J Forensic Sci, July 2006, Vol. 51, No. 4
doi:10.1111/j.1556-4029.2006.00178.x
Available online at: www.blackwell-synergy.com

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Performance Evaluation of the Scent Transfer Unit™ (STU-100) for Organic Compound Collection and Release

ABSTRACT: The Scent Transfer Unit™ (STU-100) is a portable vacuum that uses airflow through a sterile gauze pad to capture a volatiles profile over evidentiary items for subsequent canine presentation to assist law enforcement personnel. This device was evaluated to determine its ability to trap and release organic compounds at ambient temperature under controlled laboratory conditions. Gas chromatography-mass spectrometry (GC-MS) analyses using a five-component volatiles mixture in methanol injected directly into a capture pad indicated that compound release could be detected initially and 3 days after the time of collection. Additionally, 15 compounds of a 39-component toxic organic gaseous mixture (10–1000 parts per billion by volume [p.p.b.$_v$]) were trapped, released, and detected in the headspace of a volatiles capture pad after being exposed to this mixture using the STU-100 with analysis via GC-MS. Component release efficiencies at ambient temperature varied with the analyte; however, typical values of *c*. 10% were obtained. Desorption at elevated temperatures of reported human odor/scent chemicals and colognes trapped by the STU-100 pads was measured and indicated that the STU-100 has a significant trapping efficiency at ambient temperature. Multivariate statistical analysis of subsequent mass spectral patterns was also performed.

KEYWORDS: forensic science, volatiles profile, scent, odor, canine, scent transfer

The landmark Supreme Court case, *Daubert vs. Merrell Dow Pharmaceuticals* (1993) (1), significantly changed the legal admission procedures for scientific evidence in criminal cases. Previously, the 1923 decision in *Frye vs. United States* (2) stated that a procedure or method claiming to have scientific merit had to be considered reliable in the scientific community, have testimony provided by a qualified subject expert, and have proof presented that the person performing the test used correct scientific procedures. *Daubert* went further by adding specificity to the requirements that scientific claims must be verifiable, published in scientific journals, have a known error rate, and be standardized. As this decision, a number of common methods utilized by law enforcement and forensic science laboratories have undergone scrutiny in the United States judicial system. One of these methodologies involves scent-discriminating canines and the investigative tools utilized.

For admissibility purposes under *Frye* and *Daubert*, it would be beneficial for law enforcement purposes to reproduce the detection capability of canines in methodical form, with real-time and onsite capabilities. Research efforts are focusing on improvements in sampling of organic chemicals emanating from clothing or other evidentiary material, and furthering the development and use of effective trapping materials to characterize and facilitate the canine's scent-sensing ability. This research presents results from a performance evaluation of a device called the Scent Transfer Unit™ (STU-100), which is currently used by law enforcement for sampling the volatiles profile emanating from evidentiary material.

The history of using human scent-discriminating canines (*Canis familiaris*) for searching and for identifying criminal suspects in the United States is extensive. Positive scent matches are routinely accepted in the criminal justice system as probable cause and can be admitted as evidence in a trial, provided that additional evidence corroborates the canine's response (3). Unlike many European nations that have national certification and proficiency standards for scent-discriminating canines (4,5), there are no national standards in the United States. As a result, the reliability of scent-discriminating canines to correctly match and identify individuals from scent objects continues to be debated (6–11).

There are essentially six categories of detection canines being used by law enforcement: trailing, tracking, article detection, substance detection, area search, and scent identification line up. Trailing canines are trained to match the volatiles profile (scent/odor) acquired from an article of evidence to a matching trail of scent/odor present on the ground or in the field. Tracking canines are trained to follow ground disturbances, crushed vegetation, and although a human odor component may be present, they are not required to match a scent sample. Article detection canines are trained to locate items recently deposited within a search area. Substance detection canines used for detecting the presence of narcotics, explosives, arson accelerants, or human remains are typically trained on predetermined specific chemicals or mixtures and they are taught to alert when a match is located. Area search-and-rescue canines are trained to search mass disaster areas for the presence of live humans. Scent identification line-up canines are trained to use the scent/odor acquired from an article of evidence to identify the suspect of a crime from a line up of scented objects.

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Received 23 Oct. 2005; and in revised form 4 March 2006; accepted 26 March 2006; published 23 June 2006.

A human volatiles profile is more chemically complex and requires substantially different canine training scenarios compared with those used with targeted chemicals.

For the purposes of the canine and law enforcement communities, the terms scent and odor are community specific and are not necessarily interchangeable with other disciplines, such as biology, ecology, etc. Progress is being made in characterizing the variables and defining the terminology behind human scent composition and canine olfactory systems to develop a better understanding of the canine's response to mixtures of volatile and semivolatile chemicals. Curran et al. (12) developed terminology to categorize the complex mixtures that constitute human scent or odor. The first category, "primary odor," consists of constituents that are stable over time regardless of diet or environmental factors and are genetically based. A second category, "secondary odor," contains constituents that are present due to diet and environmental factors. Finally, "tertiary odor" contains constituents that are present due to the influence of outside sources such as lotions, soaps, or perfumes. For the purposes of human odor detection and analysis by canines, scent is considered the overall volatiles profile left by a human and odor consists of the elements of the volatiles profile that elicits a behavioral response. There may be dozens of compounds present in human scent, but it is unknown at this time the identity or quantity of compounds required by the canine to obtain an odor-related behavioral response for match-to-sample recognition. A volatiles profile is the terminology used here for describing a superset of both scent and odor that is independent of the human model (odor) and the canine model (scent/odor) as well as an instrumentation model (sampling chemicals) as it is known that particles are not responsible for eliciting a volatiles detection response in canines (13). The purpose of this study was to explore the ability of a specific sorbent to collect and release a combination of volatile chemicals. Although the compounds selected for this study have been previously reported to be components of human scent, the authors do not intend to imply that they are the components utilized by canines.

STU-100

There are four commonly used methods to collect a human volatiles profile for canine use: direct, swipe, adsorption/absorption, and indirect. The traditional direct method allows the canine to smell an article of evidence or volatiles source directly by bringing the item close to, or in contact with, its nose. Swiping involves wiping the surface of the evidentiary material with a sterile gauze pad and thereby transferring volatiles onto the pad. The pad is then presented to the canine as in the direct method. Adsorption/absorption involves placing a sterile gauze pad on the source surface for some time period, thereby creating a concentration effect or an aggregate from the item(s) of interest. Often, the source object(s) and a sterile gauze pad are placed into and sealed in a plastic resealable bag. After some time, the gauze pad is removed and presented to the canine as previously described (3). A major drawback to these three methods is the possible disruption and contamination of trace evidence *within* the object of evidence during scent pad contact. To address this issue, U.S. law enforcement personnel have recently been using an indirect or noncontact method of volatiles collection via the STU-100. Very little scientific data have been published that characterizes the trapping medium's (cotton pad) ability to adsorb and desorb organic compounds responsible for scent at ambient temperature.

