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Development of headspace SPME method for analysis of volatile organic compounds present in human biological specimens

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Abstract In recent years, interest has increased regarding the identification of volatile organic compounds (VOCs) for metabolic profiling, human scent identification of the living and deceased, and diagnostic potentials for certain diseases that are known for its association with distinct odor. In this study, a method has been developed that is capable of sampling, identifying, and differentiating the VOCs present in various biological specimens of forensic importance (blood, breath, buccal cells, and urine) taken from the same individuals. The developed method requires a pretreatment step to remove targeted VOCs from the sampling apparatus prior to sampling of the individual specimens. The VOCs collected from the biological specimens were characterized by solid-phase microextraction and gas chromatography/ mass spectrometry with ratios of the most abundant and frequent VOCs compared using qualitative and semiquantitative methods. Blood, breath, and buccal cells required extraction procedures ranging from 18 to 21 h in order to optimize the limit of detection, which averaged 5–15 ng across these specimens. The optimal method for measuring urine VOCs was complete in less than an hour; however, the limit of detection was higher with a range of 10–40 ng quantifiable. The demonstrated sensitivity and reproduc-

ibility of the methods developed allow for population studies of human scent VOCs from various biological specimen collection kits used in the forensic and clinical fields.

Keywords Forensic science · SPME-GC/MS · Biological specimens · Volatile organic compounds · Human scent

Introduction

Human scent is the result of the complex combination of the body's metabolism, gland secretions, hormonal control, and interaction with the residing bacterial populations. The individual odor hypothesis has triggered the interest of many researchers to investigate the fingerprint characteristics of human scent and various biological specimens including skin, sweat, blood, and breath. Odors of these specimens are made up of volatile organic compounds (VOCs) of various functional groups such as aldehydes, alcohols, alkanes, esters, fatty acids, and ketones. VOC profiling of emanations from human biological specimens has been used for various applications including toxicology, medicine, and forensic science. While there is a wide range of biological specimens from which to choose, the specimens that are easily obtainable in large quantities are favorable for profiling purposes. These specimens include saliva, breath, blood, and urine.

Human oral fluid (buccal cells and saliva) is a complex aqueous matrix that consists of numerous enzymes, glycoprotein, inorganic salts, and minerals. Analysis of odiferous volatile saliva compounds has generally been in relation to disease of the mouth (i.e., periodontitis) [1–3]. More recently, saliva analysis has gained interest for its diagnostic tool potential for cancer, infections from virus

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and bacteria, hormonal abnormality, and drug testing due to the noninvasive nature of sample collection [4–6]. In the clinical and forensic fields, buccal swab sampling is a routine sampling method for the collection of DNA. Extraction and analysis of salivary VOCs from smaller sample size (as in buccal swabs) have not yet been widely investigated.

Modern breath analysis started in 1971 when Pauling et al. determined more than 200 components in human breath using gas chromatography (GC) [7]. Since then, variations in the VOCs as well as the effect of age and gender on the profile of volatile components in normal human breath have been studied widely resulting in the detection of over 3,000 compounds [8, 9]. Additionally, there has been an increasing interest in human breath analysis for their investigation potential of biomarkers for diseases such as certain types of cancer (lung and breast), oxidative stress, pulmonary tuberculosis, diabetes mellitus, and kidney impairment [10–14]. The interest in human exhaled breath is based on the noninvasiveness and safety in the sampling process as compared to blood testing; however, a standard breath sampling and analysis protocol for clinical settings is yet to be settled. Studies on the analysis of endogenous compounds found in human exhaled breath are still challenged due to the low concentrations of the VOCs. Currently, breath-alcohol testing is the only typical routine breath analysis application implemented in the medical and forensic fields.

Blood VOCs have been studied for the health effects of occupational and environmental exposures [15–17]. While present in most human blood, increased levels of volatile aromatic compounds such as benzene, toluene, and xylene isomers are indicative of occupational or environmental VOC exposures and/or smoking. Despite the more invasive sampling method when compared to breath or urine, blood VOC analysis is more representative of the internal environment of human biological activities. For this reason, analyses of volatile biomarkers for diseases have been gaining interest in the recent years [18–21]. Previously reported headspace analyses of blood samples involve venipuncture blood drawing from subjects which is highly invasive. In this paper, we present for the first time the headspace analysis of blood samples stored on FTA cards, requiring significantly less sample volume and can be less invasively collected through finger stick procedure.

VOCs in the headspace of urine have also been studied in a variety of fields including toxicology (drug detection and recovery) [22–24], occupational and environmental exposures [25–28], and metabolic investigations [29–31]. Early studies on the urinary VOC profiles showed that there was significant variance between different individuals, but intra-person urinary VOC profiles remained constant over time even with variation factors such as diet, exercise, circadian, and seasonal changes [32]. Disturbances of volatile compounds found in urine have been associated to physiological disorders including diabetes mellitus, liver and kidney disease, and trimethylaminuria [31, 33, 34].

