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Abstract:

An original device has been developed to measure perfume release in the air above a surface. This device has proven its originality, effectiveness, and repeatability both *in vitro* on different types of model surfaces and *in vivo* directly on the skin of the forearm of volunteers. A perfume composed of eight fragrance molecules in ethanol was used to measure evaporation in the headspace with solid phase microextraction (SPME) and gas chromatography analysis. Temperature control, time effects, system dimensions, volume and seal integrity, and SPME optimizations were investigated for the measurement device and the analytical method setup. Finally, the system's effectiveness and modularity were demonstrated with evaporation studies carried out on four different surfaces: a chemically inert glass surface, the Strat-M® model, a perfume test strip, and the skin. This original device shows promising results in providing a better understanding of the evaporation phenomena of fragrance molecules and its link with the physicochemical properties of the skin.

Keywords: perfume, evaporation modeling, headspace, *in vivo*, *in vitro*

1. Introduction

Perfumes are a mixture of fragrance molecules and/or natural extracts diluted in a volatile solvent. Typically, ethanol is used in fine fragrance because it is an excellent solubilizer for fragrances. Each fragrance molecule in the perfume has its own physicochemical properties that govern its evaporation into the air and its longevity on the skin. Fragrances can be classified as top, middle (or heart), and base notes according to their volatility. Base notes consist of low-volatility, long-lasting components that give body to the perfume, while top notes provide the initial perception when using a perfume, as they are very volatile and lack tenacity [1]. Fixative molecules in perfumes, which are part of the base notes, enhance the staying power of perfumes on the skin. The volatility of each fragrance molecule, determined by its vapor pressure, influences its olfactory perception when released into the air. However, a perfume's smell is affected not only by the volatility and intrinsic odor of its fragrances but also by their chemistry and potential interactions with the skin surface. Depending on their chemical structure and hydrophilic/lipophilic affinity, their retention on the skin varies from one molecule to another, thus altering their release kinetics [2].

Few studies are available in the literature to understand the fate of fragrances after their application on the skin surface. The analysis of perfumes is usually performed by gas chromatography (GC) to separate, identify, and quantify the different components. The headspace (HS) technique can be used for sampling perfume molecules released in the gas phase above a solid or liquid layer. Vuilleumier *et al*. [2] applied this technique to characterize the diffusion rates of volatile compounds from the skin as a function of the topical matrix. Fragrances were diluted in ethanol or introduced into a soap base. After application on the skin, volatile compounds evaporated in the HS were trapped on a porous poly-diphenyl phenylene oxide resin of Tenax®. This study highlights the complexity of evaporation phenomena in relation to the chemistry of volatile molecules and their interactions with skin lipids at the surface. Moreover, it is important to consider the disadvantages of the dynamic technique, such as its lack of responsibility to other surfaces and the requirement of specific equipment for example. Additionally, the article does not provide details on the system setup, specifications, and dimensions.

Behan *et al.* [3] also used the same headspace technique to quantify fragrances on the skin. To highlight the physicochemical interactions between skin lipids and volatile molecules, the evaporation profile of a mixture of fragrances from the skin was compared to evaporation from a vitreous ceramic tile. HS concentrations of the different fragrances trapped in Tenax® adsorbent were. Results showed significant differences between the two surfaces. The authors concluded that skin is not an inert surface; it interacts with perfume, slows its evaporation, and is time-dependent. Therefore, the HS analysis should be carried out at several stages of evaporation to provide a better overview of release kinetics and interactions with the support. However, the study by Behan *et al.* (1996) does not detail the glass cell used to sample the perfumes in the HS, particularly regarding volumes, exchange surface, sampling monitoring, and temperature control. These experimental parameters are crucial and must be documented because they greatly influence the evaporation of perfumes.

Duffy *et al.* (2018), developed a valuable method for passive headspace sampling of skin volatile emissions in human participants using HS-SPME (solid phase microextraction) GC-Mass Spectrometry (MS) [4]. To achieve this, a glass housing was fixed to the skin on the volar forearm using surgical tape to create an enclosed area of skin headspace. SPME extraction profiles were monitored over time (5–30 min). This non-invasive approach has proven efficient in investigating the volatile composition of human odor. Another system developed in the literature to study evaporation *in vitro* is the use of Franz Cells. Franz Cells are designed to study the diffusion rate of actives and ingredients, particularly in terms of permeation, when applied to the skin. Almeida *et al* [5] examined the evaporation of three fragrance molecules: α-pinene, limonene, and linalool, using dynamic headspace analysis. The temperature was kept at 33°C to mimic human skin surface temperature and *ex vivo* pig skin was placed between the donor and receptor compartments. Similarly, Capetti et al. [6] monitored the

68 release of bioactive tea tree oil markers into the atmosphere on pig ear skin using modified Franz cells.
69 The systems proved effective at predicting the evaporation of fragrances on *ex vivo* surfaces, but no
70 optimization was carried out for performing *in vivo* measurements on human skin.

71 Many non-biological models have been used to study occurrences on the surface of the skin [3,7–10].
72 These surface models were employed to explore perfume evaporation [3], cosmetic product
73 application [7,8], and the penetration of active substances [9,10]. Among these models, the glass
74 surface prevents penetration and presents chemical inertia. Another commonly studied surface in the
75 literature is Strat-M®, a synthetic model specially designed to imitate diffusion through human skin.
76 Penetration experiments [9,10] have been carried out on Strat-M®, but it has never used to investigate
77 evaporation phenomena. To better understand perfume evaporation from the skin and to assess the
78 influence of specific parameters on the release of fragrance molecules, a methodology combining
79 evaporation studies of surfaces *in vitro* and on the skin *in vivo* could be the answer.