Developed by Tolhurst and Harris and patented in 1998, (14) the STU-100 device is a portable, hand-held vacuum pump with a modified inlet, that is able to hold in place a 12.5 cm × 23.0 cm Johnson & Johnson® sterile surgical gauze pad. This "scent pad" is used as a trap to collect primarily volatile or vaporized scent compounds as the STU-100 pulls air through the gauze or sorbent at a flow rate of *c*. 300 L/min while it is physically swept above or over articles of evidence or over areas that may emanate volatiles. The pad is then removed from the STU-100 device and double packaged in heat-sealed nylon envelopes. To conduct a scent check with a trailing canine, the handler first acclimates the canine to the available volatiles profiles (scents and odors) at the start location and establishes a baseline for the canine. After harnessing, the handler opens the nylon envelope and places the pad in front of the canine's nose. If a matching odor is present at the trail start, the canine commences to follow the trail. If no matching odor is present, or the level of volatile organic compounds is below the detection capability of the canine, the canine is trained to respond by refusing to trail.

As the number of criminal investigations utilizing the STU-100 has increased, challenges to its court admissibility have surfaced. Officials of the Law Enforcement Bloodhound Association (LEBA) and the National Police Bloodhound Association (NPBA) have criticized the capabilities of the STU-100, although both organizations have members who own and use it. Published statements reflect that neither LEBA nor NPBA currently endorse the STU-100's "trap-and-release" capabilities (15). To date, neither organization has scientifically tested the device. The primary concern expressed by both bloodhound organizations and recent legal proceedings is that the STU-100 does not efficiently collect scent and that the device itself possibly contaminates the scent pads. A recent double-blind study using the STU-100 with canines from the Southern California Bloodhound Handlers Coalition showed that the percentage of positive-scent matches made by bloodhounds between human scent sampled from postblast debris and respective human subjects was 78.3%, with no false-positive identifications (3). For these tests, a false-positive identification is defined as a canine alert to a human subject whose odor was not present on the STU-100 pad during scent proffer. In another study using the STU-100, Harvey and Harvey (16) evaluated eight trained bloodhounds (three novices and five veterans) for the ability to discriminate scent between two human subjects and effectively trail the scent in a battery of terrain and weather conditions. (*Note*: Bloodhounds with more than 18 months of training were considered to be in veteran status.) The STU-100 was swept over multiple areas of the test subject, without contacting the subject, to obtain a volatiles profile not specific to any body part. In field trials, canines were offered the scent pads and they proceeded to follow the scent on 48-h-old trails. The veteran bloodhounds proved successful 96% of the time, while canines in the novice category had a 53% success rate.

Nations with human scent detection canine programs all utilize various natural fiber scent pad materials (e.g., cotton) to capture and release the volatiles profile. Although anecdotal and empirical data suggest that cotton materials collect and release organic chemicals, this study provides an organic chemical trap and release data confirmation from controlled laboratory experiments. Initial experiments focused on spiking the pads directly and utilizing a chromatographic separation before mass spectrometry for identification and confirmation. Additional experiments utilized a more direct analysis approach with no separation step before mass spectrometry. This latter approach generated multiple ion patterns or ion current profiles amenable to mathematical

factor analysis. The pad used in the STU-100 sampling device was desorbed at ambient temperatures as well as elevated temperatures utilizing analytical instrumentation in controlled laboratory settings with known chemical mixtures or standards.

Experimental Methods

Matrix Spike Mix

Initial experiments were designed to determine the extent of desorption (release) of a known chemical mixture from the pads currently used with the STU-100. One microliter of a five-component, volatile organic analysis (VOA) Matrix Spike solution (EPA method 524.2, Volatiles in drinking water) prepared at 2.5 mg/mL in methanol was injected onto the inner cotton layers of a Johnson & Johnson® sterile dressing (currently used as the volatiles profile capture pad). The pad was immediately sealed inside a blanked (i.e., previously analyzed and found to contain nondetectable levels of the volatiles of interest), 3 L Tedlar™ bag. The Tedlar™ bag was then filled with 0.5 L of pure air ($\sim$ 25°C at 60% relative humidity) delivered by a Kin-Tek 491M Dynamic Gas Diluter (Kin-Tek Instruments, La Marque, TX). A negative control was made using the same procedure without addition of the VOA standard. A positive control was prepared by directly injecting 1 µL of the test mix into a blanked Tedlar™ bag containing 0.5 L of pure air and no pad. The bags were allowed to equilibrate at room temperature for 1 h and 72 h, after which 10-mL headspace samples were removed from the bags by an Entech 7100 laboratory benchtop air concentrator (Entech Instruments, Simi Valley, CA). The air concentrator used three-stage preconcentration incorporating cryogen/Tenax TA solid sorbent, cryogen/glass beads, and a final cryofocusing stage before thermal injection into an Agilent 5973 Gas chromatography-mass spectrometry (GC/MS) fitted with a low thermal mass (LTM, RVM Scientific, Santa Barbara, CA) (17) DB-5 column (30 m × 0.25 mm OD × 0.25 µm) for analysis. The GC/MS method parameters are provided in Table 1. Quantitation of recovered analyte was based on a five-point calibration curve (0.25–25 ng/L) of the VOA mix generated before analysis.

TABLE 1—*Agilent GC/MS with LTM A68 method parameters.*

Agilent 6890 GC Oven	
Temperature	210°C
LTM-GC temperature program	
Initial temperature	30°C
Initial hold time	2.00 min
Temperature rate 1	20°C/min
Intermediate temperature 1	175°C
Hold time	0.00 min
Temperature rate 2	60°C/min
Final temperature	210°C
Final hold time	0.00 min
Transfer line/inlet	
Mode	Splitless
Temperature	250°C
Carrier gas	Helium
Column program	
Mode	Programmed pressure
Initial pressure	28.00 psi
Initial hold time	2.00 min
Pressure rate 1	2.02 psi/min
Intermediate pressure 1	38.11 psi
Hold time	0.00 min
Pressure rate 2	6.01 psi
Final pressure 2	46.10 psi
Final hold time	0.00 min
Average linear velocity	53 cm/sec
Agilent 5973 MSD settings	
Acquisition mode	Scan
Scan range	29–180 *m/z*, 0–1.5 min
	34–280 *m/z*, 1.5–11 min
Scan rate	14.64 scans/sec, 0–1.5 min
	10.13 scans/sec, 1.6–9.5 min
Threshold	150
Sampling (2^n)	$n = 1$
Solvent delay	0.00 min
MS quad/source temperature	150°/230°C
MS transfer line temperature	200°C

GC/MS, gas chromatography-mass spectrometry; LTM, low thermal mass.