Solid-phase microextraction (SPME) is a simple, efficient, sensitive, and solvent-less sampling method that has gained widespread acceptance in biological, toxicological, environmental, and forensic fields since its introduction as a new preconcentration technology by Pawliszyn [35]. Headspace SPME is extremely useful for the analysis of biological specimens as interference from high molecular weight components such as proteins in the matrix is reduced. The extracts are, therefore, much cleaner. SPME coupled to GC/mass spectrometry (MS) has been used to investigate VOCs present in the headspace of biological specimens including breath, blood, urine, and skin [29, 36–41].

To date, there is limited research reported on the direct comparison of multiple specimens sampled simultaneously from the same group of subjects. Furthermore, studies on scent profiles from biological specimens focus more on the diagnostics potential of VOCs in terms of malodor or disease biomarkers rather than individual scent of specimens in general. Research comparing multiple specimens from the same group of individuals has studied a maximum of three specimens simultaneously [42, 43]. The purpose of this paper is to develop and optimize sample collection and extraction conditions of VOCs present in the headspace of collected biological specimens.

Specimen sampling utilizes collection materials pretreated to remove the presence of background compounds that could possibly interfere with the collected scent sample compounds. Specimen sampling procedures are standard forensic and medical specimen collection techniques. The quantity of blood needed for SPME-GC/MS analysis under the presented method is typical for simple finger prick procedure normally used to check for blood glucose levels of diabetic patients. FTA cards are also used to store blood samples of relatives of missing service men and Child Identification Programs [44, 45]. Buccal swab specimens are routinely collected for DNA analysis in both the medical and forensic fields. Breath and urine samples tested were from commercially available sampling kits following standard sampling procedures. Extraction conditions using SPME were optimized for buccal swab, blood, breath, and urine, followed by GC/MS analysis of the collected scent samples. This method can be used for the collection and analyses of biological specimen samples in a future population study of VOCs present in the headspace of multiple biological specimens collected simultaneously from individuals. The information obtained from such study can be used to assess the variation of the VOCs across specimens from individuals, as well as across a population for each specimen using various statistical methods, which has both medical (diagnostic) and forensic (scent profiling) implications.

Materials and methods

Materials

Sterile cotton-tipped applicators were used for buccal cells and saliva collection (Solon Manufacturing Co., Skowhegan, ME, USA). Breath samples were collected using a Teflon Bio-VOC® breath sampler (Markes International Ltd, Rhondda Cynon Taff, UK). A disposable cardboard mouthpiece was attached to the Bio-VOC® apparatus. To seal the apparatus, 11-mm crimp seals with PTFE/Silicone septa (Sun Sri, Rockwood, TN, USA) were used. Blood samples were collected on Whatman FTA® MiniCard (Whatman International Ltd, Maidstone, UK). Ten-milliliter glass, clear, screw top headspace vials with PTFE/Silicone septa were used to hold the samples (SUPELCO, Bellefonte, PA, USA). Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30-μm film thickness) SPME fibers and SPME fiber holders were obtained from SUPELCO (Bellefonte, PA, USA).

Reagent quality (200 proof) ethyl alcohol was purchased from Florida Distillers Co. (Lake Alfred, FL, USA). Acetone, 2-pentanone, dimethyl disulfide, 1H-pyrrole, toluene, hexanal, 4-heptanone, 3-heptanone, 2-heptanone, benzaldehyde, phenol, 1-octanol, ocanoic acid, and non-anoic acid used in the urine optimization study was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). NaCl, KCl, $MgSO_4$, K_2CO_3, and Na_2CO_3 were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Materials pre-treatment method

Sterile cotton-tipped swabs were pre-treated prior to sample collection to eliminate background compounds. The swabs were spiked with 200 μL of ethanol, then baked in a 105 °C oven for 1 h, and the procedure was repeated again to reassure that the swabs were analytically clean. Prior to each sample collection, the Bio-VOC® apparatus was rinsed with acetone and placed in an oven at 40 °C for at least 1 h followed by 30 min in a 105 °C oven, then pure nitrogen gas was passed through the bulb for 2 min. The breath sampler was crimp sealed until time of breath sampling. One half of the Whatman FTA® MiniCard (one circle) was inserted into a sterile 10-ml glass headspace vial and baked in a 105 °C oven for 45 min to ensure the FTA card was analytically clean. Pretreated materials were analyzed using SPME-GC/MS to make sure they were free of undesired compounds.

Sampling procedure

Each subject was asked not to eat or drink anything other than water at least 2 h prior to buccal swab and breath samplings. Buccal swabs were collected by subjects sampling themselves with a pre-treated cotton-tipped sterile swab. Subjects followed a set sampling procedure of rinsing their mouths with water for 30 s followed by rubbing a single swab on the inside of both cheek sides (rubbing 20 times up and down per side of cheek). Samples were collected in triplicates with 10-min breaks in between each sampling. Swabs were re-sealed back into the 10-ml glass headspace vial. No attempt was made to control microbial interactions with the samples as it may make contributions to the overall scent profile.

