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Advancing the analytical toolkit in the investigation of vector mosquito host biting site selection

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Abstract

High-resolution mass spectrometry and ion mobility spectrometry provide additional confidence in biological marker discovery and elucidation by adding additional peak capacity through physiochemical separation orthogonal to chromatography. Sophisticated analytical techniques have proved valuable in the identification of human skin surface chemicals used by vector mosquitoes to find their human host. Polydimethylsiloxane (PDMS) was used as a non-invasive passive wearable sampler to concentrate skin surface non-volatile and semi-volatile compounds prior to solvent desorption directly in an LC vial, thereby simplifying the link between extraction and analysis. Ultra-performance liquid chromatography with ion mobility spectrometry coupled with high-resolution mass spectrometry (UPLC-IMS-HRMS) was used for compound separation and detection. A comparison of the skin chemical profiles between the ankle and wrist skin surface region sampled over a 5-day period for a human volunteer was done. Twenty-three biomarkers were tentatively identified with the aid of a collision cross-section (CCS) prediction tool, seven associated with the ankle skin surface region and 16 closely associated with the wrist skin surface. Ten amino acids were detected and unequivocally identified on the human skin surface for the first time. Furthermore, 22 previously unreported skin surface compounds were tentatively identified on the human skin surface using accurate mass, CCS values and fragmentation patterns. Method limits of detection for the passive skin sampling method ranged from 8.7 (sulfadimethoxine) to 95 ng (taurine). This approach enabled the detection and identification of as-yet unknown human skin surface compounds and provided corresponding CCS values.

KEYWORDS
collision cross-section prediction, human surface skin compounds, ion mobility, non-invasive sampling, UPLC-MS, wearable PDMS sampler

1 | INTRODUCTION

The identification of human skin surface chemicals has proven valuable in the development of novel malaria vector mosquito lures.[1–5] Vector mosquitoes use different behavioural cues such as visual or chemical stimulants during their host-seeking activities. Skin volatiles play an important part in host-seeking and -preference for those mosquito species that specialise in a particular host.[5] Sophisticated

analytical techniques assist in structure elucidation to identify both specific semiochemicals (chemical messengers) and potential semiochemical blends. Gas chromatography coupled with mass spectrometry (GC–MS) is widely used to identify volatile and semi-volatile semiochemicals and has been employed to compare chemical profiles for mosquito attractiveness between individuals.[6,7] Various studies have focused on long-range mosquito semiochemicals, such as carbon dioxide, which evokes attraction from a distance and acts as an activator initiating a flight response.[1,8–10] Volatile organic compounds (VOCs), for example, ammonia, (*S*)-lactic acid and tetradecanoic acid,[3,4] and semi-VOCs are also used by vector mosquitoes during navigation, using odour plumes, towards the host and close range navigation near the host.[11] However, the skin volatiles geraniol and eucalyptol have recently been noted for their repellent properties.[12,13] The last steps in the vector mosquito host-seeking activity involve landing on a suitable feeding area and finally host acceptance, that is, feeding.[11] The aforementioned investigations all involve volatile or semi-volatile compounds. To date, there are no studies that have explored the chemicals, that is, non-volatile compounds, involved in the final steps during vector mosquito host-seeking activities. Landing of a vector mosquito on a human host does not always lead to host acceptance, that is, biting, and could potentially be influenced by such skin surface chemicals.

Studies involved in the detection and identification of skin surface VOCs and semi-VOCs are most commonly done with gas chromatography coupled to mass spectrometry (GC–MS).[14] The sampling and extraction methods for skin surface sampling vary from passive type sampling, such as solid-phase microextraction (SPME),[15] sorptive polydimethylsiloxane (PDMS) loops in the form of bracelets and anklets with thermal desorption into a GC[7,16] and solvent back extraction of cotton pads,[17] or glass beads[18] used to adsorb skin volatiles onto their surfaces, to active sampling types such as dynamic headspace adsorption onto various polymers,[15] air entrainment[19] and the body chamber.[20] Active sampling techniques are generally more invasive and cause discomfort to the volunteer.[20] Studies employing LC–MS for human skin surface sampling remain limited with mainly human sweat being analysed. These studies employed an electrical current to induce sweat production followed by time-consuming micro spin solid-phase extraction (μSPE).[21,22] The range of skin surface compounds is broad,[14] and consequently, it is important to use sampling and analytical techniques that would allow for the detection of various chemical classes. Furthermore, analytical sensitivity is essential when identifying compounds from complex biological matrices. Data-independent (DIA) high-resolution mass spectrometry (HRMS) offers the most pragmatic solution to compound screening; however, sample complexity and the ability to identify between isomeric species are problematic. The addition of travelling wave ion mobility spectrometry (TWIMS), which entails gas-phase separation of ions in an electrostatic inert buffer field, has shown great promise to increase selectivity and improve the overall peak capacity when analysing complex matrices.[23,24] Coupling of TWIMS with HRMS improves spectral quality in data-independent acquisition (DIA), provides the potential to separate isomeric species, enhances product-precursor alignment using drift time matching and adds an additional feature for compound identification, using collision cross-section (CCS) values.[24] It is thus of great value in marker discovery. The potential of using LC-IMS-HRMS in metabolic fingerprinting of complex wine samples was demonstrated by Causon et al. using a generic ion mobility separation workflow for non-targeted metabolomics. The authors used retention times, accurate mass and CCS information for feature alignment during statistical multivariate assessments and for putative identification of non-target metabolites.[23]

African vector mosquitoes prefer biting the lower parts of a standing human body.[9,25,26] Such preference called for another angle to investigate mosquito attraction or detraction to humans. Non-volatile contact compounds may not deter mosquitoes from landing, but rather, direct contact with these compounds on the surface of the skin could prevent the mosquito from biting the host. During this study, human skin surface regions, namely, ankle and wrist, from one volunteer were sampled daily over a 5-day period, using a passive non-invasive wearable PDMS sampler with solvent desorption and ultra-performance liquid chromatography with ion mobility spectrometry coupled to high-resolution mass spectrometry (UPLC-IMS-HRMS). The difference in chemical profiles between the different skin regions was explored with the aim to identify potential lead components as mosquito contact surface attractants or repellents. Chemometric techniques, using appropriate software tools, allowed for marker alignment and discovery with the aid of chromatographic retention time, accurate mass and CCS values. CCS values provide additional confidence in compound identification and reported CCS values can be useful in future skin surface metabolomic studies. The effect of solvent modification of the PDMS sampler for improved extraction of especially the polar compounds and increased sampler sensitivity was also investigated to broaden the range of detectable chemical compounds.

