 °C for 1.5 min, 7 °C/min to 140 °C, and 40 °C/min to 220 °C, which was then held for 8.5 min (total run time = 32 min). The transfer line to the MS was set at 225 °C. The MS detector was operated using electron ionization in the selected ion monitoring mode set to a primary quantitation ion, a confirmation ion, and an internal standard ion where dwell times ranged from 20 to 30 ms. Ions were selected to have sufficient abundance yet minimal spectral overlap where the ISTD fragment corresponded to either the primary quantitation or confirmation ions, and the ratio of primary quantitation to confirmation ions was within 25%.[24] Identification of unknowns was established by comparison of the GC retention time with that of a known standard and mass spectral data.

Data Analysis. Data processing was performed with Xcalibur 2.0.7 (Thermo Fisher Scientific, Waltham, MA). At least five calibrators were used to create calibration curves using linear regression (R^2 ≥0.995). Descriptive statistical analyses were performed using JMP (Version 13.2.0, SAS, Cary, NC). Urine specimens that had blood smoking biomarker 2,5-dimethylfuran levels below a 1 cigarette per day cutpoint (<0.014 μg/L)[6] and that were classified as nonsmokers according to their smoke VOC biomarker blood signature were also excluded (N = 2).[26]

RESULTS

We provide using sample analysis the analytical figures of merit, LODs, accuracy, and precision. We report other method parameters involving sample collection, storage, and preparation that might reduce accuracy and precision below the 15% benchmark.

LODs. LODs were identified where the 5th percentile of the distribution of samples with the analyte levels at the detection limit intersects with the 95th percentile of the distribution of blank samples.[27] The distribution of samples with analyte levels at the detection limit was deduced by extrapolation of the standard deviations from samples at three different concentrations near the LODs consisting of at least 60 samples per level. The LODs ranged from 0.001 μg/L for chlorobenzene and 2,5-dimethylfuran to 0.099 μg/L for ethyl acetate. These LODs are listed in Supplemental Table 1 for analytes.

Spiked Recovery. Spiked recovery of VOCs in urine with upstream addition was evaluated on two different urine samples at two concentrations equivalent to level 3 and level 4. Urine samples were prepared in a similar manner as the standards in which 25 mL of urine was spiked with 40 μL of either the level 3 or level 4 stock standard and 333 μL of the ISTD methanol solution. Three urine blanks were prepared in the same manner with only ISTD added and were used for background subtraction. Analysis was performed using triplicate samples. All analytes levels were within 15% of both standard formulations. The lower extreme was tetrachloroethylene, which had average recoveries for level 3 (0.107 μg/L) of 85.8% and level 4 (0.320 μg/L) of 95.4%. The upper extreme was hexane, which had average recoveries for level 3 (0.275 μg/L) of 104.7% and level 4 (1.12 μg/L) of 110.3%.

Use of Isotopically Labeled Analogues versus Surrogate ISTD. We compared VOC measurements in urine using a surrogate ISTD (i.e., 1,2-dichlorobenzene (13C6)) with those using analyte-specific isotopic analogues as listed in Supplemental Table 1. This comparison was performed on 23 of our 45 analytes in which we use deuterated analogues that do not overlap with the native compound retention time, thus eliminating the possibility of spectral interference. This experiment was performed at 11 different spiked concentrations ranging from 0.005 to 33.2 μg/L involving the same data in which the ISTD was reassigned in the processing method. The data using the isotopic analogues were considered to be the accepted concentration. Percent error from using 13C6 as the surrogate ISTD varied by concentration and among the analytes ranging from −62.1% (isobutyronitrile at 0.074 μg/mL) to 66.7% (hexane at 9.2 μg/mL).

Correcting for VOC Losses during Urine Collection. Because biases from analyte loss mainly resulted from surface adsorption and volatilization in the urine specimen cup, we set out to characterize and compensate for these VOC losses using upstream ISTD addition. The upstream ISTD addition involved spiking the urine specimens with ISTD at the point of specimen collection. Upstream samples were compared with samples spiked with ISTD after being aliquoted into analysis headspace vials (i.e., downstream ISTD addition). Samples made for upstream and downstream ISTD addition were kept in 120 mL urine cups (nominally 143 mL) filled with pooled urine leaving no headspace. The urine volume was estimated gravimetrically assigning a density of 1.0 g/mL[28] and it was spiked with 40 μL of a VOC stock standard. This spike yielded concentrations for the different VOCs ranging from 0.012 to 0.785 μg/L. For the upstream samples, ISTD was spiked directly in the specimen cup, which was then sealed and mixed.

