

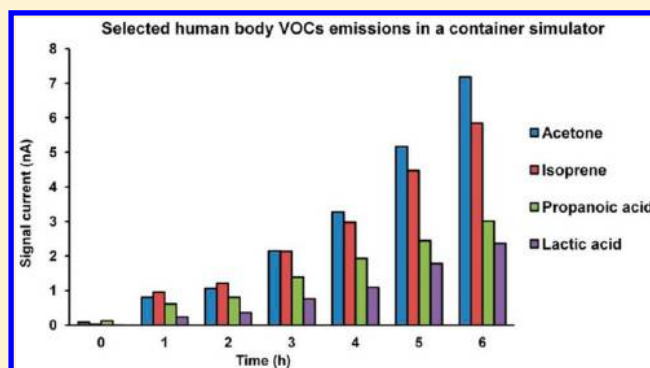
Monitoring of Human Chemical Signatures Using Membrane Inlet Mass Spectrometry

Stamatios Giannoukos,[†] Boris Brkić,^{*,†} Stephen Taylor,^{*,†} and Neil France[‡]

[†]Department of Electrical Engineering and Electronics, University of Liverpool, Brownlow Hill, Liverpool, L69 3GJ, United Kingdom

[‡]Q Technologies Ltd., 100 Childwall Road, Liverpool, L15 6UX, United Kingdom

ABSTRACT: This work is an attempt to assist border security crackdown on illegal human immigration, by providing essential results on human chemical signatures. Data was obtained using a portable quadrupole mass spectrometer coupled with a membrane probe for volunteers of both genders and under different conditions in a container simulator. During experiments, participants were asked to follow various protocols while volatile organic compounds emitted from their breath, sweat, skin, and other biological excreted were continuously being monitored. Experimental setups using different membrane materials (both hydrophilic and hydrophobic) including heating of the sampling probe and sampling flow rates were examined. From our measurements, significant information was obtained for NH₃, CO₂, water, and volatile organic compounds levels, illustrating a human chemical profile and indicating human presence in a confined space.



Border and homeland security worldwide is facing tremendous challenges due to threats from terrorism and/or national/transnational criminal organizations. During the last few decades, a continuous increase of transportation of illicit substances (drugs, explosives) and weapons as well as illegal human trafficking has been observed and is of particular concern.¹ A plethora of different possible scenarios of illegal human transportation are reported daily in media, such as the cases of hidden people in vans, big boxes, coffins and shipping containers. Most of these situations are investigated by specially trained sniffer dogs using their extremely sensitive and delicate olfactory system.^{2–5} Human chemical signatures (HCS), which basically refer to the characteristic human body odors, are an innovative and upcoming research field. Both human expired air compounds with human skin and sweat scent compose and declare an individual's characteristic odor or in other words a person's unique and distinctive chemical "odorprint" that is analogous to a fingerprint.^{6,7}

Volatile organic compound (VOC) emissions from human exhaled breath, sweat, skin, and other biological excreted have been used for a wide range of applications including diagnostic purposes in medicine, search and rescue operations, forensic and toxicological analysis.^{8–12} Human exhaled air is a complex mixture of both inorganic gases and traces of VOCs,¹³ and it depends on several factors, which are found in the daily life habits.^{14–16} It has been reported qualitatively and quantitatively that the most abundant compounds present in human breath are ammonia, acetone, isoprene, methanol, ethanol, propanol, isopropanol, butanone, 1-pentene, 1-butene, and acetaldehyde.^{17–32} Previous research proposed a "core" of volatile compounds identified in the exhaled breath of 15 volunteers

that could potentially be used for early localization of human victims in the debris of collapsed buildings after natural disasters in urban areas.⁸ In the trapped human experiment,³³ monitoring of human breath, skin volatile metabolites and inorganic gas emissions were demonstrated in a collapsed building simulator.

Human skin is the largest human organ.³⁴ It has a complex structure comprising glands that produce sweat and other metabolites. These glands can be grouped in three major categories, eccrine, sebaceous, and apocrine glands, and are situated in different regions of the human body surface.³⁵ Human sweat when secreted, in its primary form, is an odorless biological fluid produced from the above glands. Skin is colonized by a very rich microbiota that consume and metabolize this biological fluid through complex biochemical processes, concluding in the transformation of this odorless fluid to an odorous liquid.^{35–38} In total, VOCs found in human body scent can be classified in the following chemical families: short-chain and long-chain carboxylic acids, ketones, aldehydes, alcohols and phenols, esters, hydrocarbons (alkanes, alkenes), aliphatic/aromatic compounds, amines, and steroids.^{39–47}

Existing mainstream technology for laboratory analysis of expired breath and human sweat utilize mass spectrometry (MS) based techniques such as the proton transfer reaction-mass spectrometry⁴⁸ (PTR-MS) and selected ion flow tube-mass spectrometry (SIFT-MS),⁴⁹ ion mobility spectrometry

Received: August 30, 2013

Accepted: December 19, 2013

Published: December 19, 2013

techniques (IMS),^{50,51} as well as the use of electronic noses⁵² and laser spectroscopy.^{53,54} Gas chromatography/mass spectrometry (GC/MS) is considered to be the gold standard for VOC analysis and can be used to analyze sweat as well as to distinguish genders. Thermal desorption combined with GC/MS techniques has been used to identify and quantify volatile compounds in exhaled breath, sweat, urine, and other biological excreted. For volatile emissions from human body, solid-phase microextraction (SPME)-GC/MS³⁹ has been used widely. Curran et al.³⁹ by using SPME-GC/MS concluded uniqueness in human scent through both qualitative and quantitative measurements and analysis from different humans sweat samples. GC Fourier transform-infrared spectra (GC/FT-IR)⁵⁵ has been also used for underarm sweat analysis. However, in field operations (security, forensic, search and rescue), human presence detection with portable analytical instrumentation through breath and skin VOC emissions is still limited.

To overcome limitations and certain portability issues of the existing analytical technology for field chemical analysis, membrane inlet mass spectrometry (MIMS)^{56,57} coupled to a portable mass spectrometer can be used for air and aqueous analysis and monitoring. MIMS offers high sensitivity (low ppt) and fast and accurate analysis with no sample preparation requirements and can be used for both simple and multi-component mixtures simultaneously.^{58–63} Also compared to other MS techniques (e.g., PTR-MS and SIFT-MS), MIMS offers lower size, weight, and cost. This study was developed to investigate the possibility of illegal human detection in border checkpoints (airports, sea ports, and land borders). This paper reports, for the first time, the use of a portable MIMS instrument (23 kg) for monitoring human chemical signatures in a container simulator.