2 | MATERIALS AND METHODS

2.1 | Reagents and chemicals

Deionised water, acetone and methanol (MeOH) for conditioning the samplers were purchased from Merck, South Africa. Propan-2-ol (super purity solvent) was purchased from Romil-SpS™ (Waterbeach, Cambridge, United Kingdom). Ultra-purity water, acetonitrile (ACN) and MeOH were purchased from Romil (Romil-UpS™, Waterbeach, Cambridge, United Kingdom). LC–MS grade eluant mix AF2 (ACN with 0.1% formic acid) and eluant mix WF2 (water with 0.1% formic acid; Romil-UpS™, Waterbeach, Cambridge, United Kingdom) were used for the chromatographic mobile phases. A 0.5-μmole/mL amino acid standard solution in 0.2 N lithium citrate, pH 2, with 0.1% phenol and 2% thiodiglycol and sulfadimethoxine (analytical standard, purity 98.5%) were purchased from Sigma-Aldrich (Pty) Ltd. Kempton Park, South Africa. A caffeine solution (1.0 mg/mL in MeOH) was purchased from Fluka® Analytical, Sigma-Aldrich, South Africa. L-carnitine, taurine, L-tyrosine, L-phenylalanine (Holistix) and L-lysine (Dis-Chem Gold) were obtained from Dischem (Pretoria, South Africa).

2.2 | Standard solutions

A 100-ng/μL stock solution of sulfadimethoxine was prepared and a final working standard solution containing a mixture of the target analytes was prepared at 1 ng/μL in ACN:isopropanol (1:1, v/v). Details on the preparation of standard solutions can be found in the Supporting Information (S1).

2.3 | Sampling apparatus

A non-invasive passive approach was applied to sample the human skin surface. An in-house developed PDMS sampler provided sorptive extraction of organic compounds from the skin surface. The sampler was employed by Roodt et al. and Wooding et al. as a sorptive passive sampler for the absorption of skin surface VOCs and semi-VOCs in the investigation of malaria vector control applications.[7,16,27] The samplers (0.060 ± 0.003 g) were made by forming a loop with an 18-cm length of a silicone elastomer medical grade tubing (0.64 mm OD × 0.3 mm ID, Sil-Tec®, Technical Products, Georgia, USA). The sampler was formed into anklets or bracelets by joining the ends of the tubing with a 1-cm piece of uncoated silica capillary column (250 μm ID) (SGE Analytical Science, Separation Scientific (Pty) Ltd, Roodepoort, South Africa).[28] The sorption volume of the loop was 43.87 μL, and the internal volume was 13.33 μL. The PDMS samplers were cleaned and conditioned using the method as described by Triñanes et al. for cleaning silicone sampling disks.[29]

2.4 | Human skin surface sampling

The wearable PDMS samplers were modified with isopropanol to improve the recoveries of polar (log K_{ow} < 2.5) compounds.[30] The PDMS sampler was modified specifically with isopropanol, a nontoxic solvent, to comply with ethical considerations during human sampling. The sampler was opened and sonicated in 12 mL of isopropanol, whereafter the filled samplers were fashioned back into a loop. The skin sampling area on the human volunteer was wiped clean with medical-grade alcohol cleansing pads (70% isopropanol, Dischem, South Africa) prior to sampling. Skin organic compounds collected from a non-smoking, Caucasian female, age 31, were concentrated into the PDMS sorptive sampler. The volunteer's right wrist and ankle were sampled daily at the same time on five consecutive days, using three loops per sampling period of 1 h (sampling time previously optimised[16]). The samplers were worn as anklets ($n = 3$) and bracelets ($n = 3$) by the volunteer. The sampler was placed in direct contact with the skin, using a tweezer, for easy sampling and reduced invasiveness.[7,16] The sampler was covered with aluminised Mylar® (Hydroponic, South Africa) reflective sheeting (20 cm × 1.5 cm and 25 cm × 1.5 cm for wrist and ankle skin surface regions, respectively) to aid in concentrating the compounds into the sampler and to reduce background compounds during sampling. Details on the sampling procedure can be found in the Supplementary Information (S1). In order to observe compounds on the human skin surface as would normally feature in a real-life situation, the volunteer was asked to continue with her daily routine during the sampling. No effort was made to control the environmental parameters during the sampling.

After the 1-h sampling period, the sampling loops were removed from the skin with a clean stainless-steel tweezer, followed by the opening of the sampler by detaching the uncoated capillary from one end of the loop to ensure complete immersion of the sampler in the extracting solvent and placing each sampler individually in 200-μL glass conical tip inserts (Figure 1) (Macherey-Nagel GmbH & Co, Separations, South Africa). The glass inserts were put into 1.5-mL glass screw neck LC vials (Macherey-Nagel GmbH & Co, Separations, South Africa) filled with 1 mL of deionised water to enhance energy transfer during the extraction process.[31] Solvent desorption was done using the method as outlined by Margoum et al.[32] The procedure entails the addition of 200 μL of ultra-purity MeOH:ACN (1:1, v/v) to each glass insert containing the individual samplers. The LC vials were then capped with PTFE pre-slit screw caps (Macherey-Nagel GmbH & Co, Separations, South Africa) and sonicated for 15 min at room temperature. The LC vials were uncapped, and the samplers were removed with a clean stainless-steel tweezer (Figure 1). The LC vials were recapped and transferred to the sample manager, at 4°C, of an LC system for LC–MS analysis.

2.5 | Ethical considerations

An information leaflet was provided to the volunteer, explaining the study design, risks and outcomes. Written informed consent was given by the volunteer to participate in the study. No dietary or special hygiene requirements were made. Ethical clearance was provided by the ethics committee of the Faculty of Natural and Agricultural Sciences at the University of Pretoria, South Africa (Reference number EC171109-159).

