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Mass spectrometric study of cutaneous volatiles by secondary electrospray ionization

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ABSTRACT

We investigated the volatile organic compounds emanated from the hand of two individuals, on-line by secondary electrospray ionization-mass spectrometry in positive ionization mode. The background ambient air is continuously sampled, ionized and readily mass analyzed. When the probe samples above the headspace of the hand of two subjects, several peaks (63 for one subject and 37 for the other) arise above the background with masses reaching up to m/z 348. In spite of the different patterns, they share 30 common peaks. Some of these compounds have been assigned by collision-induced-dissociation, most of them as amines, including the amino acid ornithine.

1. Introduction

The analysis of volatile organic compounds from the skin is of intrinsic interest in different fields. For instance, on the recognition of individual fingerprints in body odorants [1,2], identification of mosquito attractants [3] or as non-invasive diagnostic approach [4,5]. The most extended method of analyzing skin volatiles is gas chromatography (GC) [6], usually coupled to mass spectrometry (GC–MS), which requires a concentration stage of trapping before injection into the GC column, owing to the low concentrations of volatiles typically found. The main disadvantage of this approach is its high labor intensity. It is hence desirable to develop technologies providing analysis of volatiles in real time. Indeed, proton transfer reaction-mass spectrometry (PTR-MS) [7] and selected ion flow tube-mass spectrometry (SIFT-MS) [8] have achieved this goal and proven their soundness for the analysis of skin volatiles [9,10]. An alternative approach originally suggested by Fenn and co-workers [11–13] consist in ionizing trace volatile species by contact with an electrospray (ES) cloud at atmospheric pressure. This approach has been successfully implemented also by other groups to detect by mass spectrometry the eluents exiting a GC column [14,15]. Hill and co-workers have also demonstrated the merit of this ionization technique coupled to ion mobility spectrometers [16]. They have illustrated the potential of this combination on the detection of drugs [17] and explosives [18] and have dubbed this charging technique secondary electrospray ionization (SESI). It has been also demonstrated that an ES plume can effectively ionize compounds contained in aerosol particles [12]. Chen et al. described extractive electrospray ionization (EESI), in which liquid samples were nebulized in the vicinity of an ES source [19]. The interaction of these two sprays resulted in droplet–droplet extraction and ionization of neutral analytes. This method has also been successfully applied to the analysis of skin components [20]. In this case, an aerosol is generated by an impinging gas jet onto the skin.

We have recently reported the potential of SESI-MS for the analysis of breath [21,22] and urine [23], and measured its response towards known concentrations of explosives vapors, revealing limits of detection as low as 0.2 parts-per-trillion (ppt) [24]. An initial investigation in negative ionization mode for one subject has demonstrated that SESI-MS is a suitable approach to measure skin fatty acids along with other volatile species [25]. The purpose of this investigation is to extend the study in the positive ion detection mode to two individuals.

2. Experimental

The experimental scheme (Fig. 1) is similar to that described elsewhere [22,23]. We modified the entrance of a quadrupole time of flight mass spectrometer (QqTOF; Sciex's Q-Star) to place a stainless steel chamber upstream the sampling pinhole. The chamber holds a homemade nanospray source and two 1/4 in. (o.d.) tubes. One of the tubes was connected to a pump to establish a continuous flow of 6 L/min of ambient air circulating in and out the chamber.

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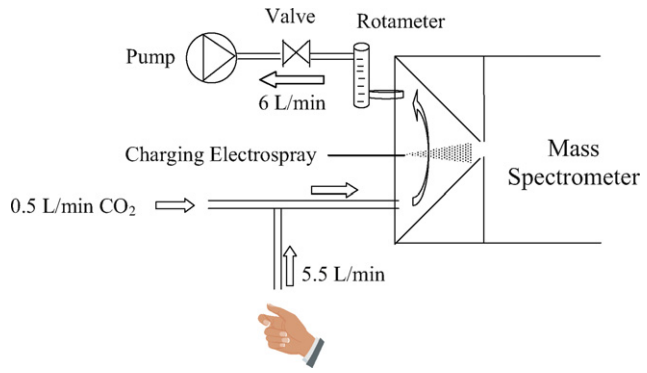

Fig. 1. Sketch of the experimental set-up to detect volatile species from the skin by SESI-MS. Laboratory air spectrum is compared to that obtained when approaching the hand to the sampling probe.