80 Some works have studied penetration *in vitro* [5,11, 6], evaporation *in vitro* [5, 6], or *in vivo* [2,4], but
81 very few studies have focused on both approaches using the same device [3]. Moreover, such studies
82 are relevant as they could enable the development of model surfaces that mimic the release and,
83 therefore, the olfactory perception of perfumes on the skin. Ideally, a modular device allowing dual
84 measurements *in vitro* on different surfaces and *in vivo* on the skin, with the potential to control key
85 experimental parameters, would be developed.

86 This current paper aims to establish this measurement device and an analysis method for evaporation
87 studies both *in vitro,* on different types of model surfaces, and *in vivo,* on the skin of volunteers. This
88 device should enable the modulation of several parameters, such as temperatures, headspace
89 volumes, measurement times, and exchange surfaces, simultaneously. The system was developed
90 based on Franz cells used for transdermal diffusion testing. A perfume composed of eight fragrance
91 molecules in ethanol was used to measure evaporation in the HS with SPME and GC-FID analysis.
92 Temperature control, time measurements, system seal integrity, volumes, and SPME optimizations
93 were investigated. Finally, the effectiveness and modularity of the system were demonstrated with
94 evaporation studies carried out on four different surfaces: a chemically inert glass surface, the Strat-
95 M® model, a perfume test strip, and the skin.

96 **2. Materials and methods**

97 2.1 Perfume composition and preparation

98 The first objective was to choose a mixture of molecules that differed in chemical structure and
99 physical properties to represent the chemical complexity in a perfume-type mixture. A summary table
100 detailing the selection criteria is given in Table 1. All the chosen molecules are soluble in ethanol, the

solvent for this mixture, and vary in their hydrophobicity (log P; P is the n-octanol-water partition coefficient), chemistry (ester, alcohol, or terpene), boiling temperature, vapor pressure, and different verified retention indices relative to C9-C30 n-alkanes on the DB5 column (RI).

Ethyl butyrate (CAS No. 105-54-4), ethyl heptanoate (CAS No. 106-30-9), and ethyl octanoate (CAS No. 106-32-1) were obtained from Sigma Aldrich. Ethyl decanoate (CAS No. 110-38-3) was obtained from Fluka, limonene from across Organics (CAS No. 5989-27-5), hexyl cinnamaldehyde (Jasmonal ®) (CAS No. 101-86-0), and myrcene (CAS No. 123-35-3) from Prodasynth, citronellol (CAS No. 106-22-9) from DRT and ethanol (CAS No. 64-17-5, purity 96%) from VWR.

Table 1: Summary table of the physicochemical properties (RI, logP, boiling point, vapor pressure, solubility, and molecular weight) of the eight molecules composing the perfume mixture, ranked according to their RI

Molecule	RI [12]	logP [13]	Boiling point (°C) [14]	Vapor pressure (mmHg at 25°C) [13]	Molecular weight (g/mol) [13]
Ethyl butyrate	804	1.85	121	14	116.16
Myrcene	992	4.17	167	2.1	136.23
Limonene	1030	4.57	176	1.55	136.24
Ethyl heptanoate	1080	3.33	188	0.68	158.24
Ethyl octanoate	1198	3.84	208	0.020	172.26
Citronellol	1233	3.91	225	0.020	156.27
Ethyl decanoate	1398	4.86	245	0.034	200.32
Hexyl cinnamaldehyde	1754	5.33	308	0.002	216.32

Each component was present at 1% (w/w) except citronellol and hexyl cinnamaldehyde, which were at 2% (w/w) in a 96% ethanol solution to best represent an “eau de toilette”, where fragrance ingredients are solubilised in the solvent and account for 10% of the total mixture. The perfume is kept at 4-6 °C between and after each application. The mixture of volatile molecules complies with IFRA51 regulations, with concentrations corresponding to the IFRA standard, and in thus *authorized in alcoholic perfumery (cat 4 IFRA) for skin application.*

2.2 Preliminary validation of the SPME experimental and analytical protocol for perfume evaporation: Semi-quantification of different fragrances

Parameters studied at this step include the equilibration time with a fixed trapping time, and then the trapping time with a fixed equilibration time, optimized in the previous step.

To achieve this, the optimization was performed in a classical closed 20mL amber glass vial. The mixture of molecules was applied to the bottom of the vial, and the system was sealed with a septum. The SPME technique was then used to extract and concentrate the volatile compounds present in the HS. The stationary phase grafted was a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) stationary phase. This coating was chosen for its affinity with volatile and semi-

volatile compounds from C3 to C20 [15,16], after preliminary tests, not shown here. After the equilibration time, which corresponds to the time given to molecules in the liquid phase to evaporate into the HS, the fiber was positioned in the HS above the solution during a second step, called the trapping time, which corresponds to the equilibrium between the solid phase of the fiber and the gaseous phase.

The compounds present after equilibration and trapping times on the fiber were then analysed using an Agilent 8860 GC with a Flame Ionization Detector (FID). They were desorbed in the injector with a split ratio of 1:25, at 260°C for 2 minutes for effective and complete injection. Compounds were separated on an HP-5 column (30 m x 0.32 mm x 0.25µm film thickness) with hydrogen as the carrier gas at a constant flow rate of 1.5 mL/min. The oven temperature program was initially set at 60°C, then raised to 160°C at a rate of 25°C/min, and then increased to 250°C at a rate of 40°C/min. The FID detector was maintained at 250°C, with an airflow rate of 300 mL/min, an H_2 flow rate of 30 mL/min, and an N_2 gas makeup flow rate of 25 mL/min. The components were separated with a resolution greater than 1.5 and identified according to their respective retention indices. The obtained chromatogram will enable the quantification of the compounds released into the headspace above the surface studied. Automatic integration of the peaks is performed by the Agilent software "Agilent OpenLab Control Panel", which provides the peak areas, enabling the quantification of each compound in the perfume mixture. Note that after each analysis, a fiber injection blank was performed to confirm the absence of residues.