2.6 | Quality control protocols

Three method blanks were analysed to account for any laboratory background compounds. An instrument blank was analysed prior to the LC analysis. All samplers were kept in either the autosampler or fridge at 4°C awaiting analysis. A pooled sample was prepared by combining 10 μL from each extract to be analysed as a single sample. All samples were analysed in a randomised order and were interspaced with injections of a QC standard and the pooled sample. The QC sample was measured after every 12th injection.

2.7 | Evaluation of solvent modification of sampler

The extraction efficiency of polar compounds from the skin was evaluated by spiking analytes on the sampler with and without prior loading with isopropanol. The PDMS sampler with solvent modification

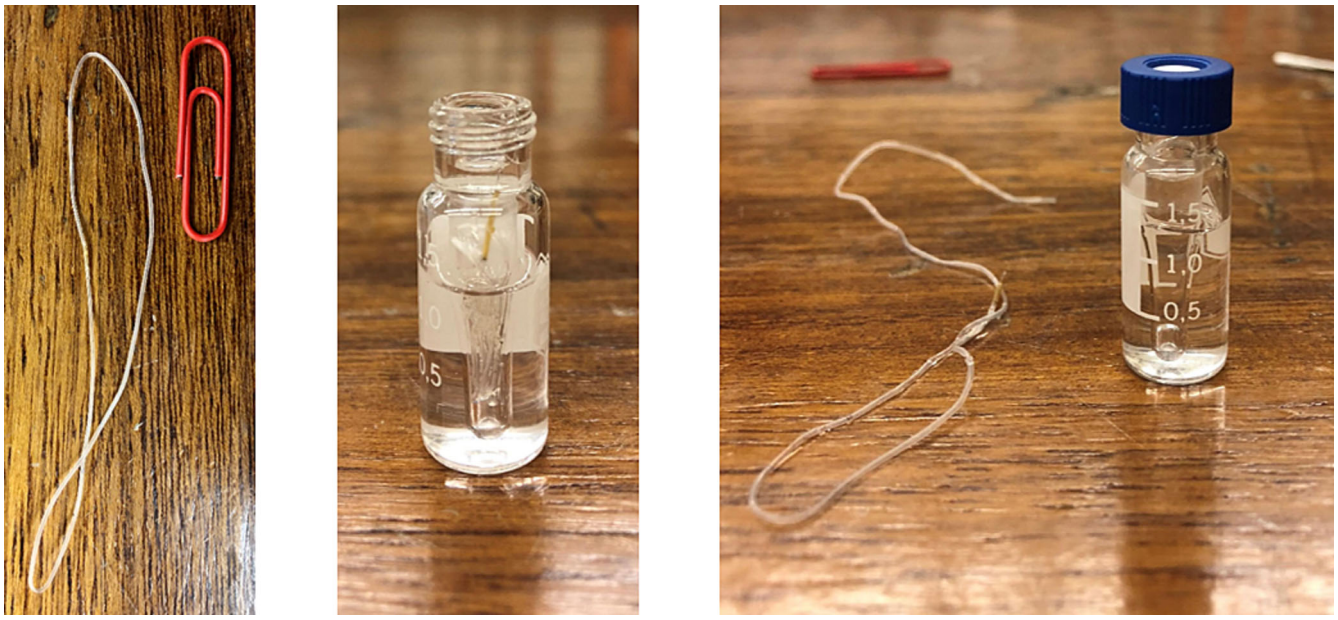

FIGURE 1 The PDMS sampler before extraction (left). The opened PDMS sampler was placed in a 200-µL glass insert in a 1.5-mL LC vial for solvent desorption with 200 µL of MeOH:ACN (1:1, v/v). Water was added between the insert and vial to enhance energy transfer during sonication (middle). The sampler was removed after sonication and the extract immediately placed in the autosampler of an LC for analysis (right).

was placed on top of a Mylar® reflective sheet (25 cm × 3 cm) pre-cleaned with a medical-grade alcohol cleansing pad. The 1-ng/µL working standard mixture solution was spiked onto the Mylar® sheet, next to but not touching the sampler, at three different volumes (100, 200, 400 µL). This resulted in a spiking mass of 100, 200 and 400 ng of each analyte. Spiking was done in triplicate for each of the two methods at the three concentration levels. The Mylar® sheet was folded and closed into a parcel using 3M dressing tape and placed in a 100-mL Schott bottle suspended in a water bath at 31°C, simulating human skin temperature.[33] After 1 h the sampler was removed from the Mylar® package and solvent desorbed. The solvent extraction was done in 200 µL of MeOH:ACN (1:1) resulting in analysis concentrations for the target analytes of 0.5, 1 and 2 ng/µL. Of the reconstituted extract, 5 µL was injected onto the LC column giving a final on-column spike of 2.5, 5 and 10 ng of each analyte. The procedure was repeated for the PDMS sampler not modified with isopropanol by omitting the additional step of solvent modification prior to spiking with the target analytes. The three method blanks were prepared using the procedure excluding the step of spiking the target analytes on the sampler.

2.8 | Instrumentation

Compound separation and detection were performed using a Waters® Synapt G2 high-definition mass spectrometry (HDMS) system (Waters Inc., Milford, Massachusetts, USA). Instrumental details can be found in the Supplementary Information (S1). The source conditions were as follows: the capillary voltage for ESI was 3.0 and 2.4 kV for positive and negative ionisation modes, respectively. The source temperature was set at 120°C, the sampling cone voltage at 40 V, the extraction cone voltage at 4.0 V and the cone gas (nitrogen) flow at 10.0 L/h. The desolvation temperature was set at 400°C with a gas (nitrogen) flow of 600.0 L/h. Mass spectral scans were collected every 0.1 s. The raw data were collected in the form of a continuous profile. Mass range was set between 50 and 1200 *m/z*. DIA was acquired using two alternating acquisition functions with low and high collision energy with ion mobility enabled (HDMS^E approach). Tandem MS (HDMS^E) fragmentation was performed using high-energy collision-induced dissociation (CID) with argon gas. Transfer collision energy was set to 6 V for the low energy function and the ramp transfer collision energy was set from 15 to 45 V for the high energy function.