The flow rate was metered with a rotameter and a needle valve. The sampled air enters the ionization region through the second tube of approximately 7 cm long (sampling tube). Yamashita and Fenn [26] suggested that the introduction of a high electron affinity gas over the ES tip would be helpful in suppressing discharge. For example SF_6 has been demonstrated to be an effective discharge suppressor [27]. In our case, we mixed the sampled ambient air with 0.5 L/min of CO_2 to reduce the likelihood of sparking from the ES. This gas mixture of ambient air (5.5 L/min) and CO_2 (0.5 L/min) entered the ionization region. Some of the neutral vapors are then ionized upon getting in contact with the ES charged drops (or eventually with evaporated ions) and mass analyzed. The ambient laboratory air is thus continuously monitored and eventually a subject approaches the palm of his/her hand at about 1 cm from the sampling tube. During our previous work on breath [22] and urine [23] we noted the precautions to be taken with highly moisturized samples, as water vapor can enhance some ions signal and this can lead to erroneous conclusions. This is not the case here since the humidity increase from the skin is negligible and consequently we defined here the background level as that obtained from the ambient air without further modifications. We operated the QqTOF in positive mode, and the ES buffer was 0.1% acetic acid in 1:1 MeOH/H_2O (v/v) infused at 65 nL/min thorough a 20 μm i.d. fused silica capillary

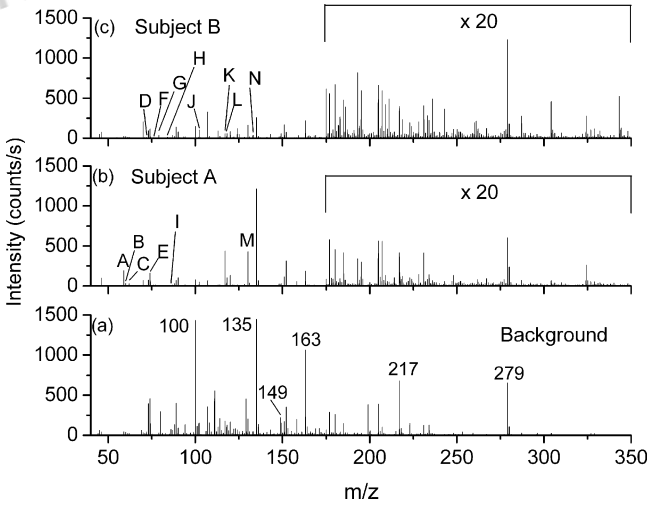

Fig. 2. (a) Mass spectrum recorded from the ambient air from the laboratory. (b) and (c) Mass spectra (background subtracted) from both subjects. Note that the region above *m/z* 175 has been magnified by a factor of 20. Labeled peaks: (A) acetone; (B) trimethylamine; (C) ethanolamine; (D) 3-buten-1-amine; (E) isobutylamine; (F) 1-amino-2-propanol; (G) dimethyl sulfoxide; (H) piperideine; (I) piperidine; (J) hexylamine; (K) heptylamine; (L) valine; (M) octylamine; and (N) ornithine.

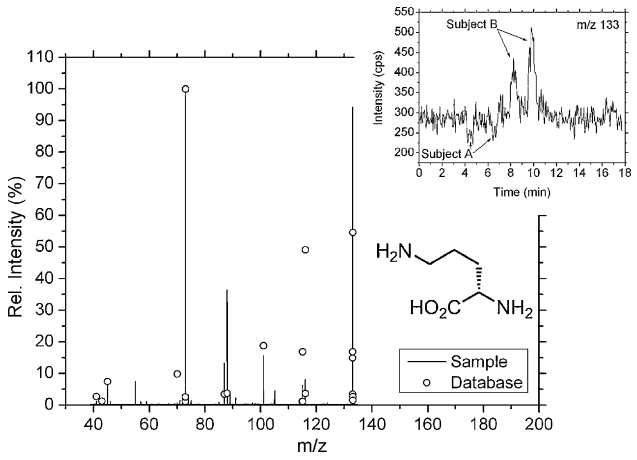

Fig. 3. Overlaid MS/MS spectra at *m/z* 133 and that of the database for ornithine at a collision energy of 10 V. The periodic change of the signal at *m/z* 133 resulting from approaching the hand of both subjects at the sampling tube can be seen in the inset. It shows a clear intensity increase associated to subject B.

with its tip sharpened. The electrospray was operated with a voltage of 3.8 kV and the flow lines and ionization chamber were kept at room temperature. All the ions identified (mainly amines) were in the form of a protonated vapor (molecular weight + 1). In this study, neither subject followed any particular protocol prior to sampling to minimize personal care products interference. Nonetheless, they refrained of using hand creams or other consumer products for at least 1 h before the analysis.