Volatiles Gas Loading to the STU-100 Pad

To characterize adequately the adsorptive properties of the pads, a standardized NIST traceable gas mixture was used to simulate realistic sampling of low parts per billion by volume (p.p.b.$_v$) levels. A U.S. EPA compendium toxic organic compound (TO-14A) gas mixture (prepared at 1 part per million by volume [p.p.m.$_v$]) containing 39 target analytes was used as the test mixture (Restek Corp., Bellefonte, PA). According to Vass et al. (18) 14 of the 39 analytes in this mix have been identified as major components in decomposition of 1-year-old buried human remains. The implementation of this TO-14A gas mixture with this study will therefore also be beneficial to future scent determination research in conjunction with cadaver detection canines.

For the analysis (performed in triplicate), a volatiles capture pad was placed onto the STU-100, which was then positioned to face a $\frac{1}{8}$ in. diameter TO-14A gas stream situated 0.5 cm from the middle of the pad (Fig. 1). A diluted, humidified, 20 p.p.b.$_v$ TO-14A gas mixture (at 25°C) was then loaded onto the pad at a flow rate of 0.5 L/min for 2.0 min (20 ng nominal mass loading per analyte), during which time the STU-100 was operating at full power ($\sim$ 300 L/min). After gas loading, the scent pads were individually sealed inside blanked, 3 L Tedlar™ bags and filled with 0.5 L of humidified pure air (25°C). The pads were allowed to equilibrate inside the bags for 3 h. Analysis consisted of concentrating the headspace (0.5 L) via the Entech laboratory air concentrator, followed by thermal desorption onto a GC-MS for TO-14A target analyte identification and quantitation. Quantitation of recovered analyte was based on a four-point calibration curve (0–20 ng) of the TO-14A gas mixture performed before analysis. This process was repeated with undiluted (1 p.p.m.$_v$) TO-14A gas mixtures as well.

Thermal Desorption with Atmospheric Chemical Ionization Mass Spectrometry (TD/APCI-MS)

A different analytical technique was utilized for analysis of the volatiles capture pads to provide additional supportive data for this evaluation. The alternate method used TD/APCI-MS. This technique offers the ability to rapidly obtain rapidly a mass spectrometric profile (or the volatiles profile in an ion current form) from analytes present on the pad that would ionize by this method. By using multivariate analysis on the acquired data, the reproducibility of the volatiles profiles can be compared and important discriminators can be determined.

A PE Sciex 365 triple quadrupole mass spectrometer (MDS Sciex, Concord, ON, Canada) equipped with a thermal desorption atmospheric pressure chemical ionization source (Mass Spec Analytica Inc., U.K.) was used for all of the experiments involving

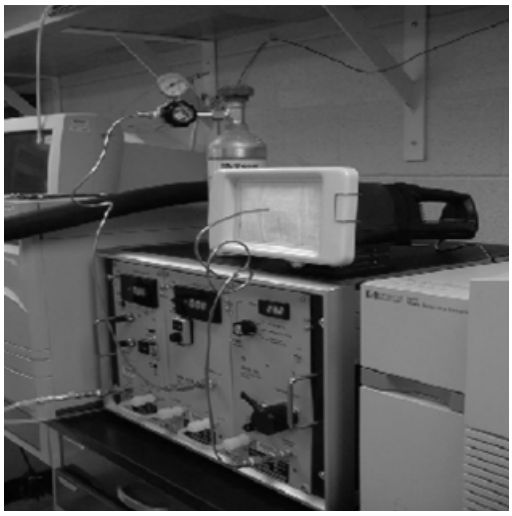

FIG. 1—*Scent Transfer Unit™ (STU-100) positioned to accept a TO-14A gas stream from the Kin-Tek 491M Dynamic Gas Diluter.*

direct analysis with no prior chemical separation of the components emanating from the pads. Figure 2 illustrates how the pad was inserted into the desorption block of the TD/APCI-MS for direct analysis. The pads were used with, or without, the STU-100 to collect volatiles from the headspace of selected chemicals. The pads were desorbed at ambient temperature or elevated temperatures to assist with the release process and mass spectra were collected from *m/z* 30 to 500. The resultant data were smoothed, centroided, and imported into a multivariate analysis software package (Pirouette Lite Explore v. 3.11) where principle component analysis (PCA) and hierarchical cluster analysis (HCA) were performed.

Experiments were conducted to demonstrate that chemicals were being adsorbed, absorbed into, or trapped onto the volatiles capture pads, and that the chemicals were released from the pads at room temperature. Approximately 1 mL of benzaldehyde was placed in a beaker and covered with a watch glass. An uncapped bottle of 2-nonenal was also placed in a beaker and covered with a watch glass. The headspace of each was sampled with the STU-100 for 5, 15, and 30 sec, with about 6–8 h between sampling. Only single measurements were acquired and the first 10 scans, unless otherwise noted, were averaged to yield the average ion current for selected compounds. In negative ion mode, a few milliliters of isovaleric acid was placed in a chamber and covered with a glass plate. The compound 2-ethylhexanoic acid was placed in a beaker and also covered with a watch glass. Sampling was performed for periods of 5, 15, and 30 sec. A binary mixture composed of approximately equal volumes of isovaleric acid and 2-ethylhexanoic acid was also prepared in a beaker and covered with a watch glass. Only semiquantitative analysis has been achieved via this technique thus far.

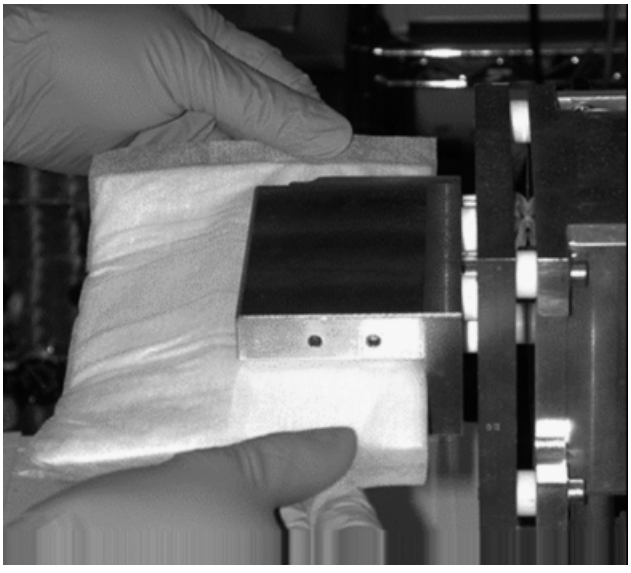

FIG. 2—*The volatiles capture pad was placed into the desorption block of the thermal desorption followed by atmospheric pressure chemical ionization mass spectrometry (TD/APCI-MS) for mass spectral analysis of the components that were successfully trapped by the pad.*

An example of the experimental data sets that can be generated via the TD/APCI-MS technique is shown in Fig. 3. In this particular data set, multiple ions are monitored as the analysis is executed. An example of two compounds that are reported to be human odor components, isovaleric acid and 2-nonenal, and their respective mass spectral patterns and selected ion profiles resulting from a headspace analysis, are illustrated. This experiment shows that when operating under these conditions, the molecular ions or the protonated molecules are primarily observed.