Nitrogen was used as drift gas with a flow rate of 90 mL/min for ion mobility spectrometry (IMS). A helium flow of 180 mL/min was used in the helium cell. The mobility t-Wave was operated at variable IMS wave velocity; the wave velocity was set at 650 m/s with a wave velocity ramp of 1000 to 300 m/s. The IMS wave height was set to 40.0 V. The transfer wave velocity was set to 224 m/s (optimised to prevent pusher phasing) and transfer wave height to 4.0 V. The trap DC bias and helium cell DC voltages were set to 45.0 and 35.0 V, respectively. A mobility separation delay was employed with an IMS wave delay of 1000 µs. The IMS was calibrated using Waters Major Mix Calibration Sample with Driftscope (version 2.8) to determine experimental CCS values (Ω). A CCS error of <3.7% (0.66 ± 0.8%) was obtained.

2.9 | Chromatographic conditions

The injection volume was 5 µL, and the autosampler was kept constant at 4°C. Separation was completed using a reverse phase step gradient H_2O with 0.1% formic acid in mobile phase A and acetonitrile

with 0.1% formic acid in mobile phase B. The gradient started with an isocratic hold of 0.1 min at 3% B followed by a linear increase to 100% B to 14.0 min; subsequently, the column was washed for 2 min, mobile phase transitioned to starting conditions over 0.5 min followed by reconditioning and re-establishing of initial conditions. The column temperature was kept constant at 40°C, and the flow rate was set at 0.4 mL/min for the entire run giving a total run time of 20 min. The positive and negative ESI mass spectra were collected in separate chromatographic runs (employing the same separation conditions and columns). Analytical columns used included a Waters UPLC® C_{18} Ethylene Bridged Hybrid (BEH) 1.7-µm particle size (2.1 mm ID × 100 mm length) column and two Phenomenex columns, namely, a Kinetex® 1.7 µm Biphenyl (2.1 mm ID × 150 mm length) column and a Luna Omega 1.6 µm Polar C_{18} (2.1 mm ID × 100 mm length) column (Separations, South Africa).

2.10 | Data processing and statistical analysis

Method performance was evaluated by determining precision (% relative standard deviation [%RSD]), limit of detection (LOD) and limit of quantification (LOQ) using QuanLynx Method Editor V4.1. The retention time window criterion was set at 0.5 min, and the mass window criterion was set at 0.5 Da. LODs and LOQs were calculated as those amounts giving a signal-to-noise ratio (S/N) of 3 and 10, respectively. All further data processing was performed using UNIFI® Scientific Information System (Waters Inc., Milford, Massachusetts, USA). The retention time (RT) tolerance was set to ±0.1 min, target mass tolerance to ±10.0 ppm, fragmentation match tolerance to ±10.0 mDa and the CCS tolerance to 5.0% for library matching. All data were lock mass corrected with UNIFI® before processing.

Features were generated in UNIFI® using the Marker Matrix software function. Features were exported into a .csv format and imported into EZinfo (version 2.0.0.0) for statistical analysis and biological marker discovery. Compounds contributing to the differences between the two surface regions sampled were tentatively identified using accurate mass, isotope fit values and fragmentation patterns, by comparison with online ChemSpider databases and the Human Metabolome Database (HMDB) (version 3.6). Tentative identification of additional skin surface compounds, utilising UNIFI®, was made by accurate mass, isotope fit values, CCS values and fragmentation patterns, by comparison with the Waters® Metabolic Profiling CCS ESI +/ESI− Libraries. Additional information on data processing can be found in the Supplementary Information (S1).

2.11 | Collision cross-section confirmation using machine learning

Further confirmation of identified molecules was cross-validated using a β-version of a CCS prediction tool created by Waters®. This prediction tool uses molecular files to predict CCS values based on physiochemical properties. $^{TW}CCS_{N2}$ predictions were calculated using a model trained through machine learning similar to the method used by Zhou et al.[34] The model was trained with acquired $^{TW}CCS_{N2}$ data of many known molecules to fit an appropriate model. The building of the model is described in a poster by Bouwmeester et al.[35]

3 | RESULTS AND DISCUSSION

3.1 | Evaluation of the solvent-modified PDMS sampler

Seven analytes, namely, sulfadimethoxine, caffeine, L-phenylalanine, L-tyrosine, taurine, L-lysine and L-carnitine, from highly polar to the mid-polar range (log K_{ow} −5.48 to 1.63) were selected to investigate the impact of PDMS solvent modification on extraction efficiency. Amino acids were selected as these have previously been found in human sweat samples[21,22]; caffeine and sulfadimethoxine were selected for their ionisation efficiency using ESI. The highly polar amino acids proved challenging to separate using reverse-phase chromatography. Consequently, different columns were investigated to improve the separation of these compounds, namely, L-lysine, L-phenylalanine, L-tyrosine and L-carnitine (Table 1). All the investigated amino acids, namely, L-lysine (RT: 0.56 min), L-tyrosine (RT: 0.69 min), L-phenylalanine (RT: 0.71 min), taurine (RT: 0.59 min) and L-carnitine (RT: 0.61 min), were detected individually (ESI+); however, when a mixture (10 ng/µL) of the amino acids was analysed, severe matrix effects, that is, ion suppression, occurred. In the mixture, only L-carnitine and L-phenylalanine were detected when using a Waters UPLC® C_{18} BEH column with ESI+ mode. Band broadening and poor retention of the very polar analyte, L-carnitine, compounded the ion suppression. The different polar columns investigated did not improve the separation of the amino acids investigated, namely, L-lysine, L-phenylalanine, L-tyrosine and L-carnitine. Adversely, the biphenyl and polar C_{18} columns increased the ion suppression resulting in only L-carnitine being detected (Table 1). Chromatographic conditions were not investigated further as a generic method allowed for the detection of a broader range of compounds potentially present on the human skin surface. It was consequently decided to use L-carnitine, as it showed a good response with ESI+ mode, L-phenylalanine, taurine (using ESI− mode as L-carnitine did not ionise in ESI− mode thus ion suppression will not be problematic in ESI− mode), caffeine and sulfadimethoxine with the BEH C_{18} column (Figure S1) to further investigate solvent modification of the PDMS sampler.