■ EXPERIMENTAL SECTION

Concept. The basic concept of this work was the chemical detection of human presence in an enclosed space such as a trailer or a container after several hours of human confinement. This can correspond to the concealment of an illegal immigrant or other hidden personnel. A small room was used to simulate a container used in cargo services at airports, ports, and land borders. During experiments, environmental weather conditions (temperature, humidity, wind velocity) and the temperature inside the container simulator were recorded on a daily basis. The tests ran for over a month and were done for volunteers of both genders, all healthy and under the age of 30. Participation was developed under a voluntary basis and individuals agreed to follow instructions regarding their personal food diet and hygiene before sampling and during the period of the experiments. This was done in order to investigate detection of human chemical signatures under different conditions. During sampling, the participants were asked to follow a 6 h protocol in which their body scent was filling the container simulator. VOCs emitted from human sweat, skin, breath, and other biological excreted were being monitored during time with a MIMS probe coupled to a quadrupole mass spectrometer (QMS). All the sampling experiments were repeated three times to ensure reproducibility and consistency of the results.

Human Subjects. Two young, healthy volunteers (one male and one female) were recruited to participate in the experiments. Table 1 gives details regarding volunteers' phenotype. The diluted body scent of the participants in the container's air was monitored continuously throughout the

Table 1. Summary of Participants' Phenotype

ref no.	gender	age (year)	mass (kg)	height (cm)
1	male	25	75	177
2	female	29	70	168

scheduled experimental day in a systematic way. Spectra were recorded every 1 h using a MIMS instrument. For safety reasons another member of the research group checked the participant's condition every 2 h to ascertain any needs and well being and also to determine whether or not to carry on with the experimental procedure. In this case, the experiment could be paused or halted if any of the volunteers were dissatisfied for any reason. The study has been approved by the Ethics Subcommittee of the University of Liverpool (ref no. RETH000650).

Test Environment and Experimental Setup. The tests for monitoring human chemical signatures (HCSs) using a MIMS were developed and completed in a container simulator in the facilities of the University of Liverpool, U.K. The container simulator was a safe and isolated small room with dimensions of 3.37 m × 5.00 m × 2.50 m. The size of the container simulator was found to be similar to a standard container size.

Before the start of the experiments, the room was properly purified, ventilated, and sealed. All the exogenous sources of volatile emissions were eliminated and removed to other spaces in order to avoid background interferences during experiments. Mass spectra of the simulator's ambient air using the MIMS were taken systematically every 1 h during the 3 days prior to the start date of the experiments as well as one measurement every morning during the experimental process to ensure the absence of exogenous analytes. The temperature of the simulator was stable at 25 °C. Figure 1a shows a schematic diagram for the MIMS experimental setup was built specifically for the human detection tests. Figure 1b shows the experimental setup for the Liverpool MIMS HCS monitoring system. The monitoring results were recorded and analyzed on a laptop computer.

Sample Introduction. During tests, two different sample introduction techniques were used. The first technique was performed by a membrane probe manufactured by the University of Liverpool. The probe was connected directly to the vacuum valve, sampling the ambient air of the container simulator. It is schematically described in Figure 1a,i. The probe and membrane were heated at 70 °C through heat transfer from a 100 W aluminum housed resistor provided by TE Connectivity, Berwyn, PA. The probe was heated to allow volatile compounds to pass through the membrane material, and it was found that 70 °C was safest maximum heating temperature for the membrane to gain maximum sensitivity. The membrane surface temperature was monitored using a glass laboratory thermometer.

The second technique, described in Figure 1a,ii, used a fused silica capillary inlet connected with a membrane sampling probe. The capillary column used for transferring the gas samples into the QMS was a 2 m long fused silica, housed within a stainless steel and heat insulating outer cover, provided by European Spectrometry Systems Ltd., U.K. A heater unit for the capillary inlet was used to heat it to 110 °C. The front side end of the capillary inlet was connected directly with the QMS, whereas the back side end of the capillary was connected with a 1/4 in. Swagelok stainless steel tee ring coupling. A membrane