Breath samples were collected following a set sampling procedure. Subjects were asked to perform a single slow vital capacity breath into a disposable cardboard mouthpiece connected to a 150-ml Teflon®-bulb, which traps the end-expired air. The Teflon®-bulb was immediately sealed after sampling.

Human whole blood containing anticoagulant EDTA was used for the blood optimization studies. To each individual FTA card blanks, 200 μL of whole blood was added, where blood was evenly distributed within the circle. FTA cards were then resealed in their respective vials.

Urine samples were prepared in sterile 10-mL glass headspace vials with 2 mL urine, 0.5 mL deionized water, 1.5 g NaCl (except during salting-out study), and spiked with 25 μL of 200 ppm standard mix solution. Samples were made in triplicates, and each sample was vortexed for 30 s.

Collected buccal swabs, breath, and blood specimens were stored at room temperature until time of analysis (no longer than 24 h after time of specimen collection). Urine was stored at 4 °C.

Instrumentation

GC/MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973N mass selective detector with a quadrupole analyzer operated in electron ionization mode (EI; Palo Alto, CA, USA). The column used to separate the analytes was an HP5-MS, 30 m, 0.25 μm, and 0.25 mm with helium as the carrier gas with a flow rate of 1.0 mL/min. The extracted VOCs were desorbed in the injection port of the GC with a temperature of 250 °C for 5 min. For buccal swab and breath samples, the GC method begins with an initial oven temperature of 40 °C for 5 min, then ramped at 10 °C/min to a final temperature of 270 °C, and held at 270 °C for 2 min for a total run time of 30 min. For blood, the GC method began with an initial oven temperature of 30 °C ramped at 10 °C/min to a final temperature of 200 °C where it was held for 2 min, for a total run time of 19 min. For urine samples, the initial oven temperature of 40 °C was held at 5 min, and then ramped at 15 °C/min to a final temperature of 280 °C where it was held for 2 min, for a
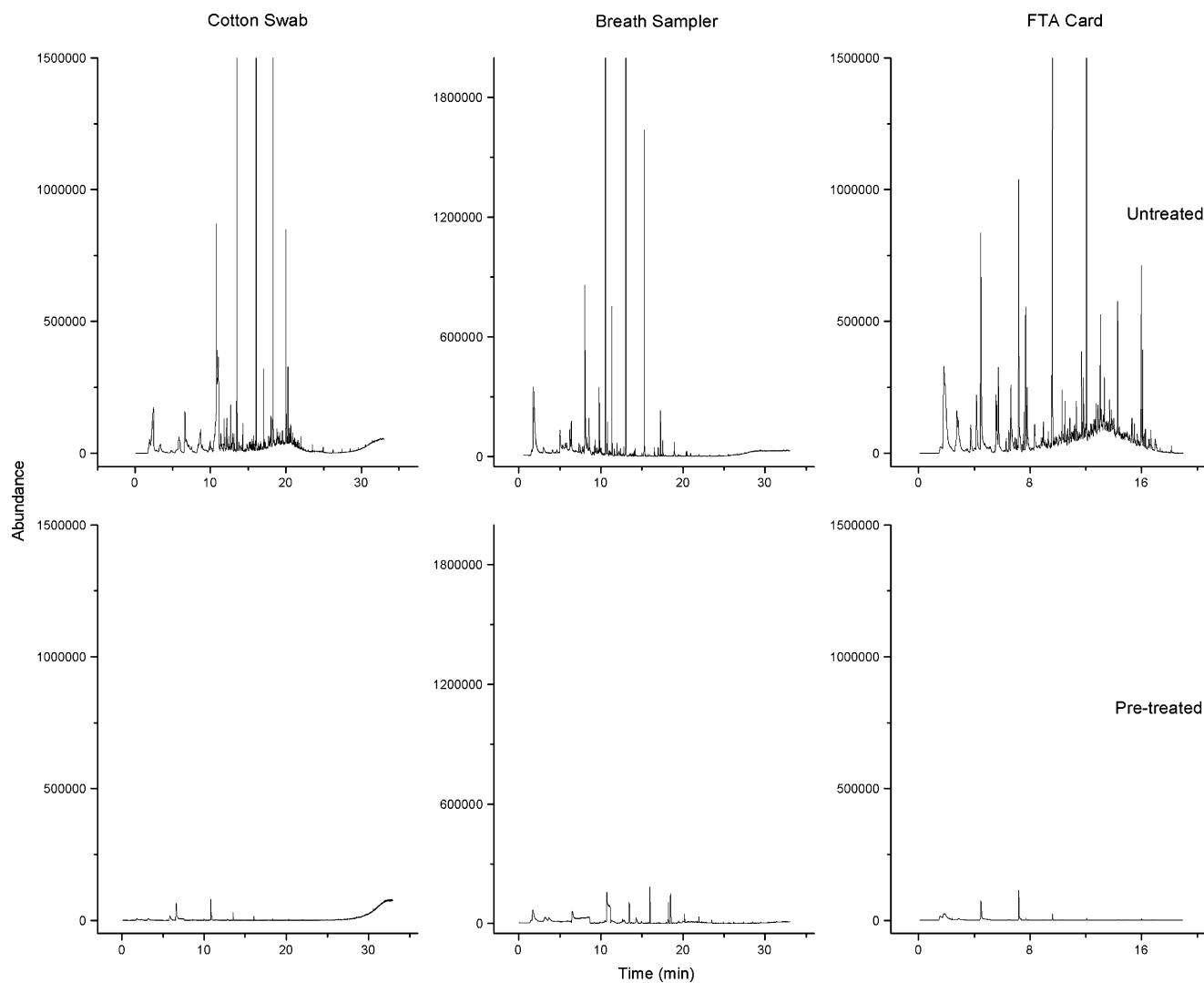
Fig. 1 Comparison of untreated (*top*) and pre-treated (*bottom*) materials