Results and Discussion

VOA Matrix Spike Mix

The results listed in Tables 2 and 3 indicate that volatile chemicals were released from the capture pad(s) and could be detected in a volume of as little as 10 mL of air at 1 and 72 h after loading. The positive controls resulted in a moderate recovery for four of the five analytes studied, with a high-recovery observed for benzene in the 1-h equilibration and for toluene in the 72-h equilibration. Although the positive control components at 72-h sampling exhibit, in general, higher recoveries than at the 1-h sampling, this was not observed in the actual pad samples. The recoveries in all three sampled scent pads were less at the 72-h mark than at the 1-h sampling (except for trichloroethylene in Sample #2), indicating that analyte may be readsorbing onto the pads over this time period, efficiently adhering to the inner surface of the Tedlar™ bag, or permeating through the bag. Nevertheless, this study indicates that measurable amounts of chemicals injected or spiked onto the scent pad in liquid form can be released over a 3-day period of time by the scent pad fibers into the headspace surrounding the pad.

Toxic Organics (TO-14A) Gas Mixture

The results along with the relative standard deviations (standard deviation/mean expressed as a percent [RSD]) are listed in Table 4 and show that a maximum of 15 out of the 39 target analytes were quantitated above the minimum detectable level (0.01 ng) when the scent pads were loaded at a concentration of 10 p.p.b.$_v$ (20 ng nominal mass). Most of these analytes detected were the heavier substituted aromatics, with the exception of two types of freons (dichlorotetrafluoroethane and trichlorofluoromethane), and 1,1-dichloropropane. Total recoveries for the remaining analytes were well below the load amount, suggesting three possibilities: (a) the pads were efficient in trapping the volatiles, but the flow rate ($\sim$ 300 L/min) of the STU-100 was too high to enable both efficient and reproducible adsorption of analyte to the pad, and/or (b) the pads are only marginally efficient in trapping or

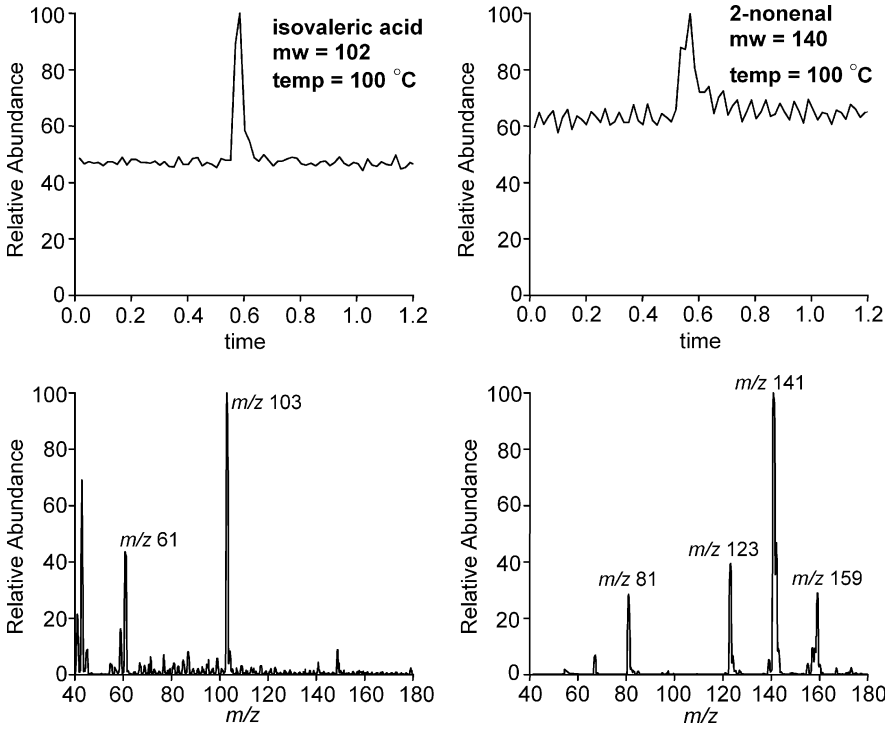

FIG. 3—*Example of the data generated by the thermal desorption followed by atmospheric pressure chemical ionization mass spectrometry (TD/APCI-MS) instrument when a volatiles capture pad is independently loaded with isovaleric acid and 2-nonenal. The total ion current (top two plots) and the individual spectra (bottom two plots) for the protonated molecule of isovaleric acid and 2-nonenal are illustrated at m/z 103 and 141, respectively.*

adsorbing the low-molecular-weight volatiles, and because of this inefficiency, analyte broke through the pads during sample loading, or (c) the pads required higher temperatures (>25–30°C) to release analyte in amounts sufficient for laboratory-based instrumentation to detect. To test the possibility of (a) above, sample loading was performed at the undiluted concentration (1 p.p.m.$_v$) of the TO-14 test mixture to load a nominal mass of 100 ng onto each scent pad, with one analysis incorporating the vacuum of the STU-100 and another analysis using only the undiluted gas stream (0.1 L/min) onto the pad. The results along with the calculated

TABLE 2—*VOA matrix spike results after 1-h equilibration.*[*]

		Analyte Recovery							
		Control		Sample #1		Sample #2		Sample #3	
Target Compound	Theoretical Yield (ng)	ng	%	ng	%	ng	%	ng	%
1,1-Dichloroethene	12.76	2.65	21	0.12	0.9	0.11	0.9	0.2	1.6
Benzene	15.6	13.91	89	1.32	8.5	0.67	4.3	5.64	36
Trichloroethylene	9.4	1.12	12	0.73	7.8	0.62	0.7	2.38	25
Toluene	13.28	3.35	25	2.45	18	2.42	18	6.7	50
Chlorobenzene	10.92	3.14	29	1.57	14	1.99	18	3.09	28

[*]All blanks yielded nondetects.
VOA, volatile organic analysis.