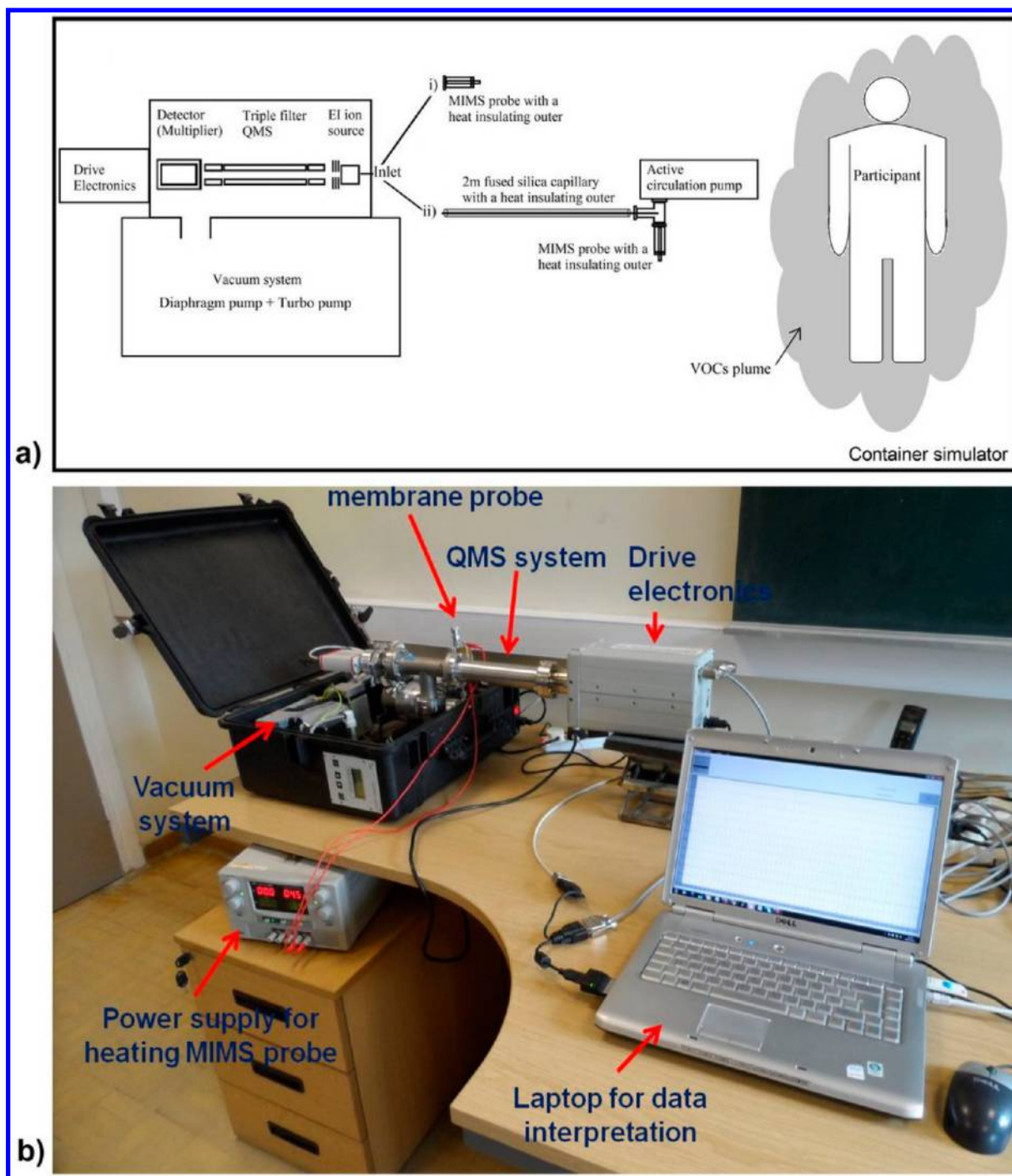


Figure 1. (a) Schematic diagram for the MIMS system used for human VOCs monitoring. Two different sample introduction techniques were used for detecting odorous emissions from human skin, breath, and sweat. The first technique includes direct sampling through a heated sampling membrane inlet, whereas the second technique uses a fused silica capillary inlet connected with a membrane probe for sampling. (b) The portable MIMS setup in the container simulator.

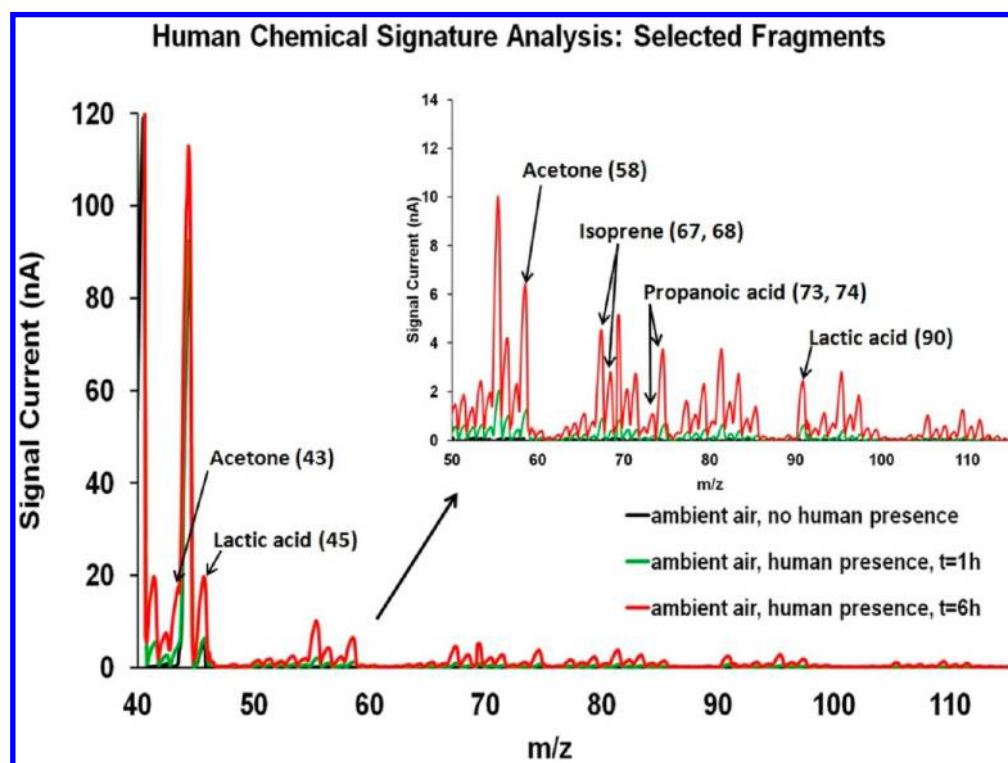
probe heated at 70 °C was attached to the one side of the tee ring coupling for sampling of simulator air. From the other side of the tee ring coupling, an active circulation pump for gases (Rietschle Thomas Ltd., U.K., model SMG4 24 V DC) was providing an air flow rate of 0.1–1.1 L/min. Both heating and airflow were used to achieve an intensive suction of the molecules from the membrane material.

The membrane probe assembly contained two thin (i.d. 0.40 mm, o.d. 1.60 mm) stainless steel tubes mounted into a thicker (i.d. 4.00 mm, o.d. 6.35 mm) stainless steel tube with a loop of

cross-linked membrane tubing made from polydimethylsiloxane (PDMS). The PDMS capillary membrane was provided by Helix Medical Inc., Carpinteria, CA. The total length of the stainless steel probe was 10 cm, whereas the PDMS membrane tubing was approximately 7 cm long with 0.55 mm wall thickness. A second, in-house developed membrane sampling probe was used additionally for the measurements, and it consists of stainless steel tubing coupled with a membrane sheet supported in the one end side with a $\frac{1}{4}$ in. Swagelok stainless steel vacuum fitting union. Table 2 shows all the

Table 2. Membranes Tested with MIMS Instrument to Evaluate Their Performance in Human Chemical Signatures Analysis and in Human Detection in a Confined Space

no.	membrane name	material	form	hydrophobicity/hydrophilicity	pore size (μm)	thickness (mm)
1	TF-200	PTFE	sheet	hydrophobic	0.2	0.139
2	Supor-100	PES	sheet	hydrophilic	0.1	0.1016
3	High Consistency Silicone Rubber	PDMS	sheet	hydrophobic	NA	0.3
4	Mitex membrane	PTFE	sheet	hydrophobic	10	0.13
5	Nylon membrane	nylon	sheet	hydrophilic	0.45	NA
6	Porelle microporous, 345	PU	sheet	hydrophobic	<1	0.045
7	SIL-TEC membrane sheeting	PDMS	sheet	hydrophobic	NA	0.12
8	Standard silicone tubing	PDMS	tubing	hydrophobic	NA	0.28

**Figure 2.** Mass spectra of the ambient air in the container simulator including no human presence and human presence for 1 and 6 h.

membranes (hydrophilic and hydrophobic) that were tested in order to examine and to achieve maximum VOCs detection with two types of sampling probes coupled to our portable MIMS instrument. Eight different membrane materials with different porosities and various membrane wall thicknesses were tested.

Mass Spectral Analysis. Mass spectral analysis of the ionized sample gas passing through different types of membranes was done using a triple filter quadrupole mass spectrometer (QMS) system supplied by Q-Technologies Ltd., U.K. The main components of the portable mass spectrometer are the electron impact (EI) ion source, the mass filter, and the detector. The enclosed EI ion source has dual Thorion filaments assembly at about 1.68 mA electron emission current. The mass analyzer contains a prefilter, a main filter, and a post filter and has a mass range of m/z 1–200 with a unit resolution over the entire mass range. The sensitivity of the quadrupole analyzer is 1×10^{-4} A/mbar. The detector comprises of a Faraday cup for detecting usual ion currents and a Channeltron type electron multiplier for detecting very low currents like those produced from low level concentration VOCs emitted from the human body. During data acquisition, 10 acquisition points were

recorded per unit mass with an average number of 20 scans per measurement throughout the whole mass range. In order to eliminate possible false-positives with interferences, peaks with relative abundance >3% above baseline were examined.

