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Comparative Study of Odours Present in Twin Fragrances by GC-sniffing-ToFMS

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Fragrances have the power to generate an identity and evoke memories and sensations based on their characteristic raw materials. These raw materials correspond to volatile organic compounds (VOCs) present at concentrations above their odour threshold value in gaseous phase. Knowledge about the fragrance formula allows create modifications of the fragrance or to launch an imitation. These products try to evoke the smell of the original fragrance whereas being commercialized at more affordable prices to the end consumer. Consequently, it is important to establish limits of sensory identity, or put in another way, to quantify the limits where the imitation fragrance does not jeopardize the identity of the original. Defining sensory delineations is a difficult task because of the subjective nature of sensorial experience. However, it is becoming increasingly possible to devise unambiguous methods due to recent technological advancements in the sensitivity of instruments. It can be done by means of instruments capable of acting as human noses, or rather, acting as a powerful combination of nose-instrument complexes. These complexes take advantage of the complementary capacities existing between the high sensitivity of the human olfactory system and robust instrumental analysis techniques. GC-Sniffing, a human-instrument complex, is the most powerful analytical technique for odour identification. It can obtain chemical and sensory information in a single chromatographic analysis, with an experimented analyst detecting odours at the same instant in which the chemicals responsible for those odours are being chemically detected by the instrument.
This study analyses two similar samples by GC-Sniffing-ToFMS, one of them commercialized as an imitation of the other, with the purpose of finding those sensory and chemical differences that make each one of them unique. The results show different concentrations of odour compounds and sensory perception by GC-Sniffing-ToFMS in the original and its perfume-twin. Some odour compounds were identified as key markers to understand sensory differences between the twin fragrances under study.

1. Introduction

The global market for Fragrances and Perfume is forecast to exceed US$40 billion by 2020 (GIA, 2015). 83% of women wear perfume occasionally and 36% daily. The successful of this powerful industry is due to its extensive marketing, high profit margins and a careful choice of the target audience. Retail price of perfumes give to the company a 95% profit and only 3% of the cost spent on the production and ingredients. Because perfumes can be associated with a brand, large establishments (clothing stores try that people can recognize its own perfume to enter) or companies with environmental awareness, want to strengthen their image by creating a perfume for their consumers without animal testing or synthetic chemicals as part of their business strategy. Increasingly, applications multiply and the market increases, so it becomes more necessary the existence of protocols to describe uniquely fragrances of each brand, and even to patent its formulation, and also to differentiate between an original perfumes and its copy.
Determination of the chemical composition of a fragrance is a complex task. On average, 30 to 50 (and sometimes up to 200) ingredients, synthetically manufactured, as well as natural extracts or essences, are used to create a fragrance composition (Curtis, et al., 2001). Fragrance analysis has radically changed over the last 15–20 years, this is mainly to the rapid evolution of analysis techniques and the fast increase in the number of chemical analyses a routine laboratory is required to run. The analysis of aroma compounds in

fragrances, based on detection and identification of volatile organic compounds (VOCs) is preferably carried out by gas chromatography (GC) in combination with mass spectrometry (GC-MS). Although GC-MS can provide chemical identification of volatile spices in fragrances, it does not provide qualitative information about sensory perception of the aroma molecules.

GC-Olfactometry (GCO) or GC-sniffing technique improves the performance of GC-MS systems in terms of odour analysis because it allows to obtain a sensory description (by a trained assessor) of the each fragrance molecule eluted from a chromatographic run while at the same time the these molecules are identified by a chemical detector, commonly MS (Guichard, et al., 1995; Mayol and Acree, 2001). In this way, a system GCO-MS is probably the most powerful technique for chemical characterization of odorous compounds and fragrance analysis (Bratolli et al., 2013; Delahunty et al., 2006).

Sensitivity has a crucial role in this type of analysis. Odours are detected by the human nose at very low concentrations (low ppt), so it is necessary the use of highly sensitive detector in this type of instrumental configuration to avoid, to the extent possible, cases of odour detected by the nose without a spectral signal.