https://doi.org/10.1021/acsomega.1c00854
ACS Omega 2021, 6, 12684−12690

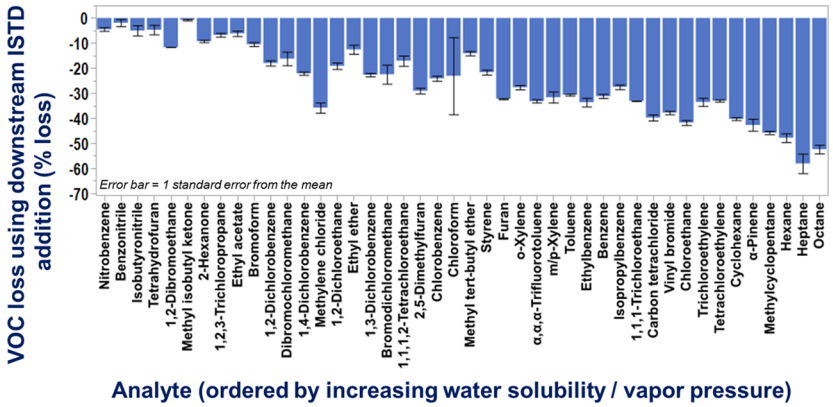


Figure 1. Percent loss of VOC concentrations from specimen collection and processing determined from use of an ISTD added at the point of collection compared with samples in which the ISTD was added at the point of analysis.

A 3 mL aliquot was then taken with a separate, clean 5 mL headspace syringe and placed in a 10 mL headspace vial. For the downstream samples, 3 mL of the spiked urine was aliquoted with a clean syringe to a headspace vial and then spiked with ISTD before sealing the headspace vial. All samples were spiked with ISTD in the same proportion based on the nominal sample volume. Final results were adjusted with a dilution factor based on the relative standard and ISTD proportions. Collection and analysis were performed in triplicate.

Percent loss was calculated where the upstream point of collection addition of ISTD was used as the accepted amount and the downstream ISTD addition as the new amount. Analyte results were averaged and plotted as shown in Figure 1 ordered by increasing water solubility divided by vapor pressure at 37 °C. Water solubilities and vapor pressure were estimated using the SPARC calculator (http://archemcalc.com/).[29]

Error bars were constructed using one standard error from the mean. For all analytes, urine samples with the ISTD added upstream had higher calculated concentrations than those samples where the ISTD was added downstream. The largest percent loss occurred for the most nonpolar VOCs, namely, the alkanes, which ranged from −42 to −58%. Minimal percent loss occurred for the most polar and nonvolatile compounds such as methyl isobutyl ketone (i.e., −0.7). The average percent loss among these VOCs was −25%.

Biases from Different Urine Collection Volumes. Figure 2 shows the efficacy of upstream ISTD addition in compensating for headspace and surface adsorption losses of VOCs for different specimen volumes. For this experiment, different amounts of pooled urine were aliquoted into separate 120 mL collection cups at 20, 40, 60, 80, 100, and 120 mL volumes. The specimen cups used had a 143 mL nominal capacity. Each cup was then spiked with a standard spiking solution that contained ISTD in proportion to the urine volume. The standard spiking solution consisted of 100 μL of the level 7 stock and 133 μL of the ISTD stock diluted with methanol to 5 mL. This standard spiking solution was spiked in 40 μL aliquots in proportion to the sample volume to achieve the same VOC concentration among the different specimens. For example, the 20 mL specimen received one 40 μL spike, the 40 mL specimen received two spikes, and so forth. The concentrations for the different analytes, which were determined by their specific sensitivity, ranged from 0.073 μg/L (for chlorobenzene) to 3.6 μg/L (for nitrobenzene).