TABLE 3—*VOA matrix spike results after 72-h equilibration.*[*]

		Analyte Recovery							
		Control		Sample #1		Sample #2		Sample #3	
Target Compound	Theoretical Yield (ng)	ng	%	ng	%	ng	%	ng	%
1,1-Dichloroethene	12.76	3.85	30	ND	—	ND	—	0.17	1.3
Benzene	15.6	5.51	35	0.15	0.9	0.42	2.7	1.7	11
Trichloroethylene	9.4	5.29	56	0.14	1.6	1.05	11	1.1	12
Toluene	13.28	14.93	112	0.39	2.9	1.63	12	2.85	21
Chlorobenzene	10.92	3.35	31	0.52	4.8	0.9	8.2	1.06	9.7

[*]All blanks yielded nondetects (ND).
VOA, volatile organic analysis.

TABLE 4—*Volatiles profile capture pad results for 10 p.p.b.$_v$ gas load during STU-100 operation.*

Target Compound[++]	Recovery (ng)* Control[+]	Sample Pad #1	Sample Pad #2	Sample Pad #3	RSD (%)
Dichlorotetrafluoroethane[†]	15.84	0.13	0.2	0.22	26
Trichlorofluoromethane[†]	18.74	0.23	0.04	ND	100
1,2-Dichloropropane	22.41	0.6	ND	ND	—
Toluene[†]	29.96	ND	0.2	0.07	68
Ethylbenzene[†]	15.29	ND	0.29	0.3	2
m-Xylene[†]	15.64	ND	0.26	0.27	3
p-Xylene[†]	15.64	ND	0.26	0.27	3
Styrene[†]	15.84	ND	0.15	ND	—
o-Xylene[†]	15.74	ND	0.15	0.25	35
1,3,5-Trimethylbenzene	19.91	0.04	0.04	0.02	35
1,2,4-Trimethylbenzene	19.3	ND	0.04	0.02	47
1,4-Dichlorobenzene	9.98	0.06	0.05	ND	13
1,2-Dichlorobenzene	9.86	0.05	0.08	0.11	38
1,2,4-Trichlorobenzene	4.42	0.14	0.15	0.14	4
Hexachlorobutadiene	11.36	0.04	0.04	0.02	35

*Most analytes from the TO-14A gas mix were below minimum detection level (<0.01 ng).
[†]Denotes compounds identified as major components in 1-year-old buried human remains (16).
[‡]Nominal 20 ng analyte loading.
[§]All blanks yielded nondetects (ND).
STU-100, Scent Transfer Unit™.

RSDs are listed in Tables 5 and 6, respectively. The analytes and recoveries at the higher concentration of 1 p.p.m.$_v$ using the STU-100 at a high flow rate (Table 5) coincided with those from the 10 p.p.b.$_v$ study, whereas higher recoveries of a larger number of analytes were obtained by loading the scent pads without the use of a high flow (Table 6). These results suggest that although the recoveries remain comparatively low against the theoretical load, the high flow of the STU-100 may reduce the adsorptive and/or absorptive efficiency of the scent pads, resulting in lower detectable amounts. Nevertheless, the pads demonstrated the ability to *trap* a limited number of gaseous analytes from a 0.1 L/min gas stream and *release* quantities at ambient temperature into the surrounding air at levels detectable by analytical laboratory instrumentation.

TABLE 5—*Volatiles profile capture pad results for 1.0 p.p.m.$_v$ gas load during STU-100 high-flow operation.*

Target Compound[++]	Recovery (ng)*+ Sample Pad #1	Sample Pad #2	Sample Pad #3	RSD(%)
Dichlorotetrafluoroethane[†]	0.23	0.23	0.24	2
Trichlorofluoromethane[†]	0.21	0.21	0.21	0
1,2-Dichloropropane	ND	1.01	1.4	23
Toluene[†]	0.18	0.2	ND	7
Ethylbenzene[†]	0.29	0.28	0.28	2
m-Xylene[†]	0.25	0.25	0.24	2
p-Xylene[†]	0.25	0.25	0.24	2
Styrene[†]	0.13	0.13	0.15	8
o-Xylene[†]	0.15	0.15	0.15	0
1,3,5-Trimethylbenzene	0.06	0.02	0.02	69
1,2,4-Trimethylbenzene	0.1	ND	ND	—
1,4-Dichlorobenzene	0.23	0	0.05	94
1,2-Dichlorobenzene	0.09	0.05	ND	40
1,2,4-Trichlorobenzene	0.21	0.15	0.13	25
Hexachlorobutadiene	0.09	0.02	0.02	93

*Most analytes from the TO-14A gas mix were below minimum detection level (<0.01 ng).
[†]Denotes compounds identified as major components in 1-year-old buried human remains (16).
[‡]Nominal 100 ng analyte loading.
[§]All blanks yielded nondetects (ND).
STU-100, Scent Transfer Unit™.

TABLE 6—*Volatiles profile capture pad results for 1.0 p.p.m.$_v$ gas load under no flow STU-100 conditions.*

Target Compound[++]	Recovery (ng)[+] Sample Pad #1	Sample Pad #2	Sample Pad #3	RSD (%)
Dichlorodifluoromethane[†]	ND	0.1	ND	—
Chloromethane	ND	0.55	0.38	26
Dichlorotetrafluoroethane[†]	0.12	0.23	0.12	41
Trichlorofluoromethane[†]	0.29	0.22	0.28	14
1,1-Dichloroethene	ND	0.13	ND	—
Trichlorotrifluoroethane	0.24	0.21	0.22	7
(Z)-1,2-Dichloroethene	0.14	0.15	0.13	7
Chloroform[†]	0.05	0.06	0.04	20
1,2-Dichlrorethane	0.08	0.11	0.05	38
Carbon tetrachloride[†]	0.03	0.05	0.09	54
Benzene[†]	0.12	0.09	0.09	17
Trichloroethylene[†]	0.07	0.13	0.06	44
(Z)-1,3-Dichloropropene	ND	0.15	0.14	5
1,1,2-Trichloroethane	0.07	0.07	0.05	18
Toluene[†]	0.23	0.19	0.22	10
Chlorobenzene	0.34	0.4	0.32	12
Ethylbenzene[†]	0.33	0.37	0.31	9
m-Xylene[†]	0.3	0.35	0.28	12
p-Xylene[†]	0.3	0.35	0.28	12
Styrene[†]	0.24	0.31	0.19	24
1,1,2,2-Tetrachloroethane	0.28	0.33	0.19	27
o-Xylene[†]	0.15	0.16	0.15	4
1,3,5-Trimethylbenzene	0.23	0.35	0.12	49
1,2,4-Trimethylbenzene	0.28	0.82	0.17	82
1,3-Dichlorobenzene	0.31	1.12	0.15	99
1,4-Dichlorobenzene	0.62	1.73	0.35	81
1,2-Dichlorobenzene	0.44	1.15	0.28	74
1,2,4-Trichlorobenzene	0.78	2.1	0.6	71
Hexachlorobutadiene	0.69	2.06	0.53	77

*Nominal 100 ng analyte loading.
[†]Denotes compounds identified as major components in 1-year-old buried human remains (16).
[‡]All blanks yielded nondetects (ND).
STU-100, Scent Transfer Unit™.