3. Results and discussion

Fig. 2 shows three mass spectra: one corresponding to the ambient air (background) and two recorded when placing the palm of the hand of two subjects in the close vicinity (~1 cm) of the sampling tube. The majority of the peaks are greater in the ambient background than in the headspace of the hands. Nonetheless 37 peaks rise clearly above the blank for subject A (male) and 63 for subject B (female), 30 of which are common to both subjects. We

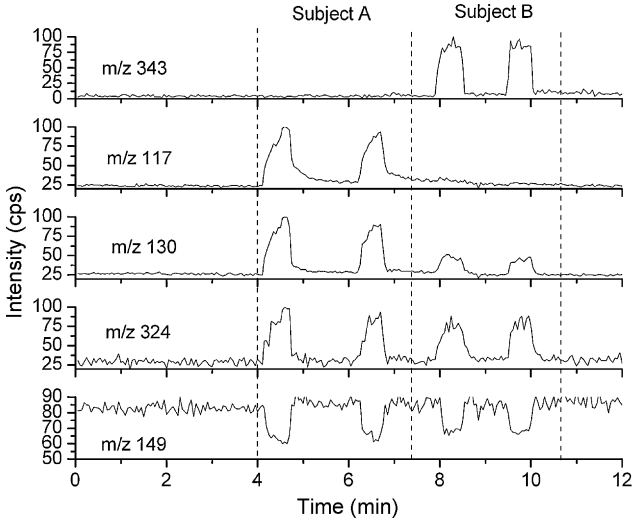

Fig. 4. Intensity of some representative peaks as a function of time (SIM trace). The peak at *m/z* 149 is a laboratory contaminant and its signal decreases when the two subjects approach their hand (min 4 and 6 subject A, and min 8 and 9.5 subject B). Both subjects show comparable signals at *m/z* 324, whereas subject A doubles the signal at *m/z* 130. The traces at *m/z* 117 and *m/z* 343 illustrate how some peaks are associated to only one volunteer (A and B, respectively).

Table 1
Peaks observed above the background for both volunteers. Identified and tentatively identified compounds, characteristic MS–MS fragments and their known metabolic pathways.

m/z	Subject	Identity	Characteristic MS–MS fragments (Da)	Metabolic pathway
41	A			
45	B			
46	Both			
55	B			
57	A			
59	Both	Acetone[A,*]	59, 41, 31	Synthesis and degradation of ketone bodies; propanoate metabolism
60	Both	Trimethylamine[B]	60, 45, 44, 43	Methane metabolism
62	Both	Ethanolamine[C,*]	62, 45, 44	Aminophosphonate metabolism; glycerophospholipid metabolism
64	B			
68	B			
70	Both			
72	Both	3-Buten-1-amine[B]	72, 44	
74	Both	Isobutylamine[C]	74, 57	
76	Both	1-Amino-2-propanol[C]	76, 58	Glycine, serine and threonine metabolism
77	A			
79	B	Dimethyl sulfoxide[B]	79, 64, 61	
84	Both	Piperideine[B]	84, 56, 42	Lysine degradation
86	Both	Piperidine[C]	86, 69, 58, 41	
87	B			
90	Both			
91	B			
96	Both			
102	Both	Hexylamine[C]	102, 74, 58, 56, 46	
104	A			
105	B			
107	B			
113a	B			
113b	Both			
116	Both	Heptylamine[B]	116, 99, 88, 74, 60	
117	A			
118	Both	Valine[D]	118, 101, 72	Valine, leucine and isoleucine degradation; valine, leucine and isoleucine biosynthesis; propanoate metabolism; pantothenate and CoA biosynthesis; aminoacyl-tRNA biosynthesis
120	Both			
124	B			
126	B			
128	Both			
130	Both	Octylamine[C]	130, 84, 57, 43, 41, 39	
133	B	Ornithine[C]	133, 116, 115, 105, 101, 88, 87, 73, 45	Urea cycle and metabolism of amino groups; arginine and proline metabolism; D-arginine and D-ornithine metabolism; glutathione metabolism
135	A			
142	Both			
143	B			
148	Both			
149	B			
151	Both			
152	A			
159	B			
165	B			
168	Both			
175	B			
182	B			
186	B			
193	Both			
195	Both			
209	B			
211	B			
228	B			
233	B			
236	B			
243	B			
250	B			