Vacuum System. The QMS was housed in a stainless steel chamber pumped by a vacuum system consisting of an Oerlikon DIVAC 0.8 LT diaphragm pump and a Pfeiffer Balzers turbomolecular pump. The diaphragm pump provides pressure down to 1×10^{-2} Torr, while the turbomolecular pump gives a base pressure of 1×10^{-7} Torr. The system pressure was continuously being monitored by a highly accurate digital pressure gauge supplied by Pfeiffer (MRT 100, DN 25 ISO-KF) that uses a Pirani/Cold cathode method of measurement. Operating pressure for mass analysis with an attached membrane sampling probe or heated GC column was 5×10^{-6} Torr.

RESULTS AND DISCUSSION

Human Chemical Signature Analysis. This experiment was done with a single volunteer to prove the principle of detection of human presence in a confined space such as a container similar to those used in cargo services. During the

tests, VOC emissions from human breath, sweat, and skin were present in the container simulator. In each test, the human VOC plume in the ambient air of the simulator was continuously monitored every hour for 6 h in total. Blank measurements of the container air were taken before the start date of the experiments as well as every experimental day to ensure the absence of exogenous compounds contamination. All the data were recorded and further analyzed using the NIST Chemistry WebBook as reference for spectral peaks of each compound.

Figure 2 shows a representative mass spectrum indicating human presence in the container simulator after 6 h of presence with the mass range m/z 40–115. The differences between no human presence and human presence in the confined space used for the experiments are clearly seen. Moreover, peak intensities after 1 and 6 h of human enclosure in the container simulator increase by a factor of 5. Key mass fragments detected during tests are shown in Table 3. CO₂ responses are

Table 3. Potential VOCs Emitted from Human Breath and Body in the Container Simulator with Their Characteristic Mass Fragments and Their Signal Intensity Changes during Time

potential human odor compounds detected			
no.	compound name	characteristic mass fragments (m/z)	intensity change
1	H ₂ O	17, 18	increased
2	NH ₃	16, 17	increased
3	CO ₂	44	increased
4	CO	28, 12	increased
5	methanol	29, 30, 31, 32	increased
6	O ₂	32, 16	decreased
7	acetaldehyde	29	increased
8	hexane	57, 86	increased
9	lactic acid	45, 90	increased
10	nonanal	57, 70, 98	increased
11	isoprene	53, 67, 68	increased
12	acetone	43, 58	increased
13	limonene	68, 121	increased
14	phenol	31, 45, 46, 94	increased
15	pentane	41, 42, 57, 72	increased
16	heptane	55, 56, 57, 70, 71, 85	increased
17	1-pentene	55, 70	increased
18	hexanal	56, 57, 58, 82	increased
19	isopropanol	27, 45, 59	increased
20	2-nonenal	55, 56, 57, 70, 83	increased
21	ethanol	27, 29, 30, 31, 45, 46	increased
22	propanoic acid	73, 74	increased

of particular concern indicating a characteristic inorganic gas of human detection in a confined space. Other organic compounds of interest are acetone (m/z 43, 58) and isoprene (m/z 67, 68), which are characteristic for human breath and skin emissions. Carboxylic acids like propanoic acid (m/z 73, 74) and lactic acid (m/z 45, 90) present in human sweat emanations were also detected.

Optimization Experiments. In order to achieve optimal sensitivity for human detection in a confined space, eight series of experimental measurements were carried out in total for a single volunteer. The first experimental set was performed to examine ambient air analysis of the container simulator directly from the vacuum valve (setup i). The second set was performed

with a heated GC column connected to the vacuum valve (setup ii). The third set examined the use of a capillary PDMS sampling probe coupled to the vacuum valve (setup iii). The vacuum valve was fully open with the operational pressure at 5×10^{-6} Torr. The fourth set was developed with a heated capillary PDMS sampling probe again directly connected to the vacuum valve (setup iv), while the fifth set used a heated capillary PDMS sampling probe coupled with a GC column as a transfer line (setup v). The probe was being heated as described above with a sampling flow rate of 1.1 L/min from a small differential pump. This was done to achieve an efficient suction of molecules from the membrane surface into the vacuum system and the MS. The sixth set involved ambient air analysis of the simulator with a sheet PDMS membrane probe connected to the MS vacuum valve (setup vi).

The seventh set examined the use of a heated sheet PDMS membrane probe (setup vii). Finally, the eighth set investigated the use of a heated sheet PDMS sampling probe coupled with a GC column, heated and with a sampling flow of 1.1 L/min (setup viii).

During the tests, oxygen levels were decreased over time as expected but remained sufficiently high for safe human life during the measurements. On the other hand, CO₂ levels slowly increased as expected. Organic compounds such as acetone, isoprene, and carboxylic acids (propanoic acid and lactic acid) were also detected and showed an upward trend. It can be seen from Figure 3 that a heated sampling probe with capillary PDMS membrane (setup iv) has the best performance for sensitivity compared to the remaining seven experimental sets. It was also found that heating affects membrane response time and that high suction flow rates (L/min) applied on the membrane materials give better results than low flow rates (mL/min).

Membrane Experiments. In order to choose the best membrane material for our experiments, a series of measurements with both hydrophilic and hydrophobic membranes were done. Membranes presented in Table 2 were tested. It has been observed that membrane nos. 7 and 8 of Table 2 have the best performance with high selectivity of volatile compounds and high sensitivity. They have fast response times in the detection of volatile compounds from the human body. Table 4 shows 90% response times for each membrane material. The 90% response time is the time required for the signal intensity to reach the 90% of its maximum value after the sample valve has been opened.⁶³

Human Gender Experiments. This section describes the difference in HCS profiles between different genders. Figure 4 presents simultaneously the differences in the chemical profiles between a male and a female volunteer in the container simulator after 6 h of enclosure. In both cases the following targeted VOCs were detected: acetone, isoprene, propanoic acid, and lactic acid. It is noticeable from the mass spectra in Figure 4 that the above compounds show greater levels of abundance for the male participant instead of the female. Characteristically the male volunteer appears to produce in the ambient air of the container simulator appreciable quantities of the above-described carboxylic acids than the female volunteer. This can be justified by the fact that men are more prone to sweating than women.⁶⁴ Moreover, skin surface plays an important role in sweat secretions. The selection of male volunteers for further study under different conditions was done because it has been found by Wagtail UK Ltd., a specialist sniffer dog company, that the percentage of gender distribution