Time of Flight-Mass Spectrometer (ToFMS) is the most sensitive chemical detector able to detect molecular traces at concentration levels of a 100 times lower than standard MS detectors (Vera et al., 2013). Linking the molecular information provided by a GC-ToFMS with the perceived intensity and odour description by the sniffing technique, allows detailed understanding of the key odour impact molecules present in the perfume.

The main objective of this study is to compare the odours perceived between perfume-twin with their respective chemical identification through TD-GCO-ToFMS (Thermal Desorption-Gas Chromatography-Olfactometry-Time of Flight Mass Spectrometry).

2. Instrumentation

The chromatographic analyses were carried out by a thermodesorption unit (TD) coupled to a Gas Chromatograph-Mass Spectrometer (GC-ToFMS) instrument. The instrumental system is composed of a Gas Chromatograph (Agilent 7890 model, Agilent, USA), Time-of-Flight Mass Spectrometer (BenchTOF-dx model, Almsco, Germany) and Thermal Desorption (Unity model, Markes International Limited, Llantrisant, UK). The column used was the mid polar DB-624; (60m, 250µm, 1.4µm; Agilent, USA).

The deconvolution process providing the chemical identification from the GC-MS data was carried out by using TargetView V3 (software developed by ALMSCO International, Germany).

The sniffing analysis was performed by an olfactory detector port OP275 (GL Sciences Inc., Japan).

The type of sorbent tubes used was TenaxTA/Carbograph5 (Markes International Limited, Llantrisant, UK). All tubes, before used were conditioned by TC20 (tube conditioning-20) (Markes International Limited, Llantrisant, UK).

A manual pump (EasyVOC, Markes International Limited, Llantrisant, UK) was used to capture the volatile compounds in gas phase to the sorbent tubes

3. Experimental procedures

3.1 Sample preparation

For this study, one women perfume-twin was selected (Perfume A and B). In two different samplings for each perfume, 25µl of perfume was direct injected on a Nalophan bag with 1 L of inert gas (nitrogen 5.0). After 10 minutes of equilibration time, 100 ml of the headspace was collected into a sorbent tube through a manual pump. Additionally, one thermodesorption tube without sample and under the same sampling conditions than real samples was collected as a blank.

3.2. Instrumental method

Desorption of the analytes retained on the TenaxTA/ Carbograph5TD sorbent tubes was carried out in a Unity Thermal Desorption system. In the primary desorption, tubes were heated to 300ºC with a helium flow rate of 50 mL min^{-1} during 8 min. This was done to desorb the analytes which were refocused on a hydrophobic general purpose cold trap, filled with Tenax TA and a graphitised carbon, cooled at 10ºC. After flash-heating of the cold trap at 320ºC during 5 min, analytes were injected into the chromatographic column.

Separation and detection were performed in a 7890N gas chromatograph and Time-of-Flight Mass spectrometer, using a mid-polar DB-624 capillary column (60m, 250µm, 1.4µm) and helium gas (6.0) as the carrier at a flow rate of 1.5 mL min^{-1}. The oven temperature of the GC was initially held at 40ºC for 5 min, then raised to 165ºC at a rate of 13ºC min^{-1} and then raised again to 230ºC at a rate of 4ºC min^{-1} and held at that temperature for 5 min. The GC-MS interface was set at 230ºC. The mass spectrometer acquired data in scan mode with an m/z interval from 47 to 400, operating at an electron impact energy of 70 eV.

The odour-active VOCs directed to the sniffing port were detected and characterized by two trained assessors at 22ºC. As soon as the assessor detects an odour, attribute, durability and intensity values (from 1 to 5) are assigned (Table 1). On an additional run, the Mass Spectrometer detects the chemical species in the sample by using the same instrumental methodology for the posterior identification of chemical compounds, both odorous and odourless. During the analysis, each assessor takes on the GC-sniffing task for 18 minutes before handing over to the other assessor, to cover the 36 minutes (approx.) of the whole chromatographic process. In this way the sniffing task is divided in 2 periods covering the entire chromatogram. Each assessor covers all chromatographic runs twice, so that each assessor covers the entire chromatogram, to confirm those doubtful odours and /or try to find other additional odours not detected in the previous sniffing. Therefore, two sample injections are necessary to analyse each sample. The analysis of the blank and the real sample were analyzed in duplicate.