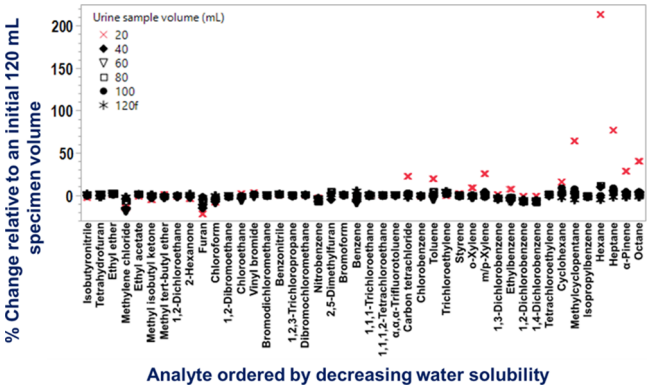


Figure 2. Comparison of concentration robustness achieved with upstream ISTD addition for different urine specimen collection volumes collected in 120 mL collection cups.

Drift in VOC levels that might occur during sample preparation was identified by comparing a 120 mL sample before (initial) and then after (final) the rest of the samples. The difference between these two 120 mL samples served as a measure of any preparation-order bias. Across the analytes, the VOC levels decreased on average by 1.4% between these initial and final 120 mL samples. Percent biases for all the volumes were determined with respect to the initial 120 mL sample. Changes in concentration for the different sample volumes were mostly within 10% of the initial 120 mL concentration, except for furan and methylene chloride, which decreased between 15 and 20% for the 40 and 60 mL volumes. For the 20 mL sample volume, several of the analyte levels were substantially biased. As shown in Figure 2, the data for the 20 mL sample volume are represented with red x's and the other specimen volumes are colored in black to emphasize the bias seen with the 20 mL volume sample.

Biases from Specimen Freezing. Use of upstream addition of ISTD to compensate for analyte loss from freezing was compared for two urine specimens in which one specimen was spiked with ISTD before freezing and the other was spiked with ISTD after freezing. Specimens were 120 mL in volume and spiked with 40 μL of the level 5 standard stock solution, which yielded concentrations among the analytes ranging from 0.049 μg/L (chlorobenzene) to 3.15 μg/L (nitrobenzene). Specimens were spiked with 1.6 mL of ISTD stock solution and analyzed in triplicate as 3 mL samples. Levels for the specimen where ISTD was added after freezing had lower

https://doi.org/10.1021/acsomega.1c00854
ACS Omega 2021, 6, 12684−12690

measured VOC concentrations ranging from −10% (tetrahydrofuran) to −80% (heptane). In addition, we quantified the contamination from the freezer environment by taking three 3 mL aliquots from the upstream specimen, removing 9 mL, and comparing analyte levels before and after freezing this specimen. Upon comparison, quantified levels were within ± 5% of prefreezing levels, with the exception of five analytes that gained in concentration from freezer storage including hexane (+6%), heptane (+38%), octane (+10%), benzene (+ 12%), and methyl isobutyl ketone (+ 6%). For this experiment, specimens were frozen and thawed over a 5 h period and all samples were analyzed within the same day.

Comparison of Urine and Blood VOC Levels from 12 Smokers. This newly developed method was applied to urine specimens collected from 12 smokers. For comparison, blood was also collected along with the urine specimens. Mean VOC levels in these two matrices were compared as shown in Figure 3. Analytes were ordered by increasing water solubility. The

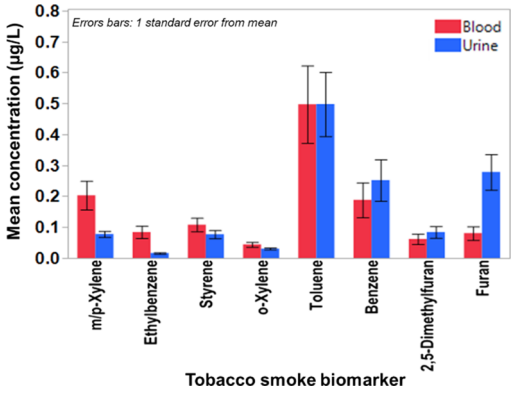


Figure 3. Comparison of mean BTEX, styrene, 2,5-dimethylfuran, and furan concentrations in blood and urine samples from 12 smokers.

water solubility for *m*/*p*-xylene was the average of *m*-xylene and *p*-xylene values. Levels for benzene, toluene, *m*/*p*-xylene, furan, and 2,5-dimethylfuran in all urine and blood specimens were above the LOD. All blood specimen levels for ethylbenzene and styrene were above the LOD, but not for all urine specimens. The mean urine level for styrene was above the LOD in which below LOD results were deduced by linear extrapolation below the LOD. The mean urine level for ethylbenzene was below the LOD.