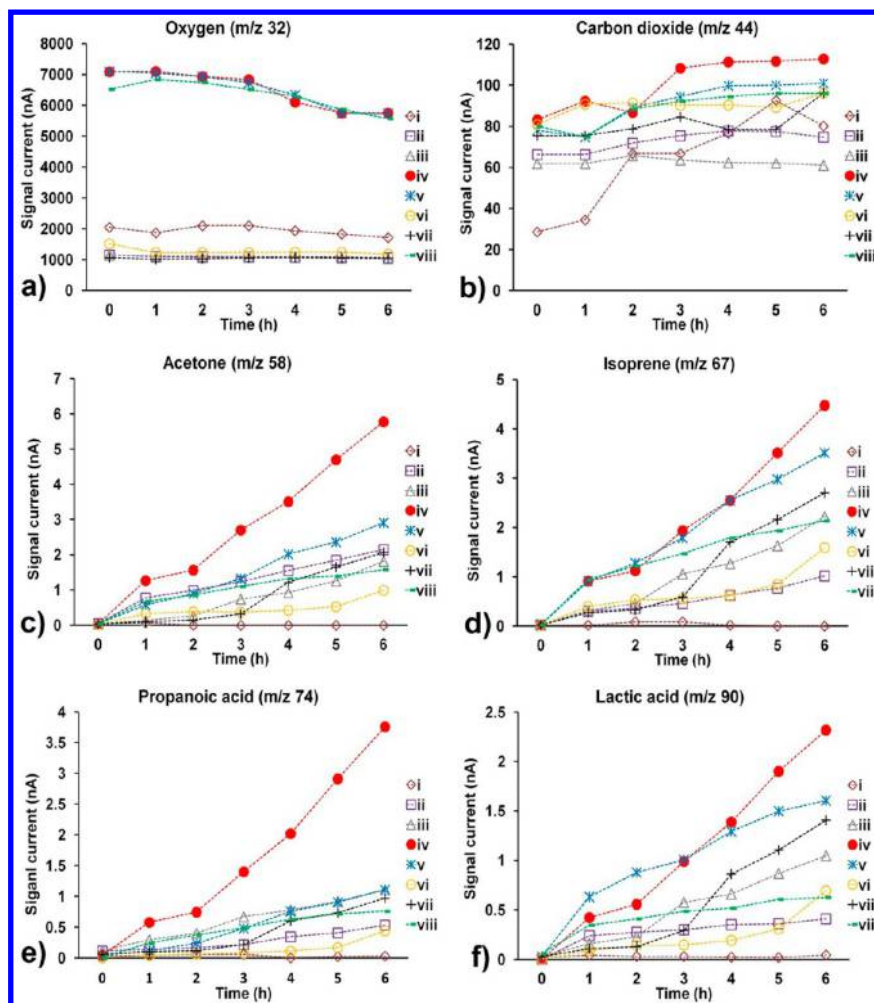


Figure 3. Signal intensity change for mass fragments of oxygen (m/z 32), carbon dioxide (m/z 44), acetone (m/z 58), isoprene (m/z 67), propanoic acid (m/z 74), and lactic acid (m/z 90) during 6 h of human presence in a container simulator using eight different experimental setups. Ambient air analysis using (i) vacuum valve, (ii) a GC column inlet, (iii) a capillary PDMS sampling probe, (iv) a heated capillary PDMS sampling probe, (v) a heated capillary PDMS sampling probe coupled to a heated GC column inlet, (vi) a sheet PDMS sampling probe, (vii) a heated sheet PDMS sampling probe, and (viii) a heated sheet PDMS sampling probe coupled to a heated GC column inlet.

Table 4. Membranes Response Times in the Detection of Human Chemical Signatures

no.	membrane name	90% response time (s)
1	TF-200	190
2	Supor-100	190
3	High consistency silicone rubber	120
4	Mitex membrane	90
5	Nylon membrane	170
6	Porelle microporous 345	120
7	SIL-TEC membrane sheeting	60
8	Standard silicone tubing	50

of immigrants who illegally pass through the borders of European Union countries is approximately 94% male and 6% female.⁶⁵

Concentration Experiments. In order to obtain an approximate estimation of the concentrations of acetone, isoprene, propanoic acid, and lactic acid of human scent in a small container, a series of concentration measurements were performed. Substance samples used for generating calibration curves were in the liquid phase with their concentrations set using a micropipet. All the chemicals that were used were of

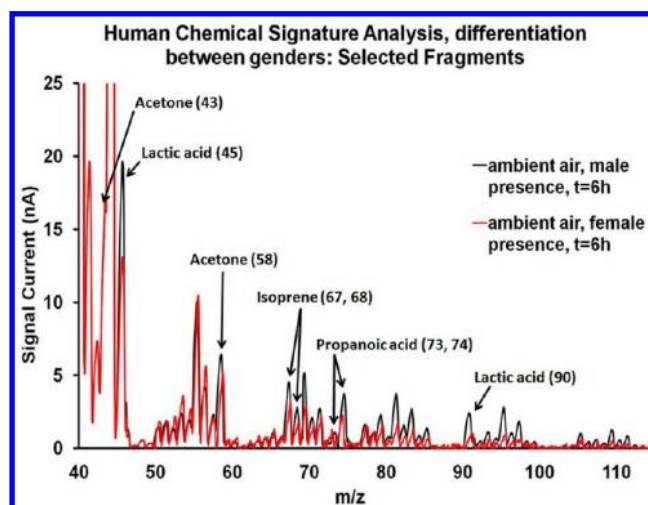


Figure 4. Representative mass spectra corresponding to the differences between male and female chemical signatures after 6 h of presence in the container simulator.

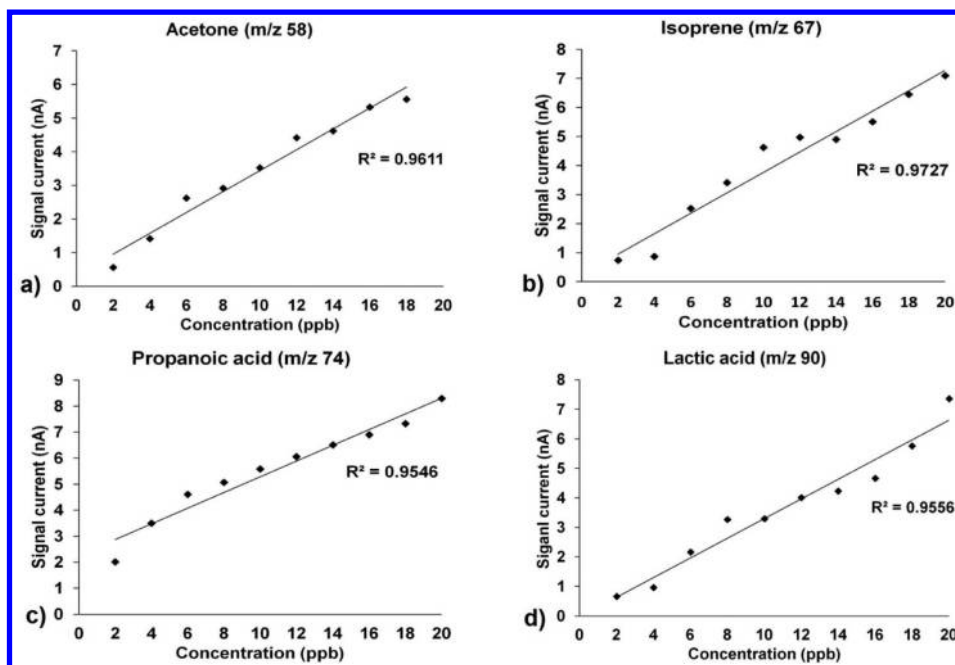


Figure 5. Calibration curves for acetone (m/z 58), isoprene (m/z 67), propanoic acid (m/z 74), and lactic acid (m/z 90) using our portable MIMS system.