Table 1: Scale of values used to describe the odours perceived

Value	Mean	Characteristic
1	Faintly perceived	Difficult to describe
2	Perceived noticeable	Noticeable
3	Strong	Very well perceived
4	Very strong	Nasal saturation
5	Extremely strong	Only extreme cases (cause interruption of sniffing process)

3.2 Data processing

All compounds detected by MS were chemically identified using TargetView software referencing to the NIST11 spectral library. Compounds above 80% of similarity were considered to be identified. Odours detected by sniffing only were confirmed after being detected at least twice out of the total 4 analyses of the sample. Those odours identified just once were discarded. Before assigning a chemical compound name (chemical structure) to each of the odours perceived, four checks were performed: 1) direct check through MS identification, 2) Candidates search based on Kovats index and odour descriptor. An additional tube containing C6-C18 hydrocarbons was analysed to find their retention times on the GC method used. These retention times are used as reference to calculate the Kovats index of the rest of compounds, from a defined Kovats index value assigned to hydrocarbons. 3) Automatic scan by using a library prepared exclusively for this project containing all relevant candidate compounds and their retention times. 4) Exhaustive manual searching based on the presence of target ions of candidate compounds in key retention times.

4. Results and discussion

In general, the results of the GCO -ToFMS analyses show that the perfume A has a similar chromatographic profile in comparison to the perfume B (Figure 1). 63 and 54 VOCs were identified in perfume A and B respectively. The comparative graph of Figure 2 shows that the highest concentration of compounds identified in the samples were terpenes and esters.

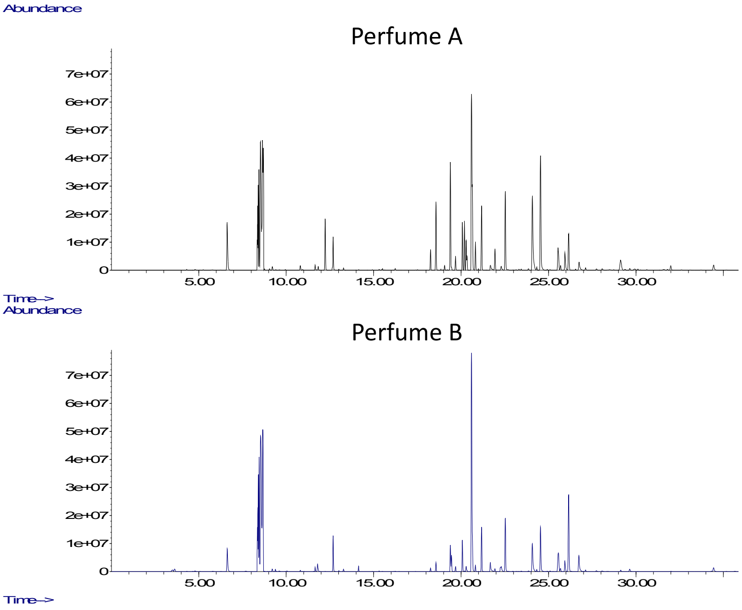

Figure 1. GC-MS chromatograms of perfume A and B.

Figure 2 shows a slight difference in the concentration of terpenes for perfume A compared to B. Out of a total of 33 terpenes identified in both samples 32 were identified in perfume A and only 22 in perfume B. Terpenes identified only in perfume A were: α-gurjunene, α-bisabolene, α-guaiene, Longifolene, (E)-beta-Famesene, Germacrene D, alloocimene, Linalool, Carveol, 3-Carene and C10H16 terpene. 4(10)-thujene was identified only in perfume B. Figure 3 shows the overlapped chromatograms in the range 30-35 minutes containing some terpenes and guaiyl acetate.