total run time of 23 min. The analytes were acquired in full-scan mode in 45–550 *m/z* range. Extracted VOCs above the scent samples were identified by spectral library (NIST) and/or by standard reference comparison.

LOD/LOQ

Limits of detection and quantitation (LOD and LOQ, respectively) were calculated for selected compounds commonly found in oral fluids, breath, blood, and urine. To determine the LODs and LOQs of analytes found in each specimen, a calibration graph was prepared using 5, 10, 20, 40, 60, and 80 ppm concentrations of standard solutions in dichloromethane and analyzed by GC/MS. The LODs and LOQs were calculated using the slopes from the calibration curves of each analyte in conjunction with the following regression equation:

$$y_{\text{lod}} = a + 3s_{x/y} \qquad (1)$$

$$y_{\text{loQ}} = a + 10s_{x/y} \qquad (2)$$

where y_{lod} is the *y*-coordinate of the limit of detection for the calibration curve, y_{loQ} is the *y*-coordinate of the limit of quantitation for the calibration curve, a is the *y*-intercept, and $3s_{x/y}$ is the standard error for the predicted *y* value for all *x* values in the regression.

Results and discussion

Materials pretreatment

Initial analyses of the collection materials revealed the presence of VOCs that have been reported as human scent
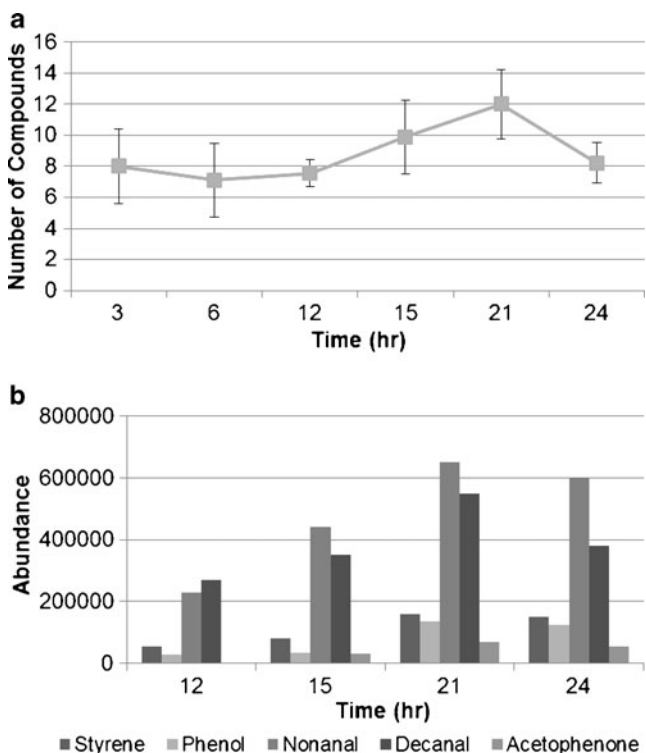
Fig. 2 a Average number of compounds extracted vs. extraction time for buccal swab odor samples. b Abundance of selected common compounds vs. extraction time for buccal swab odor sample for a single male subject