Of note is a phenomenon that some research groups termed a 'bat-o-gram' when analysing L-phenylalanine (10 ng/µL solution with a 5 µL injection on column) (Figure 2).[36] The two peaks result from the different ionised states of the compound. The compound eluting at the shorter retention time, RT: 0.71 min, will be in a more ionised state than that of the same compound at RT: 1.50. Generally, the conversion between the two states is very fast resulting in a single peak; however, as was noted for various biological molecules, long

TABLE 1 Log K_{ow} and quantification ion with corresponding adduct (*m/z*) for the selected analytes at 10 ng/μL.

Analyte	Log K_{ow}	Quantification ion *m/z* [adduct]	RT (min)		
			C_{18}	Biphenyl	Polar C_{18}
Sulfadimethoxine	1.63	311.0815 $[M + H]^+$	4.81	n/a	n/a
Caffeine	−0.07	195.0884 $[M + H]^+$	2.51	n/a	n/a
L-phenylalanine	−1.18	120.0822 $[M\text{-}COOH]^+$	0.71	n.d.	n.d.
L-tyrosine	−1.49	136.0776 $[M\text{-}COOH]^+$	n.d.	n.d.	n.d.
Taurine	−2.61	124.0089 $[M\text{-}H]^-$	0.59	n/a	n/a
L-lysine	−3.21	130.0874 $[M\text{-}COOH]^+$	n.d.	n.d.	n.d.
L-carnitine	−5.48	162.1138 $[M + H]^+$	0.61	0.91	0.71

Note: The retention times (RT) using three different columns, a Waters UPLC® C_{18} BEH column, a Kinetex® Biphenyl column and a Luna Omega Polar C_{18}, are provided for the analytes investigated. Abbreviations: *m/z*, mass-to-charge ratio; n/a, not applicable; n.d., not detected due to matrix effects (ion suppression); RT, retention time.

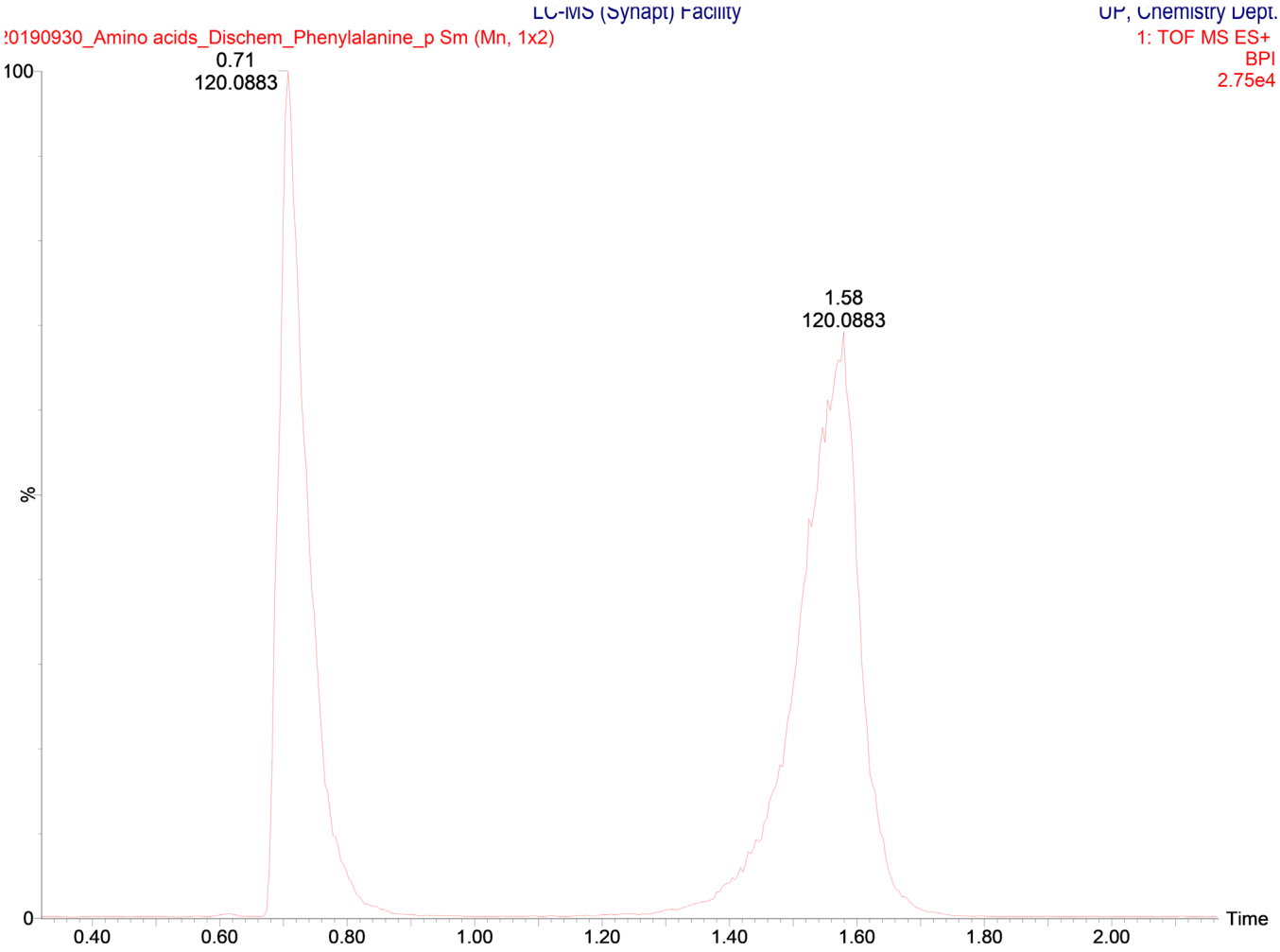

FIGURE 2 BPI chromatogram of L-phenylalanine (120.0883 *m/z*; $[M\text{-}COOH]^+$) standard (10 ng/μL with 5-μL injection on column). The two peaks are due to two different ionised states of the compound, resulting in a 'bat-o-gram'.

conversion times between the two ionised states can result in the formation on two distinct peaks.[36] The saddle between the two L-phenylalanine peaks indicates that the conversion rate between the two ionised states is similar to the average retention time of both peaks (Figure 2); thus, some compounds will travel down the column as partly one or the other state resulting in intermediate retention times.[36] Ion mobility confirmed that the two L-phenylalanine peaks were not isomers as both peaks (166.0868 *m/z*; $[M + H]^+$) detected

had an identical CCS value of 139 Å^2. Lowering the concentration of L-phenylalanine to 1 ng/μL resulted in a single distinct peak as can be seen in Figure S1 (bottom). This observation was not further investigated as skin surface compounds were not expected to occur at very high (>2-ng/μL extract) levels.