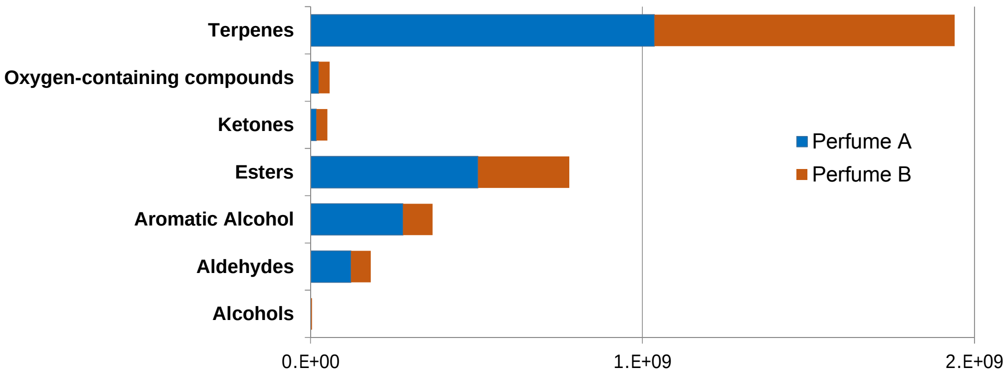

Figure 2. Comparison based on chemical families for both perfumes (A and B).

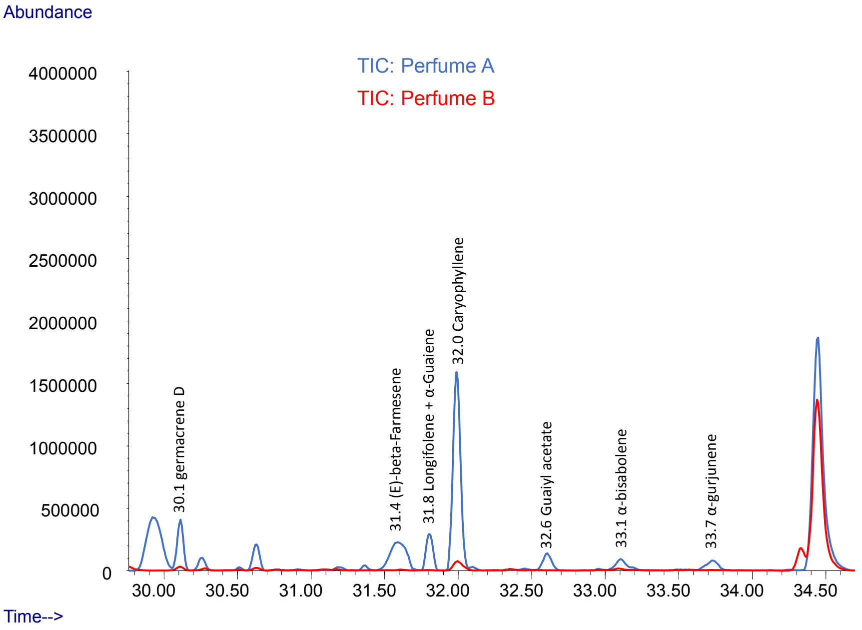

Figure 3. Overlapped chromatograms showing some of the identified terpenes and guaiyl acetate.

Of the total odours obtained in the GC-sniffing, we only show the most relevant odours with its chemical identification (Table 2). According to the information in table 2, important amount of terpenes and esters were identified in both perfumes. On the other hand, isoprenyl acetate, guaiyl acetate (esters), beta.-Myrcene, (-)-β-Pinene, Longifolene, α-Guaiene (terpenes) showed the highest intensity values in the chemically identified odours for perfume A compared to B. The assessors perceived these compounds as woody, green, minty, sweet and floral. Conversely, we have identified the terpene 4(10)-thujene at 19.5 min only in perfume B giving a turpentine and woody notes. Figure 4 shows little differences between perfume A and B in the intensities of sensory profile for floral, woody and citrus notes. These differences are key to the overall smell of each perfume.