Thermal Desorption Atmospheric Chemical Ionization Mass Spectrometry Analysis

Two chemicals that have been identified as potentially contributing to human odor were selected to evaluate alternatively the volatiles capture pads' ability to trap and release the material being sampled: benzaldehyde and 2-nonenal. The headspace over benzaldehyde, a possible odor component from hands (9), and 2-nonenal, a scent component associated with people less than 40 years of age (19), was sampled with the STU-100. The positive ion mode single measurement results are shown in the bar graphs in Figs. 4, 5*a* and *b*. The average ion signal intensity for the protonated molecule of benzaldehyde at *m/z* 107 is plotted in Fig. 4. Figure 5*a* shows the average ion signal from the protonated molecule of 2-nonenal (*m/z* 141) as a function of sampling time; Fig. 5*b* shows the average ion intensity summing the ion current generated from the protonated molecule as well as two fragment ions, *m/z* 123 and 81 from 2-nonenal. These results indicate the ability of the pad to capture chemical compounds associated with

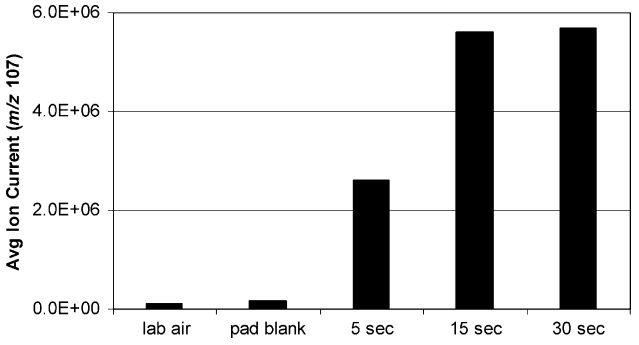

FIG. 4—*Averaged ion signal (eight scans) for the protonated molecule of benzaldehyde as a function of sampling time (and controls) with the Scent Transfer Unit™ (STU-100) at room temperature.*

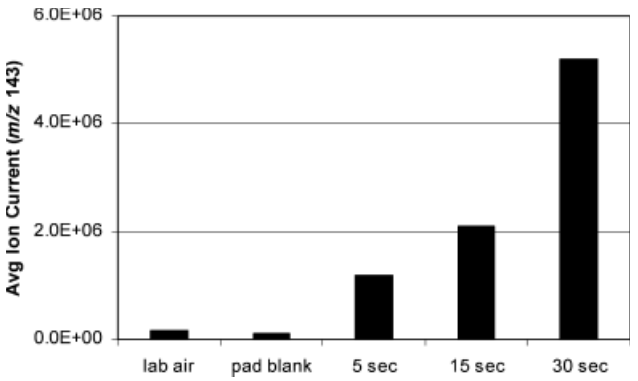

FIG. 6—*Averaged ion signal (10 scans) for the protonated molecule of 2-ethylhexanoic-acid as a function of sampling time (and controls) with the Scent Transfer Unit™ (STU-100) at room temperature.*

human odor using the STU-100 device and that these compounds can desorb from the pads at room temperature.

In negative ion mode, isovaleric acid, a foot malodor component (20), and a component found in underarm odor, 2-ethylhexanoic acid (21), were sampled using the STU-100. A binary mixture composed of *c*. equal volumes of isovaleric acid and 2-ethylhexanoic acid was also sampled for various time periods. The experimental results are shown in Figs. 6 and 7. The negative ion data, as with the positive ion data, show that chemicals are being adsorbed onto the pad and released at room temperature.

In Figs. 4–7, it is important to note that laboratory air and "blank" pads generate a measurable, albeit low, ion current via this technique. Figures 4–6 are plotted with absolute ion current on the ordinate to illustrate that "blank" pad levels for the analytes of interest are relatively low, yet present. Ideally, any pad used for evidence collection would have a nonmeasurable background level; however, in reality, this is difficult to achieve. Nevertheless, SFE has been shown to generate analytically "clean" pads to reduce and/or eliminate background levels present on virgin pads (22). The protocol for using the STU with canines mandates the use of a control presented to the canine to establish a local/scene background before sampling evidence with the volatiles capture pad and to establish a negative response to trail.

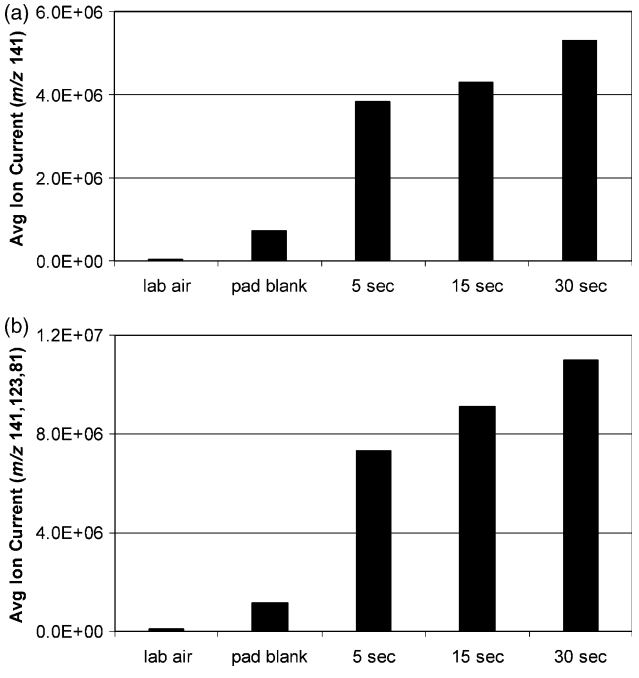

FIG. 5—*(a) Averaged ion signal (10 scans) for the protonated molecule of 2-nonenal as a function of sampling time (and controls) with the Scent Transfer Unit™ (STU-100) at room temperature. (b) Averaged ion signal (10 scans) summing the protonated molecule of 2-nonenal and fragment ions at m/z 123 and 81 plotted versus sampling time (and controls).*

Statistical Analysis

To further supplement the experimental data, statistical analysis was performed to provide mathematical visualization for the delineation of volatiles profile patterns, especially when using the rapid technique of TD/APCI-MS. Mass spectral data acquisition via TD/APCI-MS lends itself to multivariate analysis due to the multiple ion monitoring or detection that is used. In each analysis, a rapid desorption yields ion current as *m/z* measurements from all of the analytes present that can be ionized by APCI; however, using multivariate analysis or PCA, patterns within the data that arise due to the variances can be readily observed by using three-dimensional graphing.