2.3 The system adapted for *in vitro* and *in vivo* evaporation studies

To study the phenomenon of evaporation on specific surfaces, an innovative system was designed and developed to enable both *in vivo* and *in vitro* measurements, ensuring consistency of analysis and comparison of results. The principle of this setup is depicted in Figure 1 and is based on Franz cells, which are typically used to study penetration but, in the case of the present study, are adapted for evaporation studies. The Franz cell, obtained from Perme Gear.de, is composed of three different parts: the donor compartment (15 mm clear jacketed Franz Cell with a flat ground joint) where the samples are introduced, the receptor compartment (7.5 ml volume) where the diffused components are generally analysed, and the part in-between where the "model" membrane or material is inserted to conduct the permeation study. Interestingly, the temperature of the receptor compartment can be controlled. The adaptation to the purpose of the study consisted of modifying the height, the volume,

and the sealness of the donor compartment, which were the first three parameters studied and optimized.

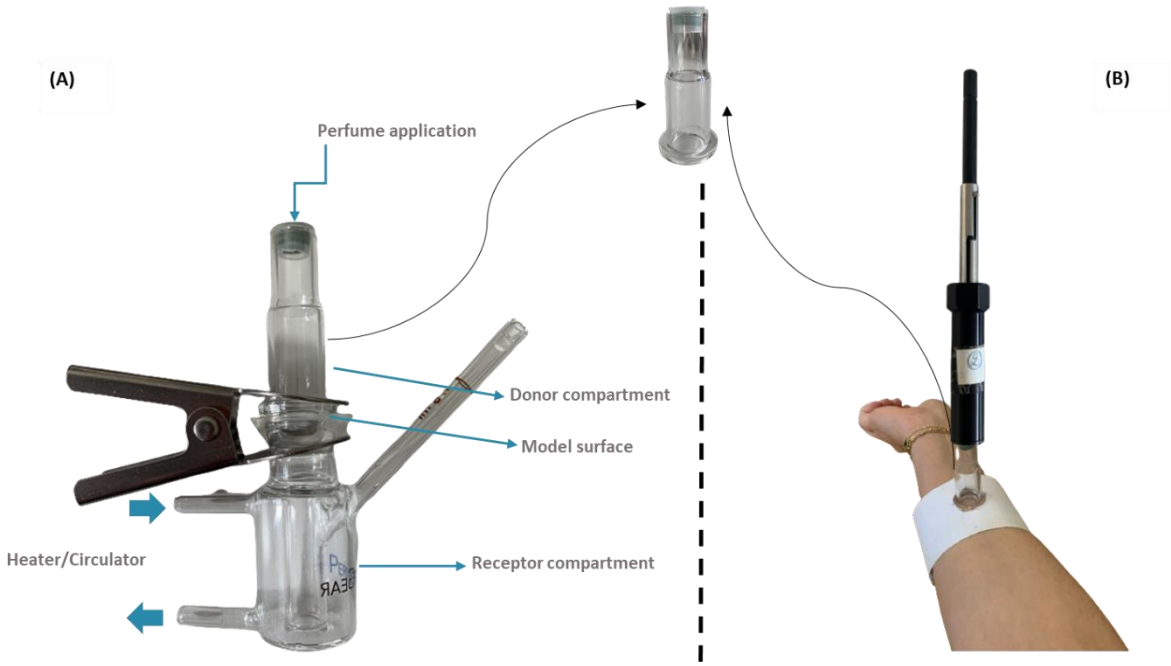

Figure 1: Set-ups: (A) *in vitro* using the Franz cell concept with the skin model positioned between the donor compartment and the receptor compartment, and (B) *in vivo*, using the same donor compartment as the Franz cell concept

Regarding sealness, based on a literature survey [5], parafilm or a 9 mm diameter Molded Thermogreen® LB-2 Septa GC septum were tested to obtain the best closed-sealed system. The donor compartment height, initially 20 mm, was also optimized and adapted for the total insertion of the SPME fiber and finally set at 50 mm with a final volume of 7 ml.

In the in-between zone, the studied perfume mixture was applied to various model surfaces, both *in vitro* (Figure 1A*)* and *in vivo* on the skin (Figure 1B), to test the system's flexibility for different kinds of supports.

Surfaces studied were:

For *in vitro* tests: i) glass lamellas from Grosseron as a chemically inert surface, ii) a perfume test strip, commonly used in perfumery iii) the Strat-M®, purchased from Merck, a model for permeation tests.

For i*n vivo* tests: the forearm of a volunteer, cleaned with ethanol before each application.

The surface was positioned between the donor compartment and the receiver, thermostated at 32°C. The mixture was applied to the 177 mm^2 surface, covered by the donor compartment, and the system was sealed at the top of the donor compartment and in the in-between zone with a clamp. The SPME technique was then used with the optimized equilibration and trapping times, enabling the analysis of the quantities evaporated from the studied surface by GC-FID. For the *in vivo* measurements (Figure 1B), the system was adapted as follows: the donor compartment was attached to a wristband, whose

tightness can be adapted to ensure a tight seal between the donor compartment and the skin. The same procedure was then followed from the application of the mixture to GC analysis, as illustrated with the setups in Figure 1.

