Supporting Information S1: Experimental Section

1. Preparation of standard solutions

A 100 ng/µl stock solution of sulfadimethoxine was prepared by dissolving 5 mg powder in 50 ml of methanol. Individual stock solutions of 1 000 ng/µl were prepared for taurine (500 mg in 500 ml), L-lysine (500 mg in 500 ml), L-carnitine (200 mg in 200 ml) and L-phenylalanine (250 mg in 250 ml) in water. A 900 ng/µl stock solution was prepared for L-tyrosine (dissolving 450 mg in 500 ml) and a 100 ng/µl stock solution of caffeine was made in water.

A 10 ng/µl mixture of the five amino acids were prepared in water by diluting 1 ml of the 1 000 ng/µl stock solutions (taurine, L-lysine, L-carnitine and L-phenylalanine) and 1.11 ml of the 900 ng/µl L-tyrosine stock solution to a final volume of 100 ml. A final working standard solution containing a mixture of the target analytes was prepared at 1 ng/µl in 50 ml of ACN:isopropanol (1:1, v/v) (5 ml of the 10 ng/µl five amino acid mixture, 0.5 ml of each 100 ng/µl caffeine and sulfadimethoxine stock solutions). All standard and working solutions were stored in glass Schott bottles and kept at 4 °C.

2. Human skin surface sampling

The PDMS samplers were modified with isopropanol to improve recoveries of polar (log K_{ow} < 2.5) compounds. Increased extraction efficiency of polar analytes from water when solvent modifying a PDMS stir bar with a range of solvents (solvent-assisted stir bar sorptive extraction (SA-SBSE)) has previously been reported by Ochiai et al.[31] The PDMS sampler was modified specifically with isopropanol, a nontoxic solvent, to comply with ethical considerations during human sampling. The sampler was opened, i.e. the uncoated silica capillary tubing used to join the ends was removed, and the now open PDMS tubing was sonicated in 12 ml of isopropanol. The samplers were taken out of the isopropanol with a clean stainless-steel tweezer, wiped dry with a lint free tissue and fashioned back into a loop as described in Section 2.3. 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 on five consecutive days, using three loops per sampling period of 1 hour. 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, in accordance with Roodt et al. and Wooding et al., for easy sampling and reduced invasiveness.[7, 14] The sampler was covered with aluminised Mylar® (Hydroponic, South Africa) reflective sheeting (20 cm x 1.5 cm and 25 cm x 1.5 cm for wrist and ankle skin surface regions, respectively) to help concentrate the compounds into the sampler and reduce background compounds during sampling. The Mylar® was kept in place with 3M Micropore medical dressing tape (Dischem, South Africa) (20 cm x 2.4 cm and 25 cm x 2.4 cm for wrist and ankle skin surface regions, respectively). 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 one-hour sampling period the sampling loops were removed from the skin with a clean stainless-steel tweezer, followed by 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 (Fig. 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.[32] Liquid/solvent desorption, i.e. back extraction into a solvent, was done using the method as outlined by Margoum et al.[33] 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 (Fig. 1.). The LC vials were recapped and transferred to the sample manager, at 4 °C, of a LC system for LC-MS analysis.

3. **Instrumentation details**

Compound separation and detection was performed using a Waters® Synapt G2 high definition mass spectrometry (HDMS) system (Waters Inc., Milford, Massachusetts, USA). The system comprised of a Waters ACQUITY UPLC® (Binary Solvent Manager a 10 µl loop installed in a Fixed Loop Sample Manager) hyphenated to a quadrupole mass filter, a Triwave™ ion mobility (IM) cell and a high-resolution time-of-flight (TOF) mass analyser. The system was operated with MassLynx™ (version 4.1) software (Waters Inc., Milford, Massachusetts, USA) for data acquisition. An internal lock mass control standard, 2 ng/µl solution leucine enkephalin (*m/z* 555.2693), was directly infused into the source through a secondary orthogonal electrospray ionisation (ESI) probe allowing alternating sampling, modulated with a baffle, every 0.3 seconds. The instrument was calibrated using sodium formate clusters and automated IntelliStart software (mass range 112.936 – 1132.688 Da). Resolution of 20 000 full width at half maximum (FWHM) at *m/z* 200 and mass error within 0.4 mDa.

The source conditions were as follows: the capillary voltage for ESI was 3.0 kV 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, extraction cone voltage at 4.0 V and cone gas (nitrogen) flow at 10.0 L/Hr. The desolvation temperature was set at 400 °C with a gas (nitrogen) flow of 600.0 L/Hr. Mass spectral scans were collected every 0.1 seconds. The raw data was collected in the form of a continuous profile. Mass range was set between 50 and 1200 *m/z*. Data-independent acquisition (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 1 000 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 1 000 µs. The IMS was calibrated using Waters Major Mix Calibration Sample with Driftscope (version 2.8) to determine experimental collision cross section (CCS) values (Ω). A CCS error of <3.7% (0.66 ± 0.8%) was obtained.

4. 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 quantification ions selected for peak area calculation were the $[M+H]^+$ accurate mass molecular ion for sulfadimethoxine, caffeine and L-carnitine, the $[M\text{-}COOH]^+$ adduct for L-phenylalanine, and the $[M\text{-}H]^-$ accurate mass molecular ion for taurine. Unambiguous identification of target analytes was achieved using retention times matching, accurate mass and MS^E fragmentation patterns of standards. 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. During the evaluation of the sampler with and without solvent modification, the peak areas were normalised using the mass (µg) of the PDMS sampler. Background laboratory contaminants from the method blank were subtracted from the peak areas of the human skin volatile samples.

All further data processing was performed using UNIFI® Scientific Information System (Waters Inc., Milford, Massachusetts, USA). The following data processing settings were employed: chromatographic peak width settings was set to 24 seconds for 0 – 3 min and 18 seconds for 3 – 7 min, and on automatic peak width selection for the remainder of the chromatographic run (7 – 20 min); the intensity threshold was set to 5.0 and 10.0 counts for the high and low energy functions, respectively. 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 was lock mass corrected with UNIFI® before processing.

Markers were generated in UNIFI® using the Marker Matrix software function. The marker tolerance for ESI+ was set to 17.70 ppm for *m/z*, 0.058 min for RT and 0.19 ms for drift time; for ESI- the tolerance was 20.80 ppm for *m/z*, 0.060 min for RT and 0.22 ms for drift time. Markers were exported into a .csv format and imported into EZinfo (version 2.0.0.0) for statistical analysis and biological marker discovery. Principal component analysis (PCA) was used to identify similarities in the chemical composition of the samples. To obtain understanding of the differences between the ankle and wrist skin surface regions sampled and to find potential biomarkers discriminating between the two skin areas orthogonal partial least squares discriminant analysis (OPLS-DA) was performed. In this supervised chemometric approach sample information, i.e. skin region, is assigned to the data. Pareto scaling was used to generate OPLS-DA models and S-plots were used to identify differentiating features between groups. 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®, were made by accurate mass, isotope fit values, collision cross section (CCS) values, and fragmentation patterns, by comparison with the Waters® Metabolic Profiling CCS ESI+ Library and Waters® Metabolic Profiling CCS ESI- Library. Discovery setting tolerance was set to ±10 mDa for *m/z*, 10 % for i-Fit confidence and SmartsScores software functionality was used for fragment matching.