Fig. 4 a Average number of compounds extracted vs. extraction time for blood odor samples at room temperature. b Abundance of selected common compounds vs. extraction time for blood odor sample for a single male subject
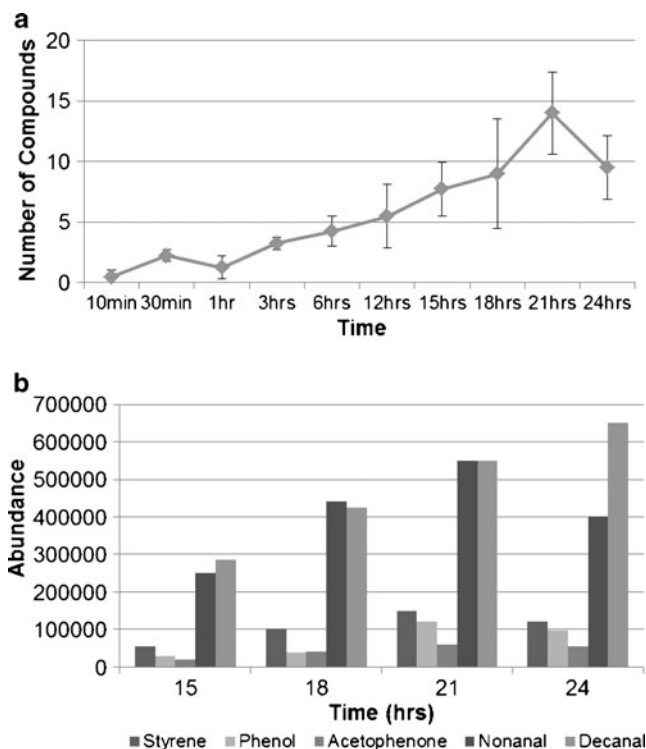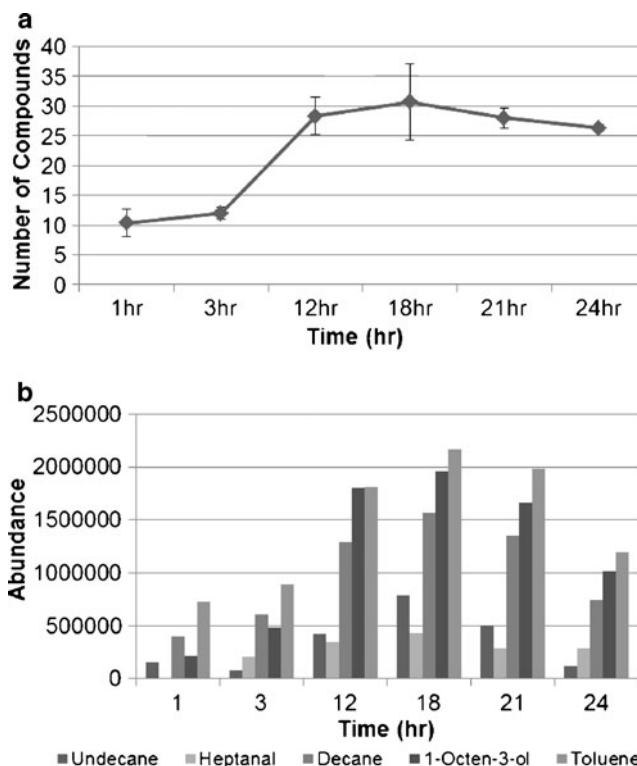

Fig. 3 a Average number of compounds extracted vs. extraction time for breath odor samples. b Abundance of selected common compounds vs. extraction time for breath odor sample for a single male subject

compounds. To remove the possible interference of such compounds in the background of the collection materials, pretreatment of each material prior to sample collection was necessary. Pretreated materials were analyzed using SPME-GC/MS to make sure they were free of undesired human scent compounds. Figure 1 shows the chromatograms of pre- and post-cleaning treatment of the cotton swabs, breath sampler, and FTA cards, respectively, prior to sample collection. The peaks remaining in the chromatogram post-cleaning treatment are SPME phase breakdown products. Materials pretreatment and SPME-GC/MC analysis of the treated materials ensures that they are analytically clean prior to sample collection.

Optimal SPME extraction conditions

Buccal swabs

Triplicate buccal swab samples were collected from unrelated subjects Male 1, Male 5, and Female 4 following the sampling method described in the previous section. Samples were stored at room temperature prior to SPME extraction. The SPME fibers were exposed to the headspace above the buccal swab samples at room temperature for 3,

**Table 1** Spiked compounds detected under different extraction times for urine specimen optimization

RT	Compound	Extraction time				
		1 min	5 min	15 min	30 min	1 h
1.89	Acetone					
3.28	2-Pentanone	x	x	x	x	x
4.44	Dimethyl disulfide	x	x	x	x	x
4.76	Pyrrole		x	x	x	x
5.07	Toluene	x	x	x	x	x
6.02	Hexanal	x	x	x	x	x
7.73	4-Heptanone	x	x	x	x	x
8.05	3-Heptanone	x	x	x	x	x
8.13	2-Heptanone	x	x	x	x	x
9.25	Benzaldehyde	x	x	x	x	x
9.58	Phenol	x	x	x	x	x
10.86	1-Octanol		x	x	x	x
12.15	Octanoic acid				x	x
12.94	Nonanoic acid			x	x	x

6, 12, 15, 21, and 24 h. The optimal extraction time for buccal swab samples was evaluated on a combination of the abundance and number of extracted odor compounds. Twenty-one hours was determined to be the optimal extraction time for the collected buccal swab odor compounds based on the evaluation criteria. Figure 2 shows the average number of odor compounds extracted as a function of extraction time between the subjects (left) and the abundance of commonly extracted compounds across the triplicate samples as a function of extraction time (right) for one individual subject (Male 1). The results demonstrate

that a 21-h extraction time is optimal, producing the greatest number of VOCs extracted by SPME with the highest abundances of these compounds for buccal swab odor samples.