2.4 Temperature control

The last parameter studied during the system optimization is temperature, which indeed impacts the evaporated quantities. Therefore, it must be controlled. To observe the influence of temperature on the evaporation of a specific molecule and compare it with that on the skin, where the average temperature is 32°C, three different temperatures were selected: 27, 32, and 37°C for the receptor compartment.

2.5 Data analysis

The statistical analyses of collected data were performed using XLSTAT software from Addinsoft (version 2012). All the measurements were carried out in triplicates, and then the average values, the standard deviations and the coefficients of variation (ratio of the standard deviation to the mean) were calculated. An analysis of variance (ANOVA) was applied to the data to determine when the temperature effect was significant ($P \leq 0.05$).

3. Results & Discussion

The objective was to set up a measurement device and an analysis method to monitor the evaporation of fragrance molecules on various surfaces, both *in vitro* on different types of model surfaces and *in vivo directly* on the skin of volunteers. This device would allow for the modulation of key parameters such as temperatures, headspace volumes, measurement times, exchange surface, and more. As a first step, SPME parameters were investigated and optimized in glass vials, which serve as a starting point of a simple setup where various optimizations were carried out to achieve efficient results and coherence with the main objective of the system: studying perfumes and fragranced cosmetics. The device was then developed and adapted to glass lamellas, with optimizations made on the system's sealness, volume, and temperature control. Finally, the effectiveness and the modularity of the system were demonstrated through evaporation studies carried out on three other surfaces: a perfume test strip, the Strat-M® model, and the skin surface.

3.1 Optimization of equilibration and trapping times using SPME

To study and understand the evaporation phenomena occurring after the application of a perfume on a surface, the first parameters investigated were the equilibration and SPME trapping times. The objective was to evaluate the influence of each time phase on the evaporated quantities and then select the most appropriate times that will enable the quantification of the different fragrance molecules. According to the literature [4], three different equilibration times, 5, 15, and 30 minutes,

were tested with a constant trapping time of 5 minutes. Then, three different trapping times, 5, 15, and 30 minutes, were tested with a constant equilibration duration of 15 minutes, optimized in the previous step. Alongside the technical aspects that guided the choices for this study, the study was also adapted for *in vivo* use on assessors. The measurement times had to maintain a balance between performance and feasibility. Indeed, for *in vivo* studies, it is challenging to exceed 30 minutes.

Results are shown in Figure 2 for ethyl heptanoate, an intermediate compound concerning volatility. Areas increase between 5 and 15 minutes and then remain constant until 30 minutes, both for equilibration time (Figure 2A) and trapping time (Figure 2B), demonstrating appropriate conditions allowing for optimized measurement duration and quality in the obtained results. For the other molecules of the perfume, results have shown that the balance is quickly established for the small, volatile molecules but it is more difficult to achieve for the less volatile molecules. A judicious choice must therefore be made to give all types of volatile molecules time to adsorb and/or absorb onto the fiber. According to the literature, it is important to operate very close to equilibrium conditions to ensure reproducibility [17]. The molecules present in a fragrance have a wide range of volatility, which is why a compromise must be made between trapping time and balance to reach the equilibrium of most molecules. In the case of this study, consistent results with good repeatability for all perfume molecules were obtained at equilibration and trapping times set at 15 minutes. These time conditions are similar to those set by Duffy *et al.*[4].

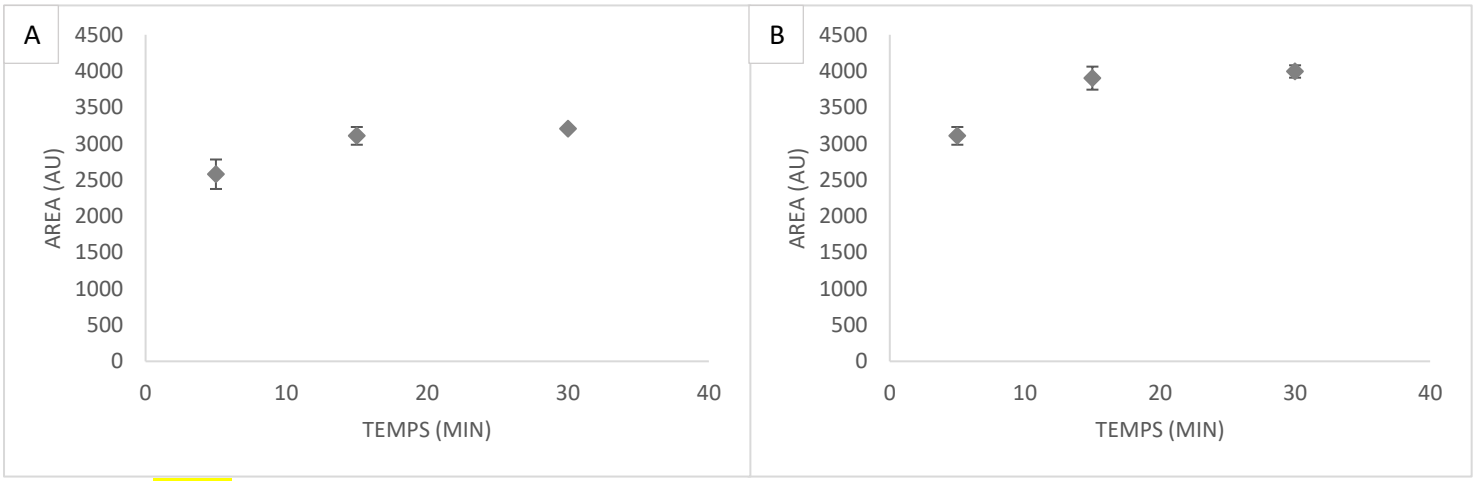

Figure 2: Areas in triplicate reflecting the quantities evaporated, obtained for ethyl heptanoate by GC-FID when applied on a glass surface A) for three different equilibration times, 5, 15, and 30 minutes, and a constant trapping time of 5 minutes, B) for three different trapping times, 5, 15, and 30 minutes, with a constant equilibration time of 15 minutes