Table 1 (*Continued*)

m/*z*	Subject	Identity	Characteristic MS–MS fragments (Da)	Metabolic pathway
252	Both			
260	B			
261	B			
278	B			
287	B			
304	B			
310	Both			
324	Both			
332	Both			
343	B			
348	B			

*Found also in consumer products. Thus its presence may be a combination of both sources. *Explanation notes:* (A) Identity confirmed by MS/MS and matching to Ref. [34]. (B) Tentatively identified based on its exact mass and fragmentation pattern interpretation. (C) Identity confirmed by MS/MS and matching to reference web database [http://www.massbank.jp/]. (D) Identity listed is known with a high degree of confidence since its MS/MS spectrum contains all characteristic fragments from the database. However several other fragments are present, most probably associated to different peaks at the same nominal mass as the primary ion.

will illustrate these differences later. Note that the mass spectra presented from both subjects are background subtracted to discriminate the peaks appearing above the ambient air, however some of the resulting peaks are artifacts as a result of this process. For example the peak at *m*/*z* 279, which dominates in the background spectrum, is a known contaminant of the family of phtalic acid [28]. Note also that the region above *m*/*z* 175 is magnified by a factor of 20. The heaviest ion above the background appears at *m*/*z* 348 (*s*/*n* ∼ 4; only for subject B) and the heaviest one common to both subjects arises at *m*/*z* 332. The mass spectra contain some labeled peaks associated to the skin, which have been identified by collision-induced-dissociation, performed with 10–20 V of collision energy. The lightest assigned component is acetone (*m*/*z* 59), which is related with the synthesis and degradation of ketone bodies, and propanoate metabolism. Noteworthy, recently it has been reported that skin acetone concentrations of patients with diabetes were significantly higher than those of the control subjects [29]. This study was performed by preconcentrating acetone vapors in a cold trap and analyzing them by GC. Note that acetone has been detected in real time previously by SIFT-MS both in breath and skin, with indications of anti-correlation with blood glucose levels [9].

Besides acetone, we also identified a family of amines, including trimethylamine; ethanolamine; 1-amino-2-propanol; piperidine; isobutylamine; hexylamine; heptylamine and octylamine. Interestingly enough we identified ornithine, although only on subject B its concentration was above the background. Ornithine is a known constituent of finger's sweat and it has been previously reported using capillary electrophoresis [30]. Fig. 3 compares its fragmentation pattern and the one extracted from the reference database [http://www.massbank.jp/]. The inset plots the single ion monitoring (SIM) trace of ornithine, showing a modest but clear signal increase associated to the hand of subject B. For reference, ornithine is an amino acid produced in the urea cycle by the splitting off of urea from arginine. It is a central part of the urea cycle, which allows for the disposal of excess nitrogen. The fragmentation pattern at *m*/*z* 118 shows all characteristic fragments of valine, although also several others. However, this is not surprising as at least two different peaks appear at *m*/*z* 118, and the MS–MS spectrum is most probably a combination of both compounds. Valine has been also reported to be a sweat constituent by liquid chromatography–MS (LC–MS) [31]. Therefore, SESI-MS–MS seems to offer an attractive approach to sense and identify these and some other volatile polar compounds released from the skin with no need of preconcentrating the sample nor actively desorb them from the skin. Also, the array of detected volatile species seem complementary to those traditionally sensed by SIFT-MS and PTR-MS.