DISCUSSION

Analytical figures of merit included detection limits in the low part per trillion range (<0.050 ng/mL) with precision of less than 25% (Supplemental Table 1) except for three analytes that were impacted by interferences. In particular, the detection of tetrahydrofuran (LOD = 0.050 ng/L) was biased by laboratory air contamination, whereas nitrobenzene (LOD = 0.056 ng/mL), benzonitrile (LOD = 0.056 ng/mL), and ethyl acetate (LOD = 0.099 ng/mL) were hindered by coeluting interferences. Nevertheless, analyte detection and precision were mainly affected by sample collection and handling biases.

The greatest losses occurred through volatilization and surface adsorption losses from specimen collection and handling, which were exacerbated by freezing the sample before the ISTD addition. Although there are some techniques that can be used to reduce volatilization loss, such as ensuring that the specimens do not remain open to air after collection, little can be done to minimize VOCs from diffusing into the headspace or adsorbing onto the cup surface. Although volatilization loss can be reduced by completely filling the collection cup, the amount of urine provided by the participant cannot always be controlled. For more nonpolar VOCs, greater loss can occur through surface adsorption than volatilization if the analyte has a low vapor pressure. Furthermore, depending on the VOC, quantitation can be substantially biased (e.g., >60%) with the use of a surrogate ISTD in place of an isotopically labeled ISTD analogue when performing the SPME headspace analysis.

VOC losses from surface adsorption and headspace volatilization were quantified by comparing the calculated concentrations from upstream and downstream addition of ISTD. Downstream addition resulted in lower measured concentrations for all analytes ranging from −0.7% (for methyl isobutyl ketone) to −58% (for heptane) (Figure 1). Although VOC losses can occur through a combination of surface adsorption and headspace volatilization, losses are attributed mainly to surface adsorption because cups were filled having no headspace. Nevertheless, similar losses occurred when the cups were not completely filled (i.e., 100 mL) and had an approximate headspace of 43 mL or 30% (data not shown). This similarity in results can be explained by equilibration of VOCs between the cup surface and air phase that is driven by a combination of solubility and vapor pressure. This conclusion is supported by comparing the relative loss of nonpolar compounds such as the alkanes (hexane, heptane, octane, methylcyclopentane, and cyclohexane) with highly volatile compounds that have BPs below room temperatures (low vapor pressure), such as chloroethane and vinyl bromide. In particular, *n*-alkanes, which were the most nonpolar (based on water solubility), had losses of approximately 50% where the lowest BP compounds had losses of a few percent. With the addition of ISTD upstream, the ISTD serves to compensate for losses that can occur from sample storage and preparation if there is a sufficient ISTD response. However, in situations where there is contamination from sample collection, storage, and preparation, the ISTD cannot be used to adjust for contamination gain, such as that demonstrated during sample freezing. In this case, an accompanying negative control sample such as blank water can be helpful in identifying any sample contamination.

Upstream ISTD addition should compensate for different collection volumes. However, at certain lower urine volumes, volatilization losses could not be offset. It is believed that these losses likely occurred before the ISTD could be added and equilibrated in the urine specimen. When the ISTD was added upstream to the urine cup, losses were reduced to within 10% for nearly all analytes for volumes ranging from 40 to 120 mL using a 120 mL cup (Figure 2). Nevertheless, as the specimen volume was decreased to 20 mL (red data points), the bias significantly increased for several analytes. In particular, the most nonpolar compounds (i.e., the alkanes) exhibited a much greater bias than the most volatile compounds (i.e., chloroethane and vinyl bromide). This larger bias against alkanes suggests that loss is more greatly influenced by solubility than vapor pressure where the most nonpolar compounds (i.e., alkanes) are likely diffusing at a faster rate from the specimen before the ISTD can be added and the cup sealed. Nevertheless, the addition of the ISTD at the point of collection maintains quantification accuracy by nearly twofold

https://doi.org/10.1021/acsomega.1c00854
ACS Omega 2021, 6, 12684−12690

for alkanes as opposed to the addition of ISTD downstream, which is more common in typical sample preparation.