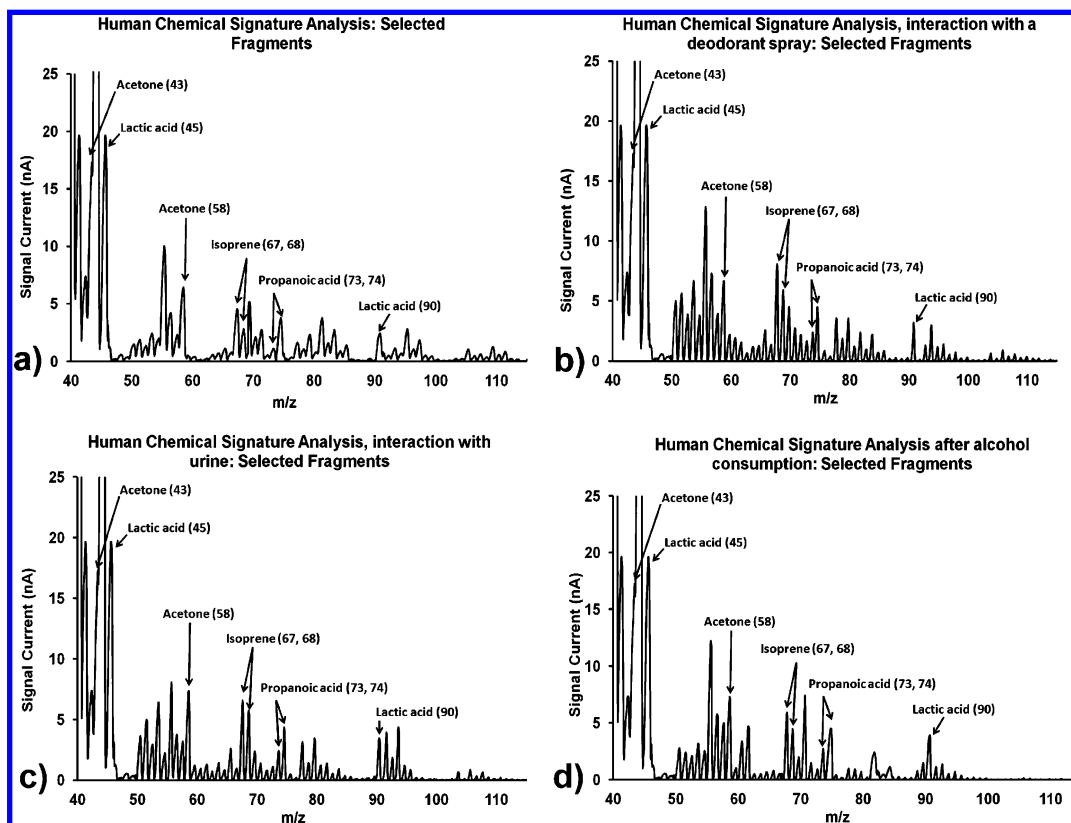


Figure 6. Mass spectra of the ambient air of the container simulator including human presence after 6 h of enclosure under four different experimental conditions: (a) ideal case for a single man-volunteer, who followed a personal hygiene and food protocol prior and during the tests without any external interferences, (b) human who has used a commercial deodorant spray in the axillary area of his body just before the start time of the experiment, (c) presence of human with urine sample, and (d) human after alcohol consumption.

high purity >99% and were obtained from Sigma Aldrich, St. Louis, MO.

Exact ppb concentrations were prepared by mixing small quantities of target substances with deionized water in a flask.

Deionized water was bought from ReAgent Chemical Services Ltd., Cheshire, U.K. A membrane probe was inserted into the liquid solution in the flask with the top cover of the flask isolated with a suitable tape to prevent evaporation. Flask was

put on a hot plate that was used to set the temperature of a substance solution and membrane probe to 70 °C as used during air sampling experiments. This was used to approximate conditions during air sampling, where the temperature of sample molecules in air becomes close to 70 °C when they approach the membrane. The substance mixture was maintained constant by using a steering rod inside the flask.

In order to achieve reliable concentration measurements, a suitable m/z with the highest linearity calibration curve was chosen for each targeted compound. For each individual substance concentration, 10 readings were taken. From these readings, the mean values were calculated for each concentration, which are the values specified in the Figure 5. Calibration curves of our MIMS instrument exhibited linearity with R^2 values in the range from 0.9546 to 0.9727 as shown in Figure 5. From the calibration curves, the following approximate concentrations were estimated for the targeted volatile compounds for a male volunteer in the container simulator after 6 h of enclosure: acetone at 18 ppb, isoprene at 11 ppb, and propanoic acid and lactic acids at around 6 ppb. For a female volunteer, approximate concentrations for acetone, isoprene, propanoic acid, and lactic acid were, respectively, 14 ppb, 7 ppb, 2 ppb, and 2 ppb.

Human Condition Experiments. In a real case scenario, the testing environment could potentially be a dirty container or a confined space with various influences from human or animal remains (urine, feces, vomit), food items, luggage, etc. Moreover, the hidden human may have used a deodorant in the axillary area, a perfume, or may have consumed a quantity of alcohol and food. In order to simulate a real situation of hidden human presence under different experimental conditions, a series of experiments involving (1) use of deodorant spray, (2) urine presence, and (3) alcohol consumption were done. During the different experimental conditions, the same volunteer was recruited. For the first series, the participant was asked to wear a generous quantity of a deodorant spray on the axillary area and over the body area prior the start of the measurements. Before the start time of the experiment, the participant was also asked not to follow any specific diet protocols or any special personal hygiene rules. During sampling, participant's body scent and the used deodorant were filling the container simulator while data from the container simulator's ambient air were recorded every 1 h of the total 6 h total duration of the experiment. Figure 6a shows an ideal mass spectrum of a human without interference of external conditions. Figure 6b shows a representative mass spectrum of a human wearing a deodorant, in the container simulator after 6 h of enclosure, with the mass range m/z 40–115. VOCs such as acetone, isoprene, propanoic acid, and lactic acid were detected at the following approximate concentrations: 20 ppb, 20 ppb, 10 ppb, and 8 ppb, respectively. The small increase of the concentration levels of the detected compounds can be justified by the presence of the deodorant peaks which interfered with human body odorous emissions. Figure 6c examines the second series of experiments, in which a male volunteer was asked to remain in the container simulator with urine present for 6 h. After 6 h, the targeted analytes were again detectable, with approximate concentrations over 20 ppb for acetone, 18 ppb for isoprene, 10 ppb for propanoic acid, and 9 ppb for lactic acid. Again a minor increase of the selected peaks can be explained by the presence of 200 mL of a urine sample in the container simulator. Figure 6d shows the third series of experiments that explores how chemical signatures