Breath

The exhaled breath VOCs were extracted by SPME fibers inserted directly into the Teflon®-bulb. The breath VOC extractions were performed at ambient room temperature on multiple samples for 10 min, 15 min, 30 min, 1 h, 3 h, 6 h,

Fig. 5 Color charts of the relative peak area ratios of VOCs extracted from buccal swab, breath, blood, and urine specimens from a single male subject
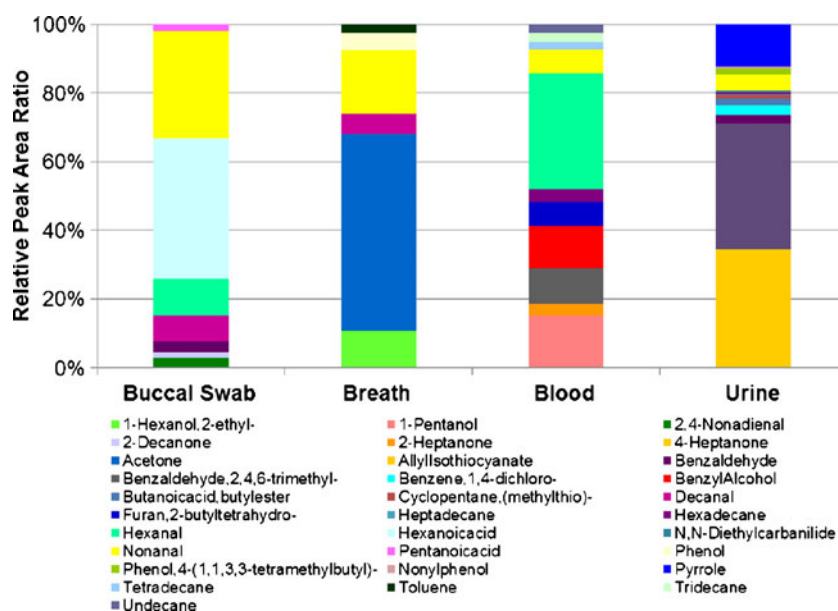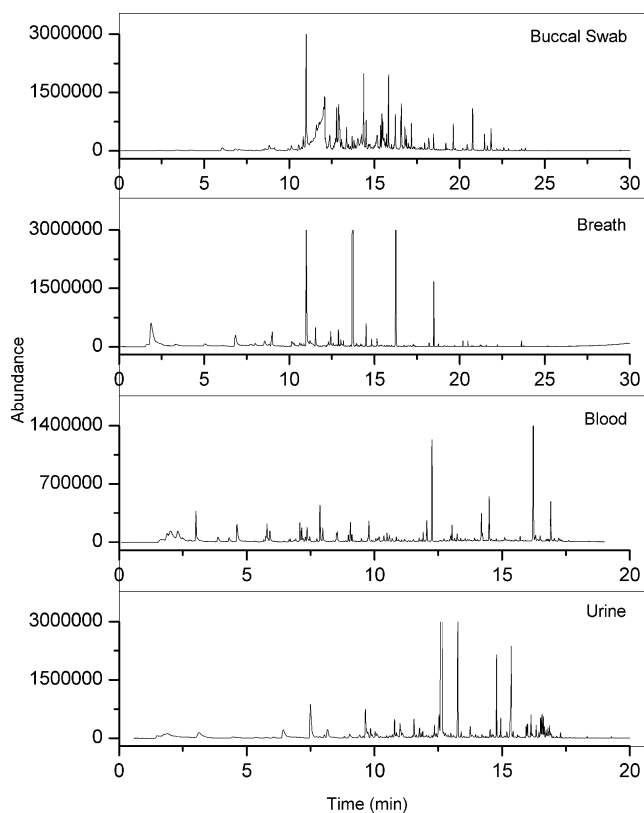
Fig. 6 Typical specimen scent profile chromatograms for a single male subject

12 h, 15 h, 18 h, 21 h, and 24 h. The optimal extraction time for breath samples was evaluated on a combination of the number of extracted odor compounds and the abundances of commonly extracted compounds from the collected breath samples. Figure 3 shows the average number of compounds extracted as a function of extraction time between the male and female subjects (left) and the abundance of commonly extracted compounds across the multiple samples as a function of extraction time (right) for
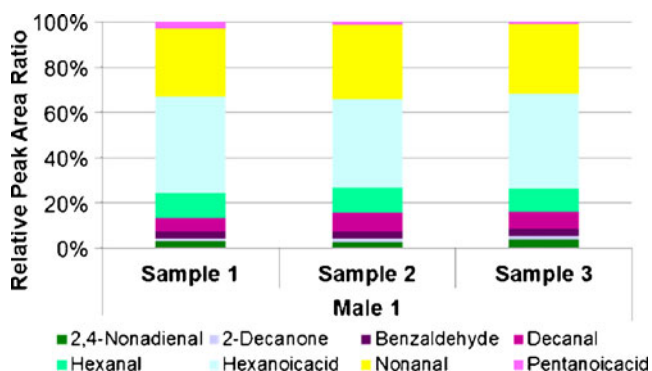