Data shown in Figure 3 compare the mean spot urine and blood levels for 12 smokers. Blood specimens, which were collected with urine specimens, helped serve as a reference for the urine VOC measurements since there exist little quantitative data on urinary VOC levels among smokers. Many of the target smoke VOCs quantified in the blood were above the LOD in the urine with the exception of styrene, for some samples, and ethylbenzene, for all the samples. More polar analytes with high water solubility, such as furan, were in a greater concentration in the urine than blood for all the participants. Based on KUB,[18,30] VOC levels should be higher in blood than in urine for nonpolar compounds, but lower in blood for more polar compounds, if fully equilibrated. However, for dynamic exposure, such as when smoking a cigarette, it is possible for nonpolar compounds to be at higher levels in urine than in blood if the VOC has had the time to be removed from the blood through exhalation or metabolism and if the urine accumulated in the urinary bladder during the exposure has not yet been voided. Nevertheless, this disproportionality was not typical on average in which blood levels were significantly higher (p <0.05 using Wilcoxon) than urine levels for the most nonpolar smoke VOCs including m/p-xylene, ethylbenzene, styrene, and o-xylene as can be inferred from Figure 3. The converse was also true for the most polar smoke VOC furan in which urine levels were significantly higher (p <0.05 using Wilcoxon). On average, toluene, benzene, and 2,5-dimethylfuran were not significantly different between the urine and blood specimens (p >0.05).

CONCLUSIONS

Although volatilization loss is an expected loss mechanism that we characterized, this work also identifies and quantifies under what circumstances surface adsorption as well as biases from freezing specimens and using a surrogate internal standard rather than an isotopically labeled analogue can cause an even greater loss. However, with the addition of an isotopically labeled analogue ISTD at the point of collection, we were able to offset these biases to achieve precision and accuracy within 15% for a broad range of VOCs. Some volatilization losses that occur during specimen collection and in the container headspace cannot be eliminated; however, having a fieldable, feasible, and accessible method to spike specimens at the point of collection as described in this work provides a means to compensate for any loss biases that might follow. In addition, the possibility of compensating for headspace losses in the urine cup prior to the addition of ISTD can be estimated using the partition theory for analytes where water solubility is low.

Despite efforts to measure unmetabolized VOC concentrations in urine to estimate dose, there has been little progress in achieving results sufficiently accurate to assist with toxicokinetic modeling, with or without the use of an internal standard (ISTD). Up until now, the most accurate method to measure VOC dose has been the analysis of whole blood. Unfortunately, it is not always practical to collect a suitable whole blood specimen. Apart from more practical aspects of this work such as identifying overlooked VOC loss mechanisms such as surface adsorption loss and quantifying bias caused by common practices such as specimen freezing or using surrogate ISTDs, we believe this work has broader implications. Analytical bias resulting from both urine and even whole blood analyses has hindered the development of toxicokinetic modeling and prediction of blood VOC levels from urine VOC levels. Combined with accurate blood VOC measurements, we believe that accurate urine VOC measurements can be used to establish and validate a viable toxicokinetic model or at least establish an accurate empirical relationship for a broad range of VOCs.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c00854.

> Analytes evaluated in this work along with their corresponding isotopically labeled ISTD analogues, LODs and their variability, water solubility, and vapor pressure. Water solubilities and vapor pressures were set at 37 °C and estimated using the SPARC calculator (http://archemcalc.com/) (PDF)

AUTHOR INFORMATION

Corresponding Author

David M. Chambers − *Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, United States*; Phone: +17704880185; Email: mzz7@cdc.gov; Fax: +17704880181

Authors

Kasey C. Edwards − *Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, United States*

Eduardo Sanchez − *Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, United States*

Christopher M. Reese − *Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, United States*

Alai T. Fernandez − *Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, United States*

Benjamin C. Blount − *Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, United States*

Víctor R. De Jesús − *Tobacco and Volatiles Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c00854

Notes

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of

https://doi.org/10.1021/acsomega.1c00854
ACS Omega 2021, 6, 12684−12690

Health and Human Services or the Centers for Disease Control and Prevention.

The authors declare no competing financial interest.

REFERENCES

(1) Agency for Toxic Substances and Disease Registry. *Toxicological profile for benzene*; U.S. Dept. of Health and Human Services, Public Health Service: Atlanta, GA, 2007. https://wwwn.cdc.gov/TSP/substances/ToxSubstance.aspx?toxid=14 (last accessed March 15, 2021).