PCA of the volatiles profile data demonstrates reproducibility achievable with the STU-100, as well as determines important discriminators in identifying individuals. PCA of unused pads showed variability between boxes; however, the data cluster together, as illustrated in Fig. 8. Replicate samples of pads used to sample the headspace of isovaleric acid (commonly observed emanating from "sweaty socks"), and those samples acquired directly over spiked isovaleric scent pads, showed similar clustering as well. Also clearly differentiated from the isovaleric volatile profiles were the pads spiked with heptanoic acid. This single plot shows clear instances of the clustering observed between isovaleric acid, heptanoic acid, background air, and virgin pads,

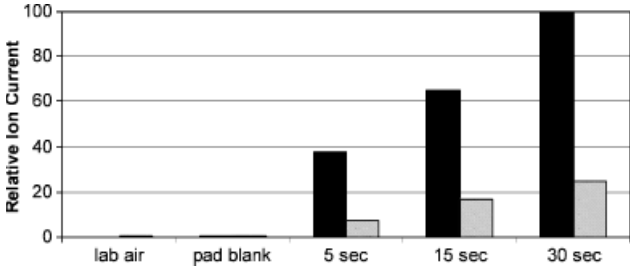

FIG. 7—*Averaged ion signal (10 scans) for the two components in a binary mixture of isovaleric acid (hatched) and 2-ethylhexanoic-acid (textured) sampled using the Scent Transfer Unit™ (STU-100) as a function of sampling time (and controls) at room temperature.*

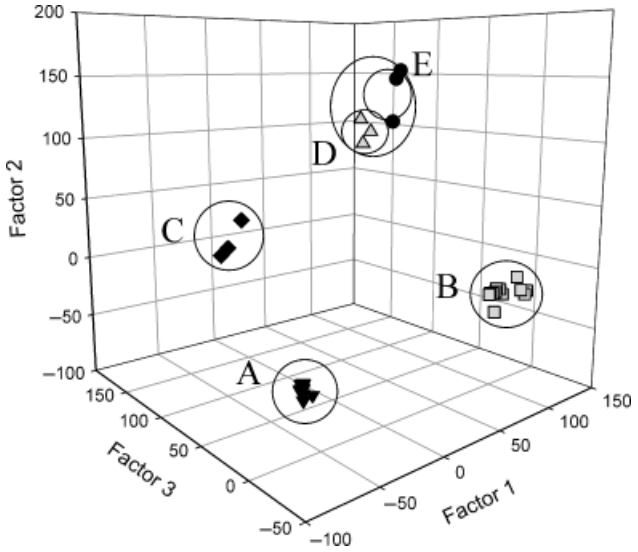

FIG. 8—*Principle component analysis (PCA) scores plot showing three factors that assist in the delineation between (A) background air, (B) virgin volatiles capture pads, (C) a pad spiked with heptanoic acid, (D) a pad loaded by sampling the headspace over a few milliliters of neat isovaleric acid, and (E) a pad spiked with isovaleric acid.*

collected using the TD/APCI-MS technique in negative ion mode. The source of the variability and thus an important discriminator for the isovaleric acid pads as identified in the PCA loading plot (not shown) was predominantly *m/z* 101 and 203, corresponding to the $(M–H)^-$ and $(2M–H)^-$ ions of isovaleric acid, respectively.

To demonstrate that the volatiles capture pads can adsorb complex mixture components and to show that PCA can help delineate these upon release or desorption, a variety of various colognes were sampled via the STU-100. The mass spectral ion current patterns resulting from the TD/APCI-MS analysis (50°C) of the pads after sampling the vapors emanating from four different colognes are shown in Fig. 9. Although the mass spectral patterns between virgin pads and the Aramis cologne are similar visually, the PCA analysis can delineate the two as shown in Fig. 10. The other complex colognes can also be distinguished in factor space. These data lend support to the fact that perhaps canines could differentiate these volatiles (scent/odor) profiles.

Conclusions

In the analytical literature, there are a host of sampling methods for measuring a wide variety of chemicals in a manner that is scientifically sound, reliable, and defensible. Before general acceptance of any sampling method, controlled testing is performed to ascertain the strengths and weaknesses of the method or the equipment used to make the measurements. In this instance, the STU-100 sampling device was evaluated to determine whether the cotton pads used in the device actually trap volatile or semivolatile chemicals and release them at room temperature. What may seem obvious to the casual observer given empirical