All five of the target analytes were detected when using either methods (Table 2). %RSD, LODs and LOQs for the two methods are given in Table 2. No significant observable difference was found in terms of precision (%RSD) and detection limits (LODs and LOQs) between the solvent-modified and unmodified PDMS sampler using a *t*-test at 95% confidence [*t* Stat = 0.15 (LODs), 0.14 (LOQs) and 1.50 (%RSD) < *t* critical = 2.36]. This finding is in line with Wooding et al. where no observable difference was noted for a range of target compounds with solvent modification of a PDMS sampler with isopropanol and direct thermal desorption of the sampler in the GC inlet liner with GC × GC-TOFMS.[37] Of interest is the increase in response for L-carnitine, L-phenylalanine and taurine at 400 ng spiked on sampler (10 ng injected on column) for the solvent-modified sampler, whereas the unmodified method appeared to reach a plateau prior to 400 ng. This finding was also noted for target VOCs and semi-VOCs by Wooding et al.[37] On sampler LOQs for L-phenylalanine (431 ng) for the without solvent modification method is higher than levels expected to be found on the human skin surface, advantageously, the solvent modified method makes quantification possible with LOQs of 288 ng on sampler. An increase in analyte capacity of the sampler when adding solvent is a likely explanation for this observation; however, further investigation is needed. All samplers were consequently solvent modified prior to human skin surface sampling to potentially increase the sampler's analyte capacity and for improved sampler sensitivity.

3.2 | Targeted analysis

Ten amino acids were unequivocally identified on the human skin from one volunteer using an amino acid reference standard mixture, a passive sampling approach and UPLC-IMS-HRMS (Table 3). The amino acids detected have previously been reported in human skin sweat samples.[21,22] The current study employed a non-invasive sampling method with minimum discomfort to the individual sampled and a simplified extraction process to detect amino acids on the human skin surface. CCSs (confirmed using a reference standard) for the 10 detected amino acids are provided in Table 3. The variation in the number of days each amino acid was detected, or not, underpins the complexity of the human skin surface chemical profile. For example, L-citrulline was detected on three out of 5 days on the wrist skin surface and only detected on one of the 5 days on the ankle skin surface. The day-to-day variance found in the skin chemical profile over the 5-day sampling period is attributed to a varied diet, and amongst others, variation in environmental exposure, sleep pattern and microbiota.

3.3 | Untargeted analysis

3.3.1 | Biomarker discovery on the human skin surface

Multivariate chemometric techniques were employed to investigate chemical differences between the two surface skin regions (ankle and wrist). Over 16 000 unique features were identified using UNIFI®'s Marker Matrix software function. Principal component analysis, an unsupervised clustering technique, showed no distinct cluster

TABLE 2 Method comparison for the PDMS sorptive sampler modified with isopropanol and without isopropanol.

	100 ng		200 ng		400 ng			
Analyte	$\bar{x} \pm \sigma_{n=3}$	**% RSD**	$\bar{x} \pm \sigma_{n=3}$	**% RSD**	$\bar{x} \pm \sigma_{n=3}$	**% RSD**	**LOD[a] (ng)**	**LOQ[b] (ng)**
PDMS sampler without solvent modification								
Sulfadimethoxine	712[c] ± 262	37	1191 ± 380	32	1760 ± 960	55	9.3	31
Caffeine	93 ± 8	9	179 ± 33	18	356 ± 112	32	80	265
L-phenylalanine	23 ± 2	7	31 ± 3	11	36 ± 5	14	129	431
Taurine	11 ± 3	28	15 ± 2	16	19 ± 3	15	67	223
L-carnitine	112 ± 15	14	125 ± 18	14	216 ± 78	36	13	43
PDMS sampler with solvent modification								
Sulfadimethoxine	769 ± 189	25	1121 ± 515	46	1638 ± 523	32	8.7	29
Caffeine	77 ± 7	9	168 ± 42	25	382 ± 70	18	62	208
L-phenylalanine	21 ± 2	8	38 ± 6	15	67 ± 2	3	86	288
Taurine	7 ± 1	18	14 ± 2	14	24 ± 3	12	95	316
L-carnitine	94 ± 14	15	142 ± 14	10	317 ± 65	20	26	86

Note: Mean, standard deviation and %RSD ($n = 3$) of the normalised peak areas at 100, 200 and 400 ng spiked on sampler (simulated method) with the selected analytes using solvent desorption with UPLC-TOFMS. LODs and LOQs are also provided.

[a]Method limits of detection (MDLs) (on sampler).

[b]Method limits of quantification (MQLs) (on sampler).

[c]Mean peak area (TIC) normalised using mass (μg) of PDMS sampler.

TABLE 3 Amino acids unequivocally identified, by comparison with an amino acid analytical standard solution using accurate mass (*m/z*), CCS values (Å^2) and retention times (min), on the human skin surface area using a sorptive PDMS sampler with solvent desorption coupled to UPLC-IMS-HRMS.