(2) Agency for Toxic Substances and Disease Registry. *Toxicological profile for tetrachloroethylene*; U.S. Dept. of Health and Human Services, Public Health Service: Atlanta, GA, 2019. https://www.atsdr.cdc.gov/ToxProfiles/tp18.pdf (last accessed March 15, 2021).

(3) Agency for Toxic Substances and Disease Registry. *Toxicological profile for toluene*; U.S. Dept. of Health and Human Services, Public Health Service: Atlanta, GA, 2017. https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=161&tid=29 (last accessed March 15, 2021).

(4) Centers for Disease Control. *Fourth Report on Human Exposure to Environmental Chemicals*; Department of Health and Human Services, Centers for Disease Control and Prevention: Atlanta, GA, 2019; Vol. 2. https://www.cdc.gov/exposurereport/index.html (last accessed March 15, 2021).

(5) Committee on Contaminated Drinking Water at Camp Lejeune, Systemic Exposures to Volatile Organic Compounds and Factors Influencing Susceptibility to Their Effects. *In Contaminated Water Supplies at Camp Lejeune: Assessing Potential Health Effects*; National Academies Press: Washington (DC), 2009; pp 68−72.

(6) Boeniger, M. F.; Lowry, L. K.; Rosenberg, J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am Ind Hyg Assoc J* **1993**, *54*, 615−627.

(7) Chambers, D. M.; Blount, B. C.; McElprang, D. O.; Waterhouse, M. G.; Morrow, J. C. Picogram measurement of volatile n-alkanes (n-hexane through n-dodecane) in blood using solid-phase microextraction to assess nonoccupational petroleum-based fuel exposure. *Anal. Chem.* **2008**, *80*, 4666−4674.

(8) Cardinali, F. L.; Mccraw, J. M.; Ashley, D. L.; Bonin, M.; Wooten, J. Treatment of vacutainers for use in the analysis of volatile organic compounds in human blood at the low parts-per-trillion level. *J. Chromatogr. Sci.* **1995**, *33*, 557−560.

(9) Song, H. N.; Kim, C. H.; Lee, W. Y.; Cho, S. H. Simultaneous determination of volatile organic compounds with a wide range of polarities in urine by headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* **2017**, *31*, 613−622.

(10) Calejo, I.; Moreira, N.; Araujo, A. M.; Carvalho, M.; Bastos Mde, L.; de Pinho, P. G. Optimisation and validation of a HS-SPME-GC-IT/MS method for analysis of carbonyl volatile compounds as biomarkers in human urine: Application in a pilot study to discriminate individuals with smoking habits. *Talanta* **2016**, *148*, 486−493.

(11) Cozzolino, R.; De Magistris, L.; Saggese, P.; Stocchero, M.; Martignetti, A.; Di Stasio, M.; Malorni, A.; Marotta, R.; Boscaino, F.; Malorni, L. Use of solid-phase microextraction coupled to gas chromatography-mass spectrometry for determination of urinary volatile organic compounds in autistic children compared with healthy controls. *Anal. Bioanal. Chem.* **2014**, *406*, 4649−4662.

(12) Kawai, T.; Okada, Y.; Odachi, T.; Horiguchi, S.; Zhang, Z. W.; Moon, C. S.; Furuki, K.; Ukai, H.; Inui, S.; Ikeda, M. Monitoring of occupational exposure to 1-butanol by diffusive sampling and urinalysis. *Int. Arch. Occup. Environ. Health* **1997**, *69*, 266−272.

(13) Pawliszyn, J. *Solid phase microextraction : theory and practice*. Wiley-VCH: New York, 1997; 247.

(14) Casas-Ferreira, A. M.; Nogal-Sanchez, M. D.; Perez-Pavon, J. L.; Moreno-Cordero, B. Non-separative mass spectrometry methods for non-invasive medical diagnostics based on volatile organic compounds: A review. *Anal. Chim. Acta* **2019**, *1045*, 10−22.

(15) Wang, Y.; Hua, L.; Jiang, J.; Xie, Y.; Hou, K.; Li, Q.; Wu, C.; Li, H. High-pressure photon ionization time-of-flight mass spectrometry combined with dynamic purge-injection for rapid analysis of volatile metabolites in urine. *Anal. Chim. Acta* **2018**, *1008*, 74−81.