3.2 System optimisation

The described system, as already mentioned, is based on Franz cells, adapted in the case of this study for evaporation studies. Once the SPME equilibration and trapping times for perfume analysis were chosen, modifications were made to the initial donor compartment concerning its sealness to ensure the repeatability of measurements, as well as its height and volume. The different modifications of this

part of the device were carried out with glass lamellas in the in-between zone. The advantage of using glass is that it is a chemically inert surface with no porosity, thus eliminating any permeation phenomena, allowing for the sole consideration of evaporation phenomena.

3.2.1 Sealness of the device

The areas of the eight fragrance compounds are presented in Figure 3. The compounds are sorted in order of retention time. Two sealness conditions were compared: parafilm and a septum aluminum cap. In the initial setup introduced by Almeida *et al* [5], parafilm is positioned at the top of the donor compartment. Parafilm makes the system sealed, which is suitable for penetration studies. However, for evaporation studies and the comparison of fragrance retention on surfaces, parafilm is not ideal. This is because it can potentially retain molecules, leading to interactions between the parafilm and the fragrances. Therefore, the parafilm was replaced with a septum aluminum cap, which is specially designed to avoid interactions with the mixture of fragrances. The peak areas shown in Figure 3 indicate higher quantities evaporated with the aluminum cap for all compounds. It should be noted, however, that the results show significant standard deviations, particularly for ethyl heptanoate, ethyl octanoate, and hexyl cinnamaldehyde. As the standard deviations are still not optimal, further improvements to the system are necessary.

3.2.2. Height of the donor compartment

Considering the feasibility of the system *in vivo*, it was necessary to ensure that the height of the system could be adjusted to maintain reproducibility of measurements. The height must be sufficient to allow for the release of the SPME fiber's full length while also maintaining a 5 mm distance from the skin. Therefore, a higher donor compartment of 55 mm (DC55), replacing the initial 20 mm one (DC20), was set up with the septum cap on top. Results are shown in Figure 3. Areas decreased significantly for most compounds except for the least volatile ones (citronellol, ethyl decanoate, and hexyl cinnamaldehyde). This decrease can be attributed to the increase in the HS volume, which modifies the equilibrium conditions. The increased volume favors the transition to the gaseous phase. Consequently, small molecules may remain in the HS without being adsorbed and/or absorbed onto the fiber, while heavier molecules, which are more concentrated in the HS, can more easily exchange with the stationary phase of the fiber. Additionally, a reduction in standard deviations was observed. In conclusion, the developed device has proven to be sealed, providing repeatable measurements

under fixed conditions, allowing for the evaporation of any type of fragrance molecules, whether volatile or less volatile.

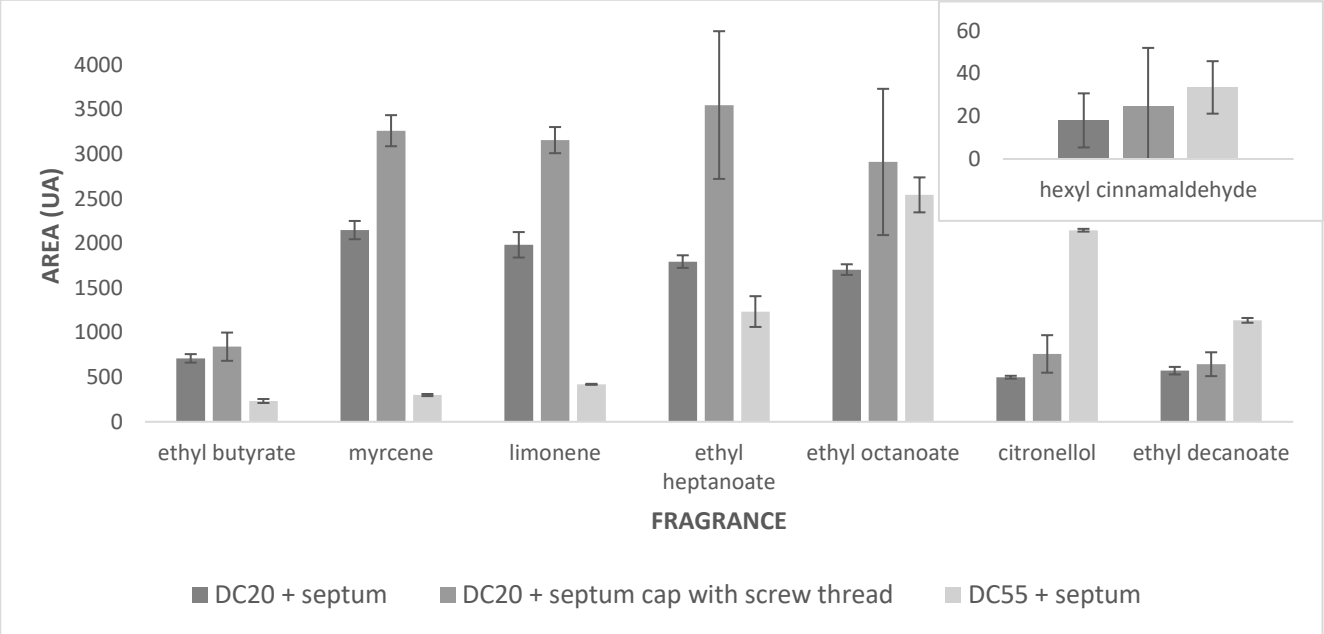

Figure 3: Average area reflecting the quantities evaporated obtained for each compound by GC-FID when the fragrance mixture is applied on a glass surface for three different systems with their respective standard deviations, ranked according to their RI