#	Compound	Observed mass (*m/z*) (mass error [ppm])	Observed adducts	RT (min)	Observed CCS (Å^2) (CCS % error)	Observed fragments (*m/z*) (mass error [ppm])	Wrist count[a] (n = 5[b], *m* = 15[c])	Ankles count[a] (*n* = 5[b], *m* = 15[c])
1	L-histidine	110.0712 (−0.3)	$[M\text{-}COOH]^+$ $[M + H]^+$ $[M + Na]^+$ $[M\text{-}H]^-$	0.58	120.9 (0.002)	70.0658 (8.89) 93.0443 (−11.34)	4	5
2	L-tyrosine	136.0753 (−3.0)	$[M\text{-}COOH]^+$	0.66	131.7 (0.15)	91.0541 (−7.7) 119.0485 (−9.72)	2	4
3	L-phenylalanine	120.0805 (−2.6)	$[M\text{-}COOH]^+$	0.71	128.3 (−0.01)	-	3	4
4	L-proline	116.0707 (−3.4)	$[M + H]^+$ $[M + Na]^+$	0.63	125.2 (1.82)	-	3	4
5	L-arginine	175.1183 (−3.7)	$[M + H]^+$	0.74	136.5 (0.52)	-	1	2
6	L-carnitine	162.1128 (1.8)	$[M + H]^+$	0.61	134.3 (0.93)	-	2	4
7	L-citrulline	198.0844 (−2.6)	$[M + Na]^+$	0.61	138.7 (n/a[d])	-	3	1
8	L-cystine	241.0302 (−3.8)	$[M + H]^+$ $[M + Na]^+$	0.59	147.2 (0.63)	-	1	n.d.
9	L-ornithine	131.0824 (−1.9)	$[M\text{-}H]^-$	0.61	127.2 (−0.60)	-	2	1
10	L-tryptophan	203.0820 (−3.2)	$[M\text{-}H]^-$	2.21	146.3 (−0.18)	-	1	4

Note: The number of days (count) the compound was detected on the wrist and ankle skin surface region is provided.
Abbreviations: n.d., not detected; RT, retention time.
[a]Number of days compound was detected.
[b]Number of sampling events ($n = 5$).
[c]Number of observations ($m = 15$); three biological replicates per sampling event.
[d]Sodium adduct not detected in reference standard.

formation between the two groups (Figure S2A). However, PCA indicated good repeatability of the quality control groups, that is, the QC standard and the pooled sample (Figure S2A). A supervised chemometric approach was used, namely, OPLS-DA, to determine putative biomarkers contributing to the differences between the chemical profiles of the two skin regions. The OPLS-DA plot showed a distinct separation between the two skin regions, ankle versus wrist (Figure S2B). The OPLS-DA model had an R^2 (cum) value of 0.961 and a Q^2 (cum) value of 0.649. An S-plot was constructed to determine the compounds contributing to the differences between the two chemical profiles. The extreme ends of the S-plot show the variables responsible for the separation into the two observed groups (Figure S2C). Compounds were paired using *m/z*, retention time (min) and drift time (ms). This approach was followed for both ESI positive and ESI negative mode data sets.

The chemometric scheme yielded 23 compounds that contributed to the difference between the two skin regions. Sixteen compounds were closely associated with the wrist skin surface area, and seven were associated with the ankle skin area. These compounds are given in Table S1. The compounds detected are from a broad range of chemical classes including phenols, ketones, terpenoids, acids, long-chain ceramides and nitrogen-containing compounds. To the best of the authors' knowledge, none of these compounds have previously been reported on the human skin surface. The CCS prediction tool confirmed tentative identification. All reported CCS values are within 6.1% of the modelled values (−2.5 ± 1.7% error). Representative chromatograms for ankle and wrist human skin surface area sampling are given in Figure S3.

Some of the compounds that were closely associated with the wrist skin surface included 5,6-*trans*-25-hydroxyvitamin D3 (a vitamin D3 derivative), ceramide (d18:1/20:0) (a long-chain ceramide, found in the subcellular fractions of the human epidermis), and obtusifoliol (a triterpenoid intermediate in the biosynthesis of cholesterol). Conversely, some of the compounds linked to the ankle skin surface region included allochenodeoxycholic acid (a bile acid), cappariloside A (a phenolic glycoside previously detected in capers, spices and herbs) and N,N-diethylbenzeneacetamide (a phenylacetamide previously detected in green vegetables).[38]

These compounds form potential lead compounds for the development of mosquito vector contact repellents and attractants. All identified markers, providing potential lead compounds in vector control strategies, are given in Table S1. The detection of plant-derived chemicals from food sources on the skin surface also reveals the potential of using this method in human dietary studies.

3.3.2 | Tentative identification of other skin compounds

CCs were included in this study as an additional feature, not only for biological marker alignment and discovery but also to aid compound

TABLE 4 Compounds tentatively identified on the human skin surface using accurate mass (*m/z*), CCS values (Å^2) compared with a CCS library and fragmentation patterns using a sorptive PDMS sampler with solvent desorption coupled to UPLC-IMS-HRMS.