may differ or vary after alcohol consumption. In this case, the targeted analytes appear to have approximate concentrations over 20 ppb for acetone, 16 ppb for isoprene, 11 ppb for propanoic acid, and 12 ppb for lactic acid.

CONCLUSIONS

The possibility of hidden human detection in a confined space such as a container has been demonstrated using a membrane inlet mass spectrometer. During monitoring of human chemical signatures, a series of different experimental scenarios were investigated. Experiments took place for both genders in a container simulator under different experimental conditions and interferences. Eight different membranes were examined to test their response times and for achieving maximum and optimum human VOC detection.

Membrane heating and different sample suction flow rates were used for improving selectivity and sensitivity. It was found that a small decrease in O_2 levels and increases of the abundances of CO_2 , acetone, isoprene, propanoic acid, and lactic acid may be potential markers of human life in a container after several hours of physical presence. Preliminary data were presented while a further study involving more human subjects with variant phenotype characteristics (race, background origin, age, gender, habits, etc.), and additional instrumentation is required for more detailed explanations on human body scent. An algorithm of the profile of the detectable human scent compounds will have to be developed and clarified. Apart from security applications, this work is also highly relevant for search and rescue operations.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: Boris.Brkcic@liv.ac.uk.

*E-mail: S.Taylor@liv.ac.uk.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The research leading to these results has received funding from the European Community's Seventh Framework Programme managed by REA Research Executive Agency (FP7/2007-2013) under Grant Agreement No. 285045. We thank our project partners TWI Ltd, Aix-Marseille University, Da Vinci Laboratory Solutions, SAES Getters Group, Envisontec GbmH, Xaar and Wagtail UK Ltd. The author greatly thanks Tom Hogan from Pathway Systems (U.K.) for his technical assistance.

REFERENCES

- (1) U.S. Department of Homeland Security. www.dhs.gov.
- (2) Schoon, G. A. A.; De Bruin, J. C. *Forensic Sci. Int.* **1994**, *69*, 111–118.
- (3) Schoon, G. A. A. *J. Forensic Sci.* **1998**, *43*, 70–75.
- (4) Komar, D. J. *Forensic Sci.* **1999**, *44*, 405–408.
- (5) Lorenzo, N.; Wan, T.; Harper, R. J.; Hsu, Y. L.; Chow, M.; Rose, S.; Furton, K. G. *Anal. Bioanal. Chem.* **2003**, *376*, 1212–1224.
- (6) Callagher, M.; Wysocki, C. J.; Leyden, J. J.; Spielman, A. I.; Sun, X.; Preti, G. *Br. J. Dermatol.* **2008**, *159*, 780–791.
- (7) Curran, A. M.; Rabin, S. I.; Furton, K. G. *Forensic Sci. Commun.* **2005**, *7*, 1607–1619.
- (8) Statheropoulos, M.; Sianos, E.; Agapiou, A.; Georgiadou, A.; Pappa, A.; Tzamtzis, N.; Giotaki, H.; Papageorgiou, C.; Kolostoumbis, D. *J. Chromatogr., B* **2005**, *822*, 112–117.