Table 2: Odorants perceived during sniffing process and chemically identified by ToFMS

					Intensity [b]	
IK [a]	RT	Odour descriptor	Compound	CAS	Perf A	Perf B
734	17.5	Woody, green, minty	Isoprenyl acetate	5205-07-2	2	1
760	18.6	Pine, turpentine	α-Pinene	80-56-8	3	3
777	19.4	Anise, fruity, green, sweet	beta-Myrcene	123-35-3	2	1
781	19.5	Turpentine, woody	4(10)-thujene	3387-41-5		2
783	19.7	Anise, minty	(-)-β-pinene	18172-67-3	3	1
795	20.3	Pine, minty	beta-Ocimene	13877-91-3	2	2
807	20.8	Minty	ß-Phellandrene	555-10-2	2	2
816	21.2	Citrus, herbaceous, turpentine	gamma-Terpinene	99-85-4	3	3
851	22.6	Floral	Linalyl anthranilate	7149-26-0	3	3
862	23.0	Fruity, herbaceous, sweet	Methyl benzoate	93-58-3	3	3
870	23.4	Fruity, sweet	Linalyl hexanoate	7779-23-9	2	2
895	24.3	Citrus, floral	β-Terpinyl acetate	10198-23-9	3	3
900	24.5	Floral, fruity, sweet	Benzyl acetate	140-11-4	3	3
914	25.1	Floral, sweet	4-Terpinenyl acetate	4821-04-9	3	3
924	25.5	Floral, minty, oily	α-Terpineol	98-55-5	1	1
941	26.1	Citrus, sweet	Nerol	106-25-2	3	3
952	26.7	Sweet, minty	Lavandulyl acetate	25905-14-0	3	3
1105	31.8	Woody	Longifolene+α-Guaiene	475-20-7 / 3691-12-1	2	
1110	32.0	Spicy, woody	Caryophyllene	87-44-5	3	3
1127	32.6	Floral, fresh	Guaiyl acetate	134-28-1	2	

[a] Kovats index: normalized ratio of the retention time eluting n-alkane. [b] Scale from 1 to 5: 1, very low; 2, low; 3, medium; 4, strong; 5, extremely strong.

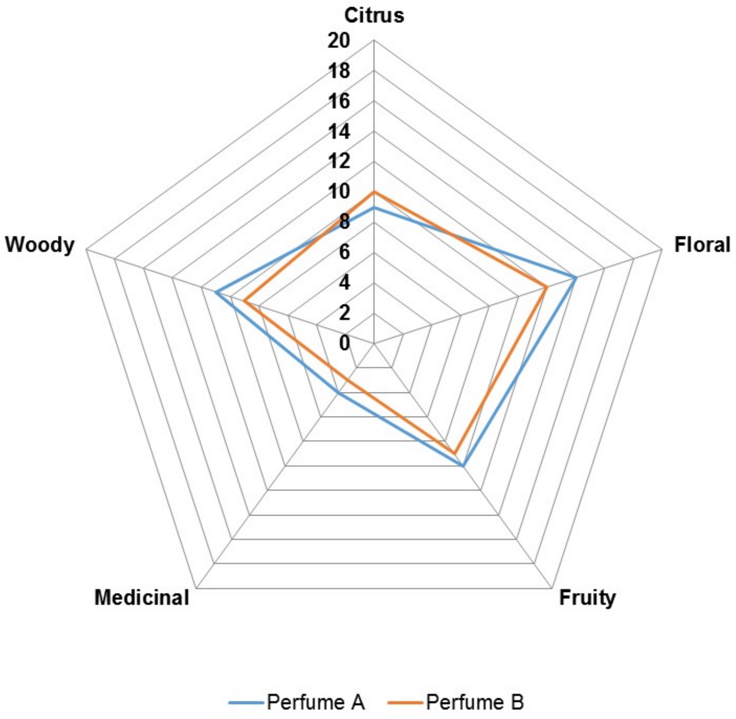

Figure 4. Sensory profile (sum of intensity) of perfume A and B based on odor descriptor and intensity.

5. Conclusions

With the made findings we conclude that GC-Sniffing methodology is a useful and powerful tool to determine differences in perfume twins. Both chemical and sensory results showed clear differences in the original perfume and its twin. Some terpenes (beta.-Myrcene, (-)-β-Pinene, Longifolene, α-Guaiene) and esters (isoprenyl acetate, guaiyl acetate) were identified as key odour impact molecules for the original perfume providing important differences in some notes as anise, floral, fruity and sweet.

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