#	Compound	Observed mass (*m/z*) (mass error [ppm])	Observed adducts	RT (min)	Observed CCS (Å^2) (CCS % error)[a]	Observed fragments (*m/z*) (mass error [ppm])	Wrist count[b] ($n = 5$[c], $m = 15$[d])	Ankles count[b] ($n = 5$[c], $m = 15$[d])
1	11Z-Eicosenoic acid	309.2794 (−1.5)	$[M-H]^-$	9.8	183 (−3.45)	-	3	4
2	4-Quinolinecarboxylic acid	174.0542 (−4.5)	$[M + H]^+$	6.32	134.40 (1.97)	-	1	n.d.
3	5-Oxo-D-proline	130.0491 (−5.5)	$[M + H]^+$	0.70	126.84 (2.04)	-	n.d.	1
4	Arachidic acid	335.2926 (1.7)	$[M + Na]^+$	11.69	191.36 (0.03)	-	3	3
5	Azelaic acid	187.0987 (6.2)	$[M-H]^-$	4.34	137.7 (0.05)	125.0984 (9.34) 141.0951 (21.29)	3	4
6	Bis(2-ethylhexyl) phthalate	413.2651 (−2.7)	$[M + Na]^+$	14.21	217.82 (0.24)	189.0139 (−22.87) 301.1389 (−15.03)	3	4
7	C20dh Cer; N-(eicosanoyl)-dihydroceramide; N-(eicosanoyl)-dihydroceramide	596.5955 (−3.6)	$[M + H]^+$	14.20	282.46 (2.71)	-	2	n.d.
8	D-(+)-rrehalose	341.1092 (0.8)	$[M-H]^-$	0.63	166 (0.02)	-	4	4
9	Dihydrotestosterone	291.2317 (−0.4)	$[M + H]^+$	10.99	180.06 (0.82)	-	2	3
10	Lactose	365.1046 (−2.4)	$[M + Na]^+$	0.64	172.27 (−0.42)	185.0391 (9.06) 203.0539 (−5.33)	2	3
11	LPC 16:0	496.3400 (0.5)	$[M + H]^+$	10.12	235.95 (0.40)	184.0727 (−6.34) 86.0954 (−18.21)	1	1
12	L-Valine	118.0860 (−2.3)	$[M + H]^+$	0.61	124.28 (0.80)	118.0856 (−10.45)	n.d.	2
13	Melibiose	365.1044 (−2.7)	$[M + Na]^+$	0.62	174.98 (−0.01)	-	2	1
14	Methyl jasmonate	225.1479 (−2.6)	$[M + H]^+$	6.97	155.21 (1.78)	-	1	2
15	Palatinose	365.1051 (−0.8)	$[M + Na]^+$	0.63	173.78 (−0.13)	203.0532 (−9.08)	n.d.	2
16	Palmitoylcarnitine	400.3410 (−2.8)	$[M + H]^+$	10.51	215.98 (−0.47)	283.2618 (−4.88)	n.d.	2
17	Phenyl acetate	175.0147 (−4.9)	$[M + K]^+$	13.62	137.05 (0.92)	-	2	3
18	Phenylacetaldehyde	121.0645 (−2.6)	$[M + H]^+$	10.97	122.38 (1.73)	-	5	3
19	Raffinose	527.1571 (−2.1)	$[M + Na]^+$	0.62	212.51 (1.20)	-	1	1
20	Sphinganine	302.3052 (−0.4)	$[M + H]^+$	9.42	194.47 (−1.18)	284.2947 (−2.04)	4	5
21	Sucrose	341.1097 (2.3)	$[M-H]^-$	0.64	165.9 (−0.09)	-	1	2
22	Urocanate	139.0499 (−2.4)	$[M + H]^+$	0.65	131.55 (1.66)	121.0392 (−8.14) 93.0450 (−2.93) 139.0501 (−5.06)	2	2

Note: The number of days (count) the compound was detected on the wrist and ankle skin surface region is provided.
Abbreviations: n.d., not detected; RT, retention time.
[a]CCS, collision cross section, error between experimental and library value (Waters® Metabolic Profiling CCS library).
[b]Number of days ($n = 5$) compound was detected.
[c]Number of sampling events ($n = 5$).
[d]Number of observations ($m = 15$); three biological repeats per sampling event.

identification. CCS is a promising technique to aid identification; however, it does have a high degree of correlation with the charge state (*m/z*) and the position of protonation (formation of different protomers).[23] To overcome these limitations, this study used known CCS values, from the Waters® Metabolic Profiling CCS ESI+ and ESI− libraries, to add additional confirmation in the identification of surface skin compounds. All 28 compounds listed in Table 4 have been tentatively identified using accurate mass, CCS matching to known values, i-Fit and fragmentation pattern where available. All reported CCS values are within 3.4% of the library values (−1.3 ± 1.3% error).

Furthermore, all fragmentation was done post-drift, allowing improved alignment between product and precursor ions and discrimination from in-source fragments. The addition of each unique feature greatly improves the reliability of analyte matching and identification. The compounds reported, to the best of the authors' knowledge, have not previously been reported on the surface of human skin. The compounds belong to a range of classes including sugars, for example, lactose and sucrose; fatty acids, for example, arachidic and azelaic acids; amino acids, including L-valine; and phthalates (bis[2-ethylhexyl] phthalate). Of interest is the detection of methyl jasmonate a repellent for the southern house mosquito, as well as ticks.[39] These results highlight the ability of the PDMS sampler, the sorptive sampling technique and simplified extraction approach to detect a range of medium to non-volatile compounds and to detect compounds associated with mosquito attractiveness. The masses detected ranged from 118.0860 to 784.5802 *m/z*.

4 | CONCLUSION

We report a non-invasive simple sampling technique that can potentially be used in mass screening of the human skin surface metabolome for the application of vector control and the potential application in human health screening, such as dietary studies and detection of disease indicators. A broad range of medium to non-volatile compounds were detected on the human skin surface using non-invasive passive sampling with a simplified solvent desorption method. Solvent desorption directly in an LC vial enabled an easy and time-efficient link between sampling and analysis. The sampling technique used was not invasive and caused minimal discomfort to the individual. Furthermore, the addition of a solvent to the sampler may increase sampler capacity leading to improved sensitivity, which is of great importance in biomarker discovery. The coupling of HRMS and CCS data provides additional confidence in marker identification, as does post-drift fragmentation alignment of product and precursor ions. The in-house developed PDMS sampler and solvent desorption with UPLC-IMS-TOFMS allowed comparison of the chemical profiles of different human skin regions. Marker alignment was strengthened using three unique features, namely, *m/z*, chromatographic retention time and ion mobility drift time. Chemometric techniques enabled the tentative identification of 23 skin surface chemicals contributing to the difference in the human surface skin regions sampled. Seven biomarkers were identified for the ankle skin surface region, and 16 were identified for the wrist skin surface area. These biomarkers can potentially be used for the development of non-volatile surface attractants and repellents in vector control applications. Limits of detection and quantification for the method were in the low to mid ng range. Ten amino acids, previously only detected in human sweat samples, were unequivocally identified on the human skin surface. This study facilitated the tentative identification of a further 22 previously unreported skin surface compounds from a broad range of chemical classes, including sugars, phthalates and fatty acids using accurate mass and CCS value matching. The CCS prediction tool (−2.5 ± 1.7% error) performed well when compared with library matching (−2.3 ± 1.3% error) of CCS values. Furthermore, CCS values reported in this study can be used to support future metabolomic skin surface studies and compound identification. The method provides an additional tool to data mine the human skin surface metabolome.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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