3.2.3. Temperature control

Experimental observations on 10 volunteers revealed that skin surface temperature can vary, even within the same zone, with temperatures ranging from 27.5 to 31.9. Additionally, a notable difference in skin surface temperature can be observed depending on the area of the body [18]. Given that fragrances can be applied to different parts of the body, temperature variation can significantly impact the evaporation phenomenon and must be considered. To highlight the influence of skin temperature on molecule evaporation, three biological temperatures were selected: 27, 32, and 37 °C for the receptor compartment.

The results presented in Figure 4 highlight the effect of temperature on the evaporated quantities of the different fragrances. Two different behaviors were observed according to ANOVA statistical tests ($P \leq 0,05$): firstly, for some compounds, no significant effect was observed with increasing temperature (Figure 4A), whereas a significant increase with temperature was noticed for others (Figures 4B and C). At a constant temperature, when equilibrium is reached, a partition is established between the solid phase of the SPME fiber and the gaseous phase on one hand, and between the residual liquid phase of the fragrance mixture and the gaseous phase on the other hand. Increasing temperature disturbs this thermodynamic equilibrium and shifts the balance towards the air. This is observed experimentally: for the most volatile compounds such as ethyl butyrate, myrcene, and limonene, the evaporated quantities remain constant since their equilibrium hardly depends on temperature.

Conversely, for less volatile molecules like ethyl heptanoate, ethyl octanoate, citronellol, ethyl decanoate, and hexyl cinnamaldehyde, their evaporation is more temperature dependent. Therefore, an increase in evaporated quantities is observed experimentally for the least volatile fragrances as the temperature rises.

In conclusion, skin temperature variations can indeed impact fragrance release into the headspace. Thus, for *in vitro* and especially *in vivo* measurements, controlling the temperature parameter during evaporation studies is essential. In the *in vitro* system, temperature is controlled with water circulation in the receptor compartment, maintaining the temperature of the donor compartment and the studied surface where the mixture is applied constant. To ensure the studied surface is at the desired temperature at the time of measurement, the surfaces are allowed to equilibrate for 15 minutes before fragrance application. For *in vivo* studies, the volunteer is kept at a controlled room temperature, and the skin temperature is measured following a strict protocol.

The *in vitro* temperature was set at 32°C to represent a realistic average skin temperature, which can easily vary depending on the area of skin.