(16) Perez Anton, A.; Del Nogal Sanchez, M.; Crisolino Pozas, A. P.; Perez Pavon, J. L.; Moreno Cordero, B. Headspace-programmed temperature vaporizer-mass spectrometry and pattern recognition techniques for the analysis of volatiles in saliva samples. *Talanta* **2016**, *160*, 21−27.

(17) Novotny, M. V.; Soini, H. A. Analysis of volatile mouse pheromones by gas chromatography mass spectrometry. *Methods Mol. Biol.* **2013**, *1068*, 29−45.

(18) Gobba, F.; Ghittori, S.; Imbriani, M.; Maestri, L.; Capodaglio, E.; Cavalleri, A. The urinary excretion of solvents and gases for the biological monitoring of occupational exposure: a review. *Sci. Total Environ.* **1997**, *199*, 3−12.

(19) Imbriani, M.; Ghittori, S. Gases and organic solvents in urine as biomarkers of occupational exposure: a review. *Int Arch Occup Environ Health* **2005**, *78*, 1−19.

(20) Guidance for Industry: Bioanalytical Method Validation. *U.S. Dept. of Health and Human Services*; F. D. A., Center for Drug Evaluation and Research, Ed. United States, Congress: 2018.

(21) Ikeda, M. Solvents in urine as exposure markers. *Toxicol. Lett.* **1999**, *108*, 99−106.

(22) Higashikawa, F. S.; Cayuela, M. L.; Roig, A.; Silva, C. A.; Sanchez-Monedero, M. A. Matrix effect on the performance of headspace solid phase microextraction method for the analysis of target volatile organic compounds (VOCs) in environmental samples. *Chemosphere* **2013**, *93*, 2311−2318.

(23) Blount, B. C.; Kobelski, R. J.; McElprang, D. O.; Ashley, D. L.; Morrow, J. C.; Chambers, D. M.; Cardinali, F. L. Quantification of 31 volatile organic compounds in whole blood using solid-phase microextraction and gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2006**, *832*, 292−301.

(24) Chambers, D. M. Laboratory Procedure Manual - Analyte: Volatile Organic Compounds (VOCs) & Trihalomethanes/MTBE, Matrix: Whole Blood, Method: Headspace Solid-Phase Microextraction with Benchtop GC/MS. https://wwwn.cdc.gov/nchs/data/nhanes/2015-2016/labmethods/VOCWB_VOCWBS_I_MET.pdf (last accessed March 15, 2021).

(25) Chambers, D. M.; McElprang, D. O.; Mauldin, J. P.; Hughes, T. M.; Blount, B. C. Identification and elimination of polysiloxane curing agent interference encountered in the quantification of low-picogram per milliliter methyl tert-butyl ether in blood by solid-phase microextraction headspace analysis. *Anal. Chem.* **2005**, *77*, 2912−2919.

(26) Chambers, D. M.; Reese, C. M.; Thornburg, L. G.; Sanchez, E.; Rafson, J. P.; Blount, B. C.; Ruhl, J. R. E., 3rd; De Jesus, V. R. Distinguishing Petroleum (Crude Oil and Fuel) From Smoke Exposure within Populations Based on the Relative Blood Levels of Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX), Styrene and 2,5-Dimethylfuran by Pattern Recognition Using Artificial Neural Networks. *Environ. Sci. Technol.* **2018**, *52*, 308−316.

(27) Armbruster, D. A.; Pry, T. Limit of blank, limit of detection and limit of quantitation. *Clin. Biochem. Rev* **2008**, *29*, S49−S52.

(28) Armstrong, L. E.; Pumerantz, A. C.; Fiala, K. A.; Roti, M. W.; Kavouras, S. A.; Casa, D. J.; Maresh, C. M. Human hydration indices: acute and longitudinal reference values. *Int J Sport Nutr Exerc Metab* **2010**, *20*, 145−153.

(29) Hilal, S. H.; Karickhoff, S. W.; Carreira, L. A. Prediction of the vapor pressure boiling point, heat of vaporization and diffusion coefficient of organic compounds. *QSAR Comb Sci.* **2003**, *22*, 565−574.

(30) Imbriani, M.; Ghittori, S.; Pezzagno, G.; Capodaglio, E. Urine/air partition coefficients for some industrially important substances. *G Ital Med Lav* **1985**, *7*, 133−140.

https://doi.org/10.1021/acsomega.1c00854
ACS Omega 2021, 6, 12684−12690