Fig. 7 Color charts of relative peak area ratios of VOCs extracted across the triplicate buccal swab specimens from a single male subject

Table 2 LOD/LOQ for buccal swab VOCs

Buccal swab compounds	LOD (ng)	LOQ (ng)
Hexanal	3.61	12.03
Benzaldehyde	6.29	20.98
2-Pentylfuran	3.51	11.71
Hexanoicacid	3.00	9.99
2-Nonenal, (E)-	5.96	19.88
2,4-Nonadienal, (E,E)-	7.52	25.06
Decanal	4.79	15.96
2-Octenal, (E)-	6.36	21.19
Nonanal	5.75	19.18
Acetophenone	5.49	18.30
1-Octen-3-ol	5.53	18.44
Nonanoic acid, methyl ester	4.18	13.94
Average	5.17	17.22

one individual subject (Male 1). Based on the evaluation criteria, Fig. 3 demonstrates that a 21-h extraction time was determined to be the optimal extraction time for collected breath odor compounds using Bio-VOC®.

Blood

The triplicate blood samples stored on FTA cards were heated in a 37 °C sand bath, and the SPME fiber was exposed to the headspace above the samples for 10 min, 15 min, 30 min, 1 h, 3 h, 12 h, 15 h, 18 h, 21 h, and 24 h. The optimal extraction time for blood samples was evaluated on a combination of the abundance and number of extracted odor compounds of various functional groups. Eighteen hours was determined to be the optimal extraction time for collected blood VOCs as made evident from results as shown in Fig. 4. Data from 15-h extractions were omitted from the graph as the results were anoma-

Table 3 LOD/LOQ for breath VOCs

Breath compounds	LOD (ng)	LOQ (ng)
Toluene	ND	ND
p-Xylene	4.57	15.22
Styrene	3.79	12.62
Benzaldehyde	2.03	6.76
Phenol	1.37	4.56
1,2-Dichlorobenzene	3.28	10.93
1-Hexanol,2-ethyl-	2.41	8.04
Nonanal	2.66	8.88
Dodecane	4.81	16.03
Decanal	3.78	12.61
Average	3.19	10.63

Table 4 LOD/LOQ for blood VOCs

Blood compounds	LOD (ng)	LOQ (ng)
Toluene	ND	ND
Undecane	10.91	36.38
1-Octen-3-ol	11.55	38.49
1-Pentanol	ND	ND
Hexanal	16.15	53.83
2-Heptanone	11.31	37.70
Benzyl alcohol	12.11	40.37
Nonanal	10.45	34.85
Tetradecane	10.66	35.52
1-Dodecene	11.03	36.77
Average	11.77	39.24

lous. No compounds were detected below 1 h extraction time. Beyond 18 h, the abundance of the compounds decreased. The 24-h extraction resulted in half the abundance of what was extracted with 18 h of fiber exposure.

Initial investigations revealed that extraction at ambient room temperature for blood samples on FTA cards was not sufficient to extract VOCs from the collected blood samples. It seemed that there needed to be an additional force to drive the VOCs to be released from the blood matrix when stored on the FTA card. This is perhaps due to the FTA cellulose matrix and the chemicals contained in the FTA cards trapping the VOCs tighter than other collection materials. Further studies are needed on the trapping mechanism and release rate of the VOCs from FTA cards.

Table 5 LOD/LOQ for urine VOCs

Urine compounds	LOD (ng)	LOQ (ng)
Acetone	ND	ND
2-Pentanone	ND	ND
Dimethyl disulfide	11.48	38.27
Pyrrole	11.63	38.75
Toluene	10.92	36.38
Hexanal	10.90	36.35
4-Heptanone	10.96	36.52
3-Heptanone	10.81	36.03
2-Heptanone	10.47	34.90
Benzaldehyde	11.72	39.08
Phenol	12.61	42.05
1-Octanol	11.63	38.77
Octanoic acid	18.80	62.65
Nonanoic acid	26.10	87.00
Average	13.17	43.89

To facilitate the releasing of VOCs from the FTA cards to the headspace above the sample, a study was performed to determine the effect of heating on the extraction efficiency for the blood samples. High temperatures degrade the blood samples, so a comparison was made between unheated samples (ambient room temperature) and samples heated to 37 °C (body temperature) in a sand bath. Greater number of VOCs were extracted from the samples heated at 37 °C regardless of the extraction time, although the difference decreased as extraction time increased (data not shown). Abundance of extracted VOCs also substantially increased for the heated samples. Similar improvements were observed in the extraction efficiency for all other VOCs extracted from the blood samples. Therefore, 37 °C was chosen to be the extraction temperature for blood odor samples.