As indicated previously, some of the peaks are present with larger strength in one subject than the other and vice-versa or even some of them are characteristic of an individual. To better illustrate this point, we present in Fig. 4 the SIM trace of five different peaks. The first two steps (min 4 and 6) correspond to subject A, and the subsequent two (min 8 and 9.5) to subject B. The peak at *m*/*z* 149 is a well-known identifier of phthalates [28] and therefore it is not expected to be observed above the background. This is actually the case here. The negative effect of the approaching hand is probably indirect, possibly due to the reduction of the ionization probability associated to competition from other species. In negative ion mode we noted that the mass spectrum was dominated by lactic acid and was possibly largely responsible of this effect. This is in contrast with the mass spectra from Fig. 2, where any of the peaks appears markedly above the rest. The SIM trace at *m*/*z* 324 shows comparable peak intensity for both subjects, suggesting that this compound is emanated in comparable concentrations. The peak appearing at *m*/*z* 130 is common to both individuals, although it appears in greater concentration (by a factor of ∼2) in subject A. The peak at *m*/*z* 117 raises above the background essentially only on subject A. Inversely, the compound at *m*/*z* 343 is characteristic of subject B. Note that even though both subjects were advised to place the palm of their hand at about 1 cm from the sampling tube there is obviously some ambiguity regarding its exact position above the headspace of the hand. In spite of this uncertainty, the signal reaches the equilibrium and varies around a mean value for some 30 s until the hand is withdrawn. The exercise was repeated twice by each subject, obtaining a fair repeatability of the measurement as illustrated in Fig. 4. Even though the system response is almost immediate, there are clear differences among different ions. For example, the one at *m*/*z* 117 has a longer memory effect. Our previous study on explosives indicated that this effect could be reduced by heating the flow lines. Note that in contrast, our system in this study was kept at room temperature.

Table 1 summarizes the information extracted from this study. It lists all the peaks observed above the background, indicating association to volunteer A, B or both. It also lists identified compounds, including their main MS–MS characteristic fragments, the procedure followed for their identification and their known metabolic pathway (extracted from www.genome.jp). Obviously, this work does not intend to be a systematic study on the inter-individual variation of skin volatiles as the control group should be increased and interday variations should be monitored taking into account the subject's history (e.g., smoking habits, medication, etc.). Nonetheless, it illustrates the potential of this approach for the analysis skin volatiles by modifying slightly the hardware of a commercial API-MS.

It should also be noted that interference of consumer products with skin emanations is a critical issue in skin volatile analyses because it can lead to wrong conclusions. Moreover, in some cases a compound can be produced naturally in the body and at the same time be a component of consumer products. The difficulty to isolate endogenous from exogenous volatiles in skin using GC–MS has been illustrated recently [32]. In this study, Gallagher et al. followed a strict protocol using odorless soap for 7–10 days. Despite these precautions they still found several exogenous components. In our case, we should obviously expect as well some peaks associated to exogenous sources. Among those identified in this study, only acetone and ethanolamine are listed in *CosIng* (http://ec.europa.eu/enterprise/cosmetics/cosing/), which is the European Commission database with information on cosmetic ingredients contained in the "Cosmetics Directive" 76/768/EEC. At the same time both of them are known metabolites, and therefore their existence may be a combination of endogenous and exogenous sources. We are unaware of the metabolic role of some of the identified amines. But interestingly, Ref. [33] has reported the existence of a second family of receptors in the mouse olfactory epithelium, which recognize volatile amines found in urine. For example, said receptors responded to isoamylamine and cyclohexylamine, but not to the corresponding alcohols, isoamylalcohol and cyclohexanol. Genes encoding these receptors, so-called 'trace amine-associated receptors', are present in human, mouse and fish, suggesting that volatile amines may play a chemosensory function.

4. Conclusions

Upon simple modification of the entrance of a commercial mass spectrometer to incorporate a SESI source, we have studied cutaneous volatiles sampled directly from the hand of two subjects concluding that:

(1) We have monitored a total of 70 volatiles from the skin of both volunteers by SESI-MS, with masses approaching 350 Da.
(2) 30 Peaks were shared by both subjects, whereas 7 peaks appeared only for one subject and 33 for the other. Note however that some of the observed peaks may be attributed to exogenous compounds from consumer products.
(3) Because SESI ionizes species directly in the gas phase, the observed differences may be attributed to the different composition of the headspace above the skin, being complementary with other surface ionization methods.
(4) We identified by MS–MS some of the compounds associated to the skin. Among several amines, we also assigned ornithine and most probably valine, which are known constituents of sweat.
(5) Because SESI-MS requires no sample preconcentration, it provides an attractive approach to detect at least some of the identified compounds, which are traditionally analyzed by slower off-line techniques.

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