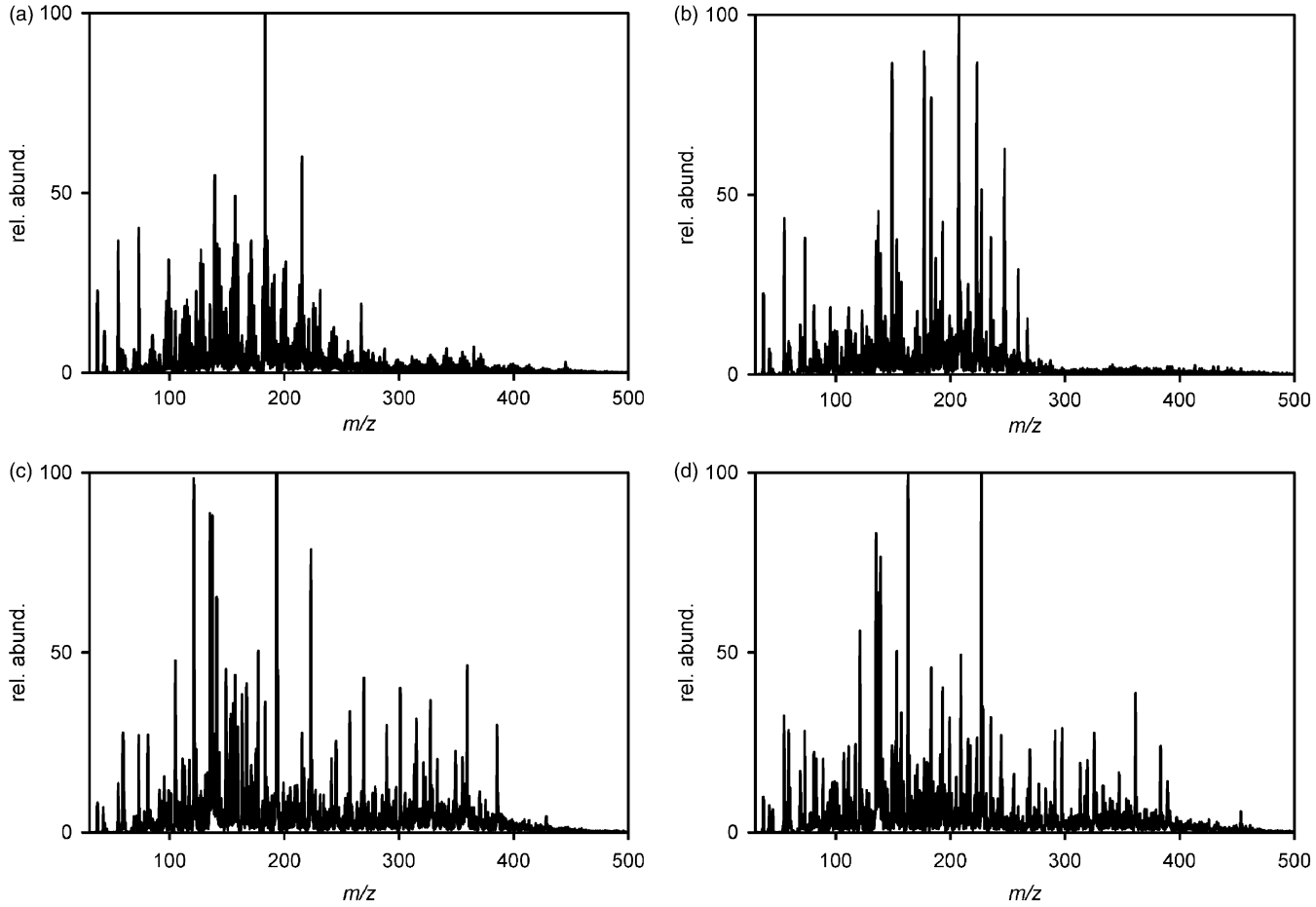

FIG. 9—*Normalized ion current (ordinate) thermal desorption followed by atmospheric pressure chemical ionization mass spectrometry (TD/APCI-MS) profiles resulting from desorption of the Scent Transfer Unit™ (STU-100) volatiles profile capture pads subsequent to sampling a variety of complex mixtures. The mass spectral ion current profiles are (A) virgin pad, (B) Davidoff cologne, (C) Tea Rose cologne, (D) Tommy cologne, and (E) Aramis cologne.*

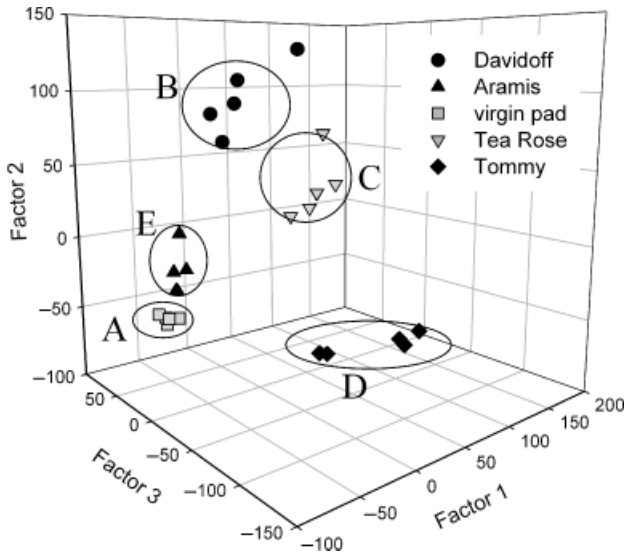

FIG. 10—*Principle component analysis (PCA) three-dimensional scores plot resulting from statistical analysis of the data generated from the thermal desorption followed by atmospheric pressure chemical ionization mass spectrometry (TD/APCI-MS) experiment utilizing four different colognes sampled by the Scent Transfer Unit™ (STU-100). Each of the different colognes cluster as: (A) virgin pad, (B) Davidoff, (C) Tea rose, (D) Tommy, and (E) Aramis.*

knowledge of cotton materials, a controlled scientific investigation into verifying these assumptions for legal precedence was still required for this device. The fact that the cotton pads used in the STU-100 sampling device can trap and release chemicals at ambient temperature has been experimentally verified. Although the trapping and release efficiencies were found to be less than optimal, these experiments were required to characterize the device and to establish a baseline for improving it.

In each of the two analytical methodologies, GC/MS and TD/APCI MS, the STU-100 performance evaluation experimental test plan consisted of a strategy using simple chemical spikes followed by more complex mixtures. With the GC/MS experiments, testing moved from a simple liquid spike mixture to a more complicated, 39-component TO-14A gaseous mixture. With the TD/APCI MS experiments, analytical measurements were performed from simple and binary mixtures as well as complex organic mixtures illustrated via the cologne analyses. Each analytical methodology used demonstrated effective loading and subsequent release of analytes with high volatility, in both liquid and gaseous form. In addition, the multivariate analysis yielded insights into the volatile and semivolatile organic compound patterns and clustering that can be differentiated in factor space. The mass spectral patterns that resulted after spiking the volatiles capture pads with various perfumes can be distinguished by the TD/APCI-MS approach both visually as well as chemometrically; however, by displaying the data in factor space, clustering of the patterns is evident and lends further support to the fact that the pads utilized in the STU-100 can trap (adsorb) and release (desorb) chemical mixtures.

This research has spawned many new ideas for adjusting, modifying, improving, and exploring further the advantages or disadvantages of the STU-100. Future directions will involve the use of test mixtures that are representative of the human odor components that have been reported experimentally (9,10) to provide a better understanding of the nature of canine olfactory detection and to exploit variation of airflow volumes for more efficient volatiles collection (adsorption). Research to improve the pad capture capabilities via surface modifications, new polymeric trapping materials, and flow modulation will be necessary to increase the levels of analyte collected for the canines as well as for laboratory analytical instrumentation. Further statistical analysis will be required to illustrate whether clusters formed in the multidimensional factor analysis plots actually can assist scientists in determining uniqueness among individuals or assist in the differentiation of individuals or groups and whether these clusters correlate with trailing canine volatiles profile (scent/odor) experiments. Finally, continued volatiles sampling of humans and potential items of evidence will be necessary to further evaluate or validate the analytical protocols and improve the overall understanding of the complexities of human volatiles profile evidence sampling and recovery.

Acknowledgments

The authors gratefully acknowledge John Luke of Mass Spec Analytica for loaning the thermal desorption APCI unit through a Cooperative Research and Development Agreement (CRADA #ORNL02-0662). S. Ramsey acknowledges support in part through the Federal Bureau of Investigation's Visiting Scientist Program, an educational opportunity administered by Oak Ridge Institute for Science and Education (ORISE).

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