Urine

Artificial urine was spiked with 14 compounds selected on the basis of varying functional groups and previously reported as being volatile components of human urine [29, 31, 32]. SPME fibers were exposed to the headspace above urine samples immersed in a 50 °C water bath with continuous stirring and a 5-min sample equilibration time for 1 min, 5 min, 15 min, 30 min, and 1 h to determine the optimal extraction time. As shown in Table 1, 13 out of the 14 spiked compounds were recovered for both 30 min and 1 h extraction times. No acetone was detected in any of the samples, as acetone elutes in the solvent front using standard solutions. From the results, 30 min was determined to be the optimal extraction time.

The temperature of the hot water bath was varied (room temperature(24±0.9 °C), 40 °C, 50 °C, 60 °C, and 70 °C) using a 5-min equilibration and a 30-min extraction time. Results demonstrated that 60 °C was the optimal bath temperature for the majority of compounds (data not shown). There was a significant decrease in the peak areas for all detected compounds at 70 °C. Raising the temperature beyond 60 °C introduces the additional concern of fiber coating swelling and powdering, which could risk damage to the fiber through fragmentation when it is withdrawn back into the needle [29].

Five inorganic salts (NaCl, KCl, $MgSO_4$, K_2CO_3, and Na_2CO_3) were tested for their salting-out abilities. Adding an inorganic salt to a sample solution improves extraction efficiency for volatile compounds in biological fluids. Salting out can be used not only to lower the detection limits but also to buffer random salt concentration in body fluids. Approximately 1.5 g of salt was added to each sample vial to completely saturate the sample solution. Samples were equilibrated for 5 min, and the SPME fiber was exposed for 30 min in a 60 °C hot water bath. No acids

were detected with the addition of K_2CO_3 or Na_2CO_3 (data not shown). Addition of $MgSO_4$ showed comparable results to no salt addition. NaCl and KCl showed similar improvement in the extraction efficiencies of VOCs from urine, with NaCl being best. Sodium chloride has been reported to show a more pronounced salting-out effect over KCl due to their respective hydration energies [46]. Therefore, NaCl was chosen as the salting-out agent for optimal extraction of VOCs.

Extraction and analysis of specimen odor samples

The VOC profile of different biological specimens is remarkably different from each other even collected from the same human subject. Figure 5 demonstrates a color odor chart compiled on the basis of relative peak area ratio calculated using the major responses obtained from each biological specimen from the same subject. The unique color bands of each specimen fairly demonstrate the differences in VOC composition and their relative ratios between different specimens. Figure 6 displays typical chromatograms obtained from the headspace above buccal swab, breath, blood, and urine odor samples. As can be seen in Fig. 6, distinct VOC profile is obtained from each of the four biological specimens. The reproducibility of the SPME-GC/MS extraction and analysis method described in this article is demonstrated in Fig. 7. Buccal swab specimens were collected in triplicate from a single male subject and analyzed under optimum SPME-GC/MS conditions. The graphical representation of the data in Fig. 7 demonstrates that the new method possesses fairly good reproducibility.

LOD/LOQ

Detection and quantitation limits were calculated from calibration data and regression statistics using Microsoft Excel. The LODs and LOQs for all analytes tested are presented in Tables 2, 3, 4, and 5. The average LOD values of the VOCs commonly found in buccal swab, breath, blood, and urine were 5.17, 3.19, 11.77, and 13.17 ng, respectively. The average LOQ values were 17.22 ng (buccal swab), 10.63 ng (breath), 39.24 ng (blood), and 43.89 ng (urine). Toluene was not detected using the current GC column for the liquid sample runs associated with the analysis of breath. Similarly, toluene and 1-pentanol were not detected for blood VOC standards, as well as acetone and 2-pentanone for urine VOC standards.

Conclusion

The headspace SPME method developed and optimized has demonstrated to be a viable method for sampling, identify-ing, and differentiating the VOCs present in various biological specimens collected with forensic and clinical sampling kits. While previous studies have generally looked at individual or pairs of biological specimens for diagnostic purposes, the current method allows for the first time direct comparison between the major samples consist-ing of blood, breath, buccal swabs (cells and saliva), and urine taken from the same individuals. The pretreatment method developed for the sample collection media allowed the removal of the targeted VOCs from the sampling kits prior to sampling, extraction, and analysis. The results of this study demonstrate the scent profiling ability and its reproducibility of different biological specimens of forensic and clinical importance. Optimized SPME-GC/MS condi-tions yielded excellent detection limits for the VOCs from blood, breath, buccal swabs, and urine with average limits of detection of 8.3 ng. VOCs from breath were detected with the lowest limit of detection, while urine samples showing the highest limit of detection, which was four times higher than that for breath samples.

The specimen collection methods described in this paper are minimally to noninvasive and only requires a small quantity of the specimens. Specimen samples utilized in this study can be easily collected by medical and forensic technicians without having special training or licenses. The quantities sampled were typical of those available from specimen collection kits for buccal swabs, breath, blood, and urine. Using the current method scent profiles of such specimen samples can be obtained and used for scent matching and individual identifications. Further population studies are underway comparing VOC profiles of multiple specimen samples collected simulta-neously from individuals.

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