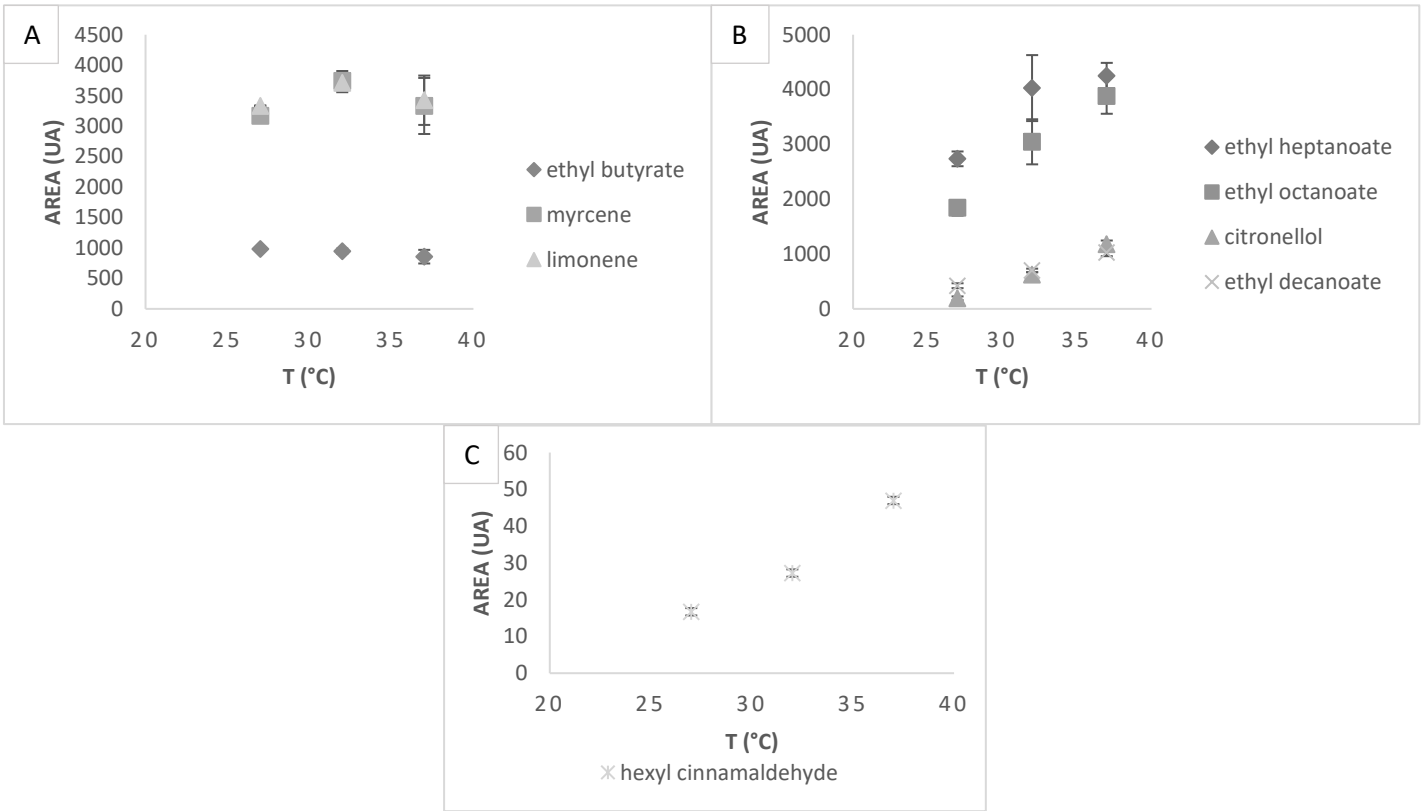

Figure 4: Average area reflecting the quantities evaporated obtained for the eight compounds by GC-FID. The fragrance mixture was applied on a glass surface for three different system temperatures: 27°C, 32°C, and 37°C

3.3 Adaptability of the system to study evaporation *in vitro* and *in vivo*

After the development of the device as presented previously, the effectiveness and modularity of the system were investigated on other surfaces, both *in vitro* and *in vivo.* The fragrance release was carried

out on three other surfaces: on the Strat-M® model, a perfume test strip, and the forearm of a volunteer. Studying these different surfaces enabled us to demonstrate the effectiveness of the system and gain a better understanding of the evaporation phenomenon.

The evaporated quantities obtained for the eight fragrance compounds are shown in Figure 5 for the four scented surfaces. Repeatability was demonstrated for each of the surfaces, proving the modularity of the device both *in vitro* and *in vivo*. The obtained results show the repeatability of the measurements (according to SPME standards) with coefficients of variation ranging from 1 to 17% for all compounds except hexyl cinnamaldehyde, for which coefficients of variation varied between 7 to 33%. It is more complicated for this compound as it has difficulty in evaporating, resulting is smaller chromatographic areas and greater fluctuation from one measurement to another, leading to slightly less accurate results.

The skin is known to have a specific surface physicochemistry due to its structure and composition [19]. Therefore, it was important to prove that the adjustments made to the system on glass were also effective on this complex biological surface. Consequently, the last part of results will be presented as follows: the results obtained and validated on the skin and complementary surfaces.

Figure 5A shows the release profile of the model perfume from the skin, with mean areas varying according to the volatility of the compounds and their affinity with the skin. Surface properties of the skin play an important role and influence skin interactions with different fragrance molecules. For example, lipids on the skin will accentuate interactions with more hydrophobic compounds with a higher logP [3]. This is probably the case of the hexyl cinnamaldehyde with a logP of 5.33 (Table 1). The presence of certain lipids on the skin, such as free fatty acids (FFA), makes the skin surface a monopolar basic surface [20]. Indeed, FFA exhibits carbonyl groups which can form hydrogen bonding and are enriched in electrons, thus increasing interactions with water and enhancing a surface monopolar basic behavior [21]. This means that some lipids of the skin could also interact with polar compounds. However, it is important to note that the release phenomena of fragrances on the skin is complex, and other factors such as intrinsic volatility of the molecules, potential absorption in the *stratum corneum* (SC), skin roughness and porosity, and water retention, also play a role in the kinetics of fragrance molecule evaporation.

Similar analyses were conducted on three non-biological surfaces: Strat-M® model (Figure 5B), perfume test strip (Figure 5C), and glass (Figure 5D), as discussed previously. Obtained areas varied according to the surface. For instance, the Strat-M®, a multilayer surface composed of polyethersulfone, polyolefin, and a lipid layer, exhibits similar areas for ethyl butyrate, myrcene, and limonene but much lower areas for the other compounds compared to *in vivo* measurements. Such

variations in the release profile of a perfume may have a strong influence on its olfactory perception both in intensity and quality. Although the Strat-M is a good candidate for imitating skin diffusion, does not exactly mimic the highly organized intercellular structure of the SC. Indeed, with a relatively thin SC layer, it provides a low-tortuosity pathway which is probably the reason for higher permeability and therefore less retention on the membrane [10]. Even though the Strat-M® has proven to be a good candidate for particular studies on the skin [9,10], it still does not exactly mimic the evaporation/absorption distribution that occurs on the skin after applying a perfumed cosmetic product. Likewise, the perfume test strip, widely used in the perfume industry, made mainly of cellulose, exhibited an evaporation behavior closer to that on the skin due to its hydrophilic and hydrophobic characteristics. Finally, the glass surface, chosen for its chemical inertia, after application of the perfume, spreading of the mixture is observed on the glass substrate, which proves that interactions do take place between the surface and the mixture of compounds. Despite being a completely different surface compared to the other surfaces studied, exhibited a similar evaporation trend to that of the skin.

While some interpretations have been provided for initial understanding of the results, the core of this article is to highlight an original innovation in terms of effectiveness, repeatability of measurements, and modularity *in vivo* and *in vitro* by adapting to any type of surface. These results underscore the device's capability to differentiate perfume evaporation on different surface types.