- (9) Vautz, W.; Baumbach, J. I. *Int. J. Ion Mobility Spectrom.* **2008**, *11*, 35–41.
- (10) Vautz, W.; Nolte, J.; Fobbe, R.; Baumbach, J. I. *J. Breath Res.* **2009**, *3*, 1–8.
- (11) Vautz, W.; Nolte, J.; Bufe, A.; Baumbach, J. I.; Peters, M. *J. Appl. Physiol.* **2010**, *108*, 697–704.
- (12) Perl, T.; Carstens, E. T. H.; Hirn, A.; Quintel, M.; Vautz, W.; Nolte, J.; Jünger, M. *Br. J. Anaesth.* **2009**, *103*, 822–827.
- (13) Buszewski, B.; Keszy, M.; Ligor, T.; Amann, A. *Biomed. Chromatogr.* **2007**, *21*, 553–566.
- (14) Ackerl, K.; Atzumuller, A.; Grammer, K. *Neuroendocrinol. Lett.* **2001**, *23*, 79–84.
- (15) Penn, D.; Potts, W. K. *Trends Ecol. Evol.* **1998**, *13*, 391–396.
- (16) Singh, D.; Bronstad, P. M. *Proc. R. Soc. B* **2001**, *268*, 797–801.
- (17) Turner, C.; Spanel, P.; Smith, D. *Physiol. Meas.* **2006**, *27*, 321–337.
- (18) Turner, C.; Spanel, P.; Smith, D. *Physiol. Meas.* **2006**, *27*, 13–22.
- (19) Turner, C.; Spanel, P.; Smith, D. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 61–68.
- (20) Turner, C.; Spanel, P.; Smith, D. *Physiol. Meas.* **2006**, *27*, 637–648.
- (21) Taucher, J.; Hansel, A.; Jordan, A.; Lindinger, W. *J. Agric. Food Chem.* **1996**, *44*, 3778–3782.
- (22) Warneke, C.; Kuczynski, J.; Hansel, A.; Jordan, A.; Vogel, W.; Lindinger, W. *Int. J. Mass Spectrom. Ion Processes* **1996**, *154*, 61–70.
- (23) Taucher, J.; Lagg, A.; Hansel, A.; Vogel, W.; Lindinger, W. *Alcohol. Clin. Exp. Res.* **1995**, *19*, 1147–1150.
- (24) Hansel, A.; Jordan, A.; Holzinger, R.; Prazeller, P.; Vogel, W.; Lindinger, W. *Int. J. Mass Spectrom. Ion Processes* **1995**, *149*, 609–619.
- (25) Smith, D.; Spanel, P. *Rap. Comm. Mass Spec.* **1996**, *10*, 1183–1198.
- (26) Lin, Y.; Dueker, S. R.; Jones, A. D.; Ebeler, S. E.; Clifford, A. J. *Clin. Chem.* **1995**, *41*, 1028–1032.
- (27) Mendis, S.; Sobotka, P. A.; Euler, D. E. *Clin. Chem.* **1994**, *40*, 1485–1488.
- (28) Phillips, M.; Greenberg, J. J. *Chrom.* **1991**, *564*, 242–249.
- (29) Gelmont, D.; Stein, R. A.; Mead, J. F. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 1456–1460.
- (30) Conkle, J. P.; Camp, B. J.; Welch, B. E. *Arch. Environ. Health* **1975**, *30*, 290–295.
- (31) Grote, C.; Pawliszyn, J. *Anal. Chem.* **1997**, *69*, 587–596.
- (32) Jones, A. W.; Lagesson, V.; Tagesson, C. *J. Clin. Pathol.* **1995**, *48*, 979–980.
- (33) Huo, R.; Agapiou, A.; Bocos-Bintintan, V.; Brown, L. J.; Burns, C.; Creaser, C. S.; Devenport, N. A.; Gao-Lau, B.; Guallar-Hoyas, C.; Hildebrand, L.; Malkar, A.; Martin, H. J.; Moll, V. H.; Patel, P.; Ratiu, A.; Reynolds, J. C.; Sielemann, S.; Slodzynski, R.; Statheropoulos, M.; Turner, M. A.; Vautz, W.; Wright, V. E.; Thomas, C. L. *J. Breath Res.* **2011**, *5*, 1–12.
- (34) Callaghan, M.; Wysocki, C. J.; Leyden, J. J.; Spielman, A. I.; Sun, X.; Preti, G. *Br. J. Dermatol.* **2008**, *159*, 780–791.
- (35) Smallegange, R. C.; Verhulst, N. O.; Takken, W. *Trends Parasitol.* **2011**, *27*, 143–148.
- (36) Jackman, P. J. H.; Noble, W. C. *Clin. Exp. Dermatol.* **1983**, *8*, 259–268.
- (37) Jacoby, R. B.; Brahms, J. C.; Ansari, S. A.; Mattai, J. *Int. J. Cosmet. Sci.* **2004**, *26*, 37–46.
- (38) Kuhn, F.; Natsch, A. *J. R. Soc. Interface* **2009**, *6*, 377–392.
- (39) Curran, A. M.; Rabin, S. I.; Prada, P. A.; Furton, K. G. *J. Chem. Ecol.* **2005**, *31*, 1607–1619.
- (40) Kanda, F. E.; Yagi, M.; Fukuda, K.; Nakajima, T.; Nakata, O. *Br. J. Dermatol.* **1990**, *122*, 771–776.
- (41) Zeng, X.; Leyden, J. J.; Lawley, H. J.; Sawano, K.; Nohara, I.; Preti, G. *J. Chem. Ecol.* **1991**, *17*, 1469–1492.
- (42) Zeng, X.; Leyden, J. J.; Brand, J. G.; Spielman, A. I.; McGinley, K. J.; Preti, G. *J. Chem. Ecol.* **1992**, *18*, 1039–55.
- (43) Zeng, X.; Leyden, J. J.; Spielman, A. I.; Preti, G. *J. Chem. Ecol.* **1996**, *22*, 237–257.
- (44) Munk, S.; Munch, P.; Stahnke, L.; Adler-Nissen, J.; Schieberle, P. *J. Surfact. Deterg.* **2000**, *3*, 505–515.
- (45) Haze, S.; Gozu, Y.; Nakamura, S.; Kohno, Y.; Sawano, K.; Ohta, H.; Yamazaki, K. *J. Invest. Dermatol.* **2001**, *116*, 520–524.
- (46) Ostrovskaya, A.; Landa, P. A.; Sokolinsky, M.; Rosalia, A. D.; Maes, D. *J. Cosmet. Sci.* **2001**, *53*, 147–148.
- (47) Bemnier, U. R.; Kline, D. L.; Barnard, D. R.; Schreck, C. E.; Yost, R. A. *Anal. Chem.* **2000**, *72*, 747–756.
- (48) Amman, A.; Poupart, G.; Tesler, S.; Ledochowski, M.; Schmid, A.; Mechtcheriacov, S. *Int. J. Mass Spectrom.* **2004**, *239*, 227–233.
- (49) Smith, D.; Spanel, P.; Davies, S. *J. Appl. Physiol.* **1999**, *87*, 1584–1588.
- (50) Marquez-Sillero, I.; Aguilera-Herrador, E.; Cardenas, S.; Valcarcel. *Trends Anal. Chem.* **2011**, *30*, 677–690.
- (51) Armenta, S.; Alcalá, M.; Blanco, M. *Anal. Chim. Acta* **2011**, *703*, 114–123.
- (52) Natale, C. D.; Macagnano, A.; Paolesse, R.; Tarizzo, E.; Mantini, A.; D'Amico, A. *Sens. Actuators, B* **2000**, *65*, 216–219.
- (53) Wang, C.; Sahay, P. *Sensors* **2009**, *9*, 8230–8262.
- (54) Wojtas, J.; Bielecki, Z.; Stacewicz, T.; Micolajczyk, J.; Nowakowski, M. *Opto-Electron. Rev.* **2012**, *20*, 26–39.
- (55) Zeng, X.; Leyden, J. J.; Lawley, H. J.; Sawano, K.; Nohara, I.; Preti, G. *J. Chem. Ecol.* **1991**, *17*, 1469–1492.
- (56) Kotiahho, T.; Lauritsen, F. R.; Choudhury, T. K.; Cooks, R. G.; Tsao, G. T. *Anal. Chem.* **1991**, *63*, 875A–883A.
- (57) Johnson, R. C.; Cooks, R. G.; Allen, T. M.; Cisper, M. E.; Hemberger, P. H. *Mass Spectrom. Rev.* **2000**, *19*, 1–37.
- (58) Mendes, M. A.; Pimpim, R. S.; Kotiahho, T.; Eberlin, M. N. *Anal. Chem.* **1996**, *68*, 3502–3506.
- (59) Riter, L. S.; Laughlin, B. C.; Nikolaev, E.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 2370–2373.
- (60) Ketola, R. A.; Kotiahho, T.; Cisper, M. E.; Allen, T. M. *J. Mass Spectrom.* **2002**, *37*, 457–476.
- (61) Cotte-Rodriguez, I.; Handberg, E.; Noll, R. J.; Kilgour, D. P. A.; Cooks, R. G. *Analyst* **2005**, *130*, 679–686.
- (62) Thompson, A. J.; Creba, A. S.; Ferguson, R. M.; Krogh, E. T.; Gill, C. G. *Rapid Commun. Mass Spectrom.* **2006**, *13*, 2000–2008.
- (63) Boscaini, E.; Alexander, M. L.; Prazeller, P.; Mark, T. D. *Int. J. Mass Spectrom.* **2004**, *239*, 179–186.
- (64) The International Dermal Institute. <http://www.dermalinstitute.com/us/>.
- (65) Collin Singer, Wagtail UK Ltd., private communication.