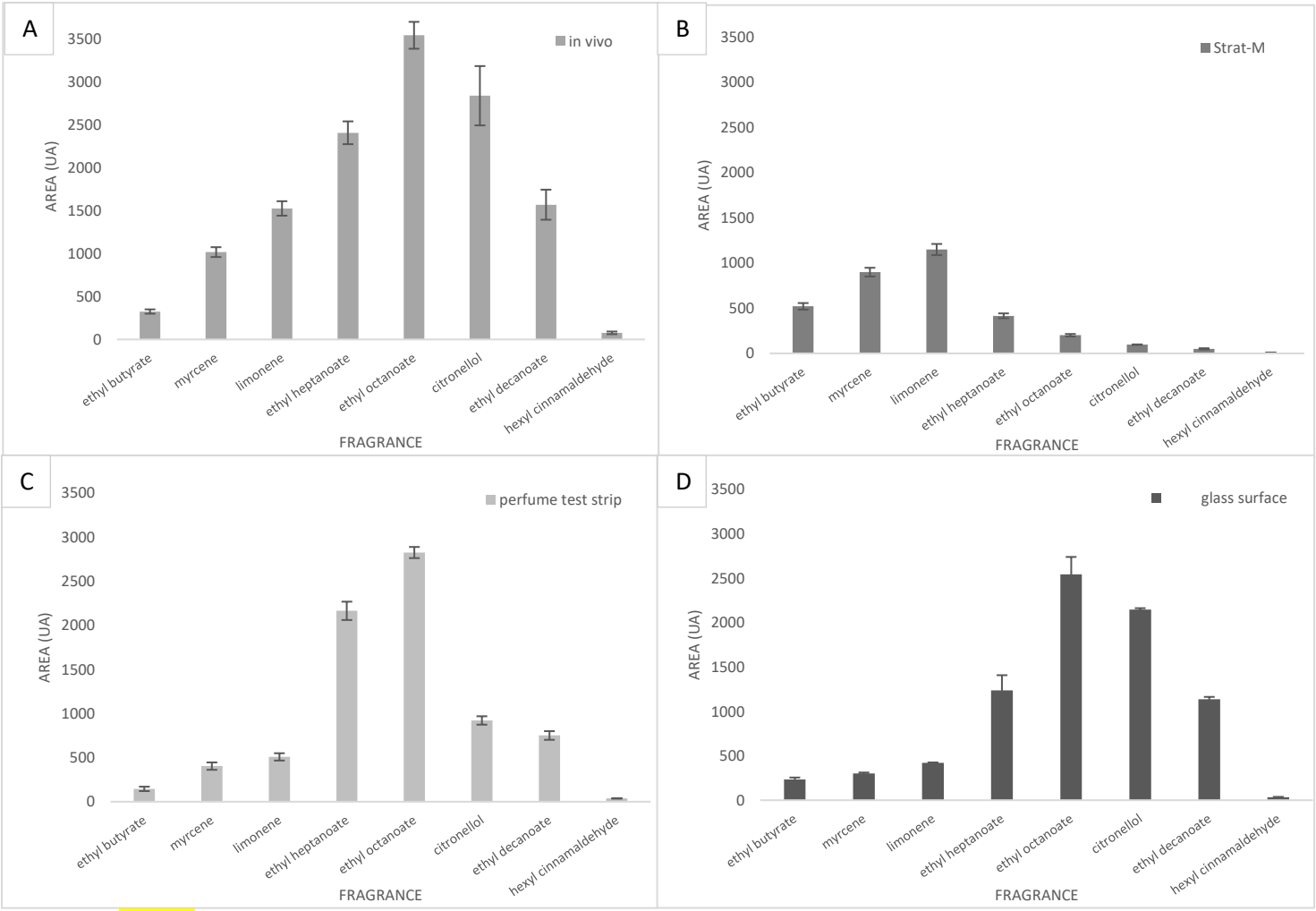

Figure 5: Average area reflecting the quantities evaporated, obtained for each compound by GC-FID, when the fragrance mixture was applied on the skin (A), on the Strat-M® surface (B), on a perfume test strip (C), and on a chemically inert glass surface (D) ranked according to their RI

4. Conclusion

This paper presents a methodological development of a novel device that makes it possible to measure perfume release in the air above a surface. This device has proven its originality, effectiveness, and repeatability both *in vitro* on different types of model surfaces and *in vivo* directly on the skin of volunteers' forearms. A model fragrance composed of eight compounds in ethanol was studied using HS analyses with SPME GC-FID. This study has shown the influence of equilibration and trapping times on the evaporated quantities, which helped us select the most appropriate conditions for semi-quantification of different fragrances. With targeted measurement times and optimizations made on the system's seal, height, volume, and temperature control, the measurement device and the analytical method were subsequently set up. The modularity of the system was then demonstrated with evaporation studies carried out on four different surfaces: a chemically inert glass surface, the

Strat-M® model, a perfume test strip, and the skin. Further studies can be conducted to evaluate the system's modularity on surfaces such as textiles [22,23] or even *ex vivo* skin.

The results obtained from this study underscore the fact that retention of fragrance molecules *in vivo* varies according to their affinity with the skin, their potential interactions, and their intrinsic volatility. The device developed in this study can provide a better understanding of the evaporation phenomena of fragrance molecules and its link with the physico-chemical properties of the skin. The study of fragrance release on *in vitro* model surfaces would offer a solution for standardized fragrance evaluation and a better understanding of retention on the skin. This approach eliminates skin inter-individual variability and facilitates the evaluation of the influence of specific parameters on the retention of fragrances. It will also be capable of determining which materials exhibit skin-like behavior in terms of evaporation phenomena. The next step will be to go even further than fragrance molecules in ethanol but also investigate in greater depth fragrance and active ingredients release in more complex cosmetic products after application on the skin.

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