

ANALYSIS OF PERFUMES WITH SYNTHETIC HUMAN PHEROMONES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Abstract

Currently there are many pheromone containing perfumes aggressively promoted by various websites. Because these products usually elude the control of any national or international authorities or regulating bodies they expose consumers to many potential frauds, and more importantly, to various health associated risks. The aim of the present study was to confirm the presence of pheromones, identify their nature and assess their relative concentration. Twelve commercially available perfumes claiming to contain human pheromones have been evaluated by gas chromatography-mass spectrometry (GC-MS). Synthetic human pheromones were identified only in seven perfume samples. Considering the obtained results and taking into account the numerous knowledge gaps regarding the effects of synthetic pheromones on human behaviour, potential gender-based effects and the absence of any quantitative study correlating hormone exposure and observed effect, the perfume manufacturers do not seem to follow any scientific protocol in the design and fabrication of these products.

Rezumat

În prezent există numeroase parfumuri cu feromoni care sunt promovate în mod agresiv de diferite *website-uri*. Aceste produse nu sunt supuse nici unui control din partea autorităților reglementate în domeniu, expunând astfel consumatorii unor potențiale fraude, dar mai ales pot pune în pericol sănătatea acestora. Scopul acestui studiu a fost confirmarea prezenței feromonilor în parfumuri, identificarea tipului acestora și determinarea concentrației relative ale acestora. Au fost evaluate 12 parfumuri, care pretind că ar conține feromoni sintetici umani, prin gaz cromatografie cuplată cu spectroscopia de masă (GC-MS). Feromonii sintetici umani au fost identificați doar în 7 din cele 12 parfumuri analizate. Având în vedere aceste rezultate, a faptului că există puține date în ceea ce privește efectele feromonilor asupra comportamentului uman și absența studiilor privind corelarea concentrației feromonilor cu efectele acestora, putem concluziona că producătorii nu utilizează nici un protocol științific la fabricarea acestor parfumuri.

Keywords: perfumes, pheromones, human synthetic hormones, gas chromatography-mass spectrometry

Introduction

The influence of pheromones on human socio-sexual behaviour represent a great interest for the scientific world and remains a subject of debate because of the lack of persuading and indisputable experimental evidences which make some authors to be sceptical regarding their action. Additionally, up to now only few well-controlled studies regarding the physiological properties of pheromones in humans have been conducted [1, 2]. Pheromones in humans are mainly produced by apocrine glands and they can induce a behavioural response or physiological change [2, 6, 7]. Some of the most important human pheromones are androstenone, androstadienone, androstenol, androsterone, and estratetraenol [12]. In the majority of the studies synthetic human pheromones are used because of their accessibility.

The aim of this study was to qualitatively and quantitatively evaluate the composition of some commercially available perfumes claiming to contain

human pheromones by gas chromatography-mass spectrometry (GC-MS).

Materials and Methods

Materials, reagents and chemicals

Twelve commercially perfumes claiming to contain human pheromones originating from different manufacturers were purchased from local stores and from the internet. Perfumes were stored at room temperature until analysis.

Androsterone (CAS 53-41-8) was used as external standard and was obtained from Sigma Aldrich (Germany).

Perfume solutions

Perfumes were diluted with methanol in a ratio of 1:10, 1:20, 1:50, 1:100 and stored at room temperature until analysis. Perfume samples were labelled from 1 to 5, distinguishing the ones dedicated to men or women.

GS-MS analysis

The GC-MS analysis was performed using an Agilent 7890A (GC system), Agilent 5975C inert

XL EI/CI MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies. Separation was carried out on a capillary column (30 m $\times$ 250 μ m, 0.25 μ m film thickness). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL/min. The GC oven temperature was increased from 60°C to 280°C with a rate of 10°C/min, and held 10 min at 280°C. A volume of 1 μ L sample was used for injection in splitless mode. Electron impact ionization mode at 70 eV was used. The MS spectra were recorded in scan mode on the range of 50 to 800 a.m.u.

Qualitative analysis

The analytes were identified by comparing the obtained mass spectra of relevant chromatographic peaks with corresponding reference spectra from the Wiley MS library.

Quantitative analysis

External standard method was used for quantitative analysis. Calibration solutions of 0.25, 0.15, 0.1, 0.075, 0.025, 0.0125, 0.005, 0.0025 mg/mL of androsterone were prepared in methanol from a freshly prepared stock standard solution. Linear regression of the recorded peak areas of the total

ion chromatograms *versus* the concentration of standards has been performed for calibration. The relative concentrations of all the identified human synthetic hormones, having closely related molecular structures, have been estimated with the aid of the standard androstenone as reference compound.

Results and Discussion

To the best of our knowledge, is the first time that human synthetic pheromones are identified and quantified in commercially available perfumes samples. A simple and fast gas chromatographic method involving minimal sample preparation using mass spectrometric detection with electron impact ionization has been developed and optimized for the determination of human pheromones in twelve perfume samples (Figure 1). By comparison of the recorded MS spectra with the existing reference spectra of the Wiley MS database, the presence of seven different synthetic human pheromones has been identified in the analysed perfume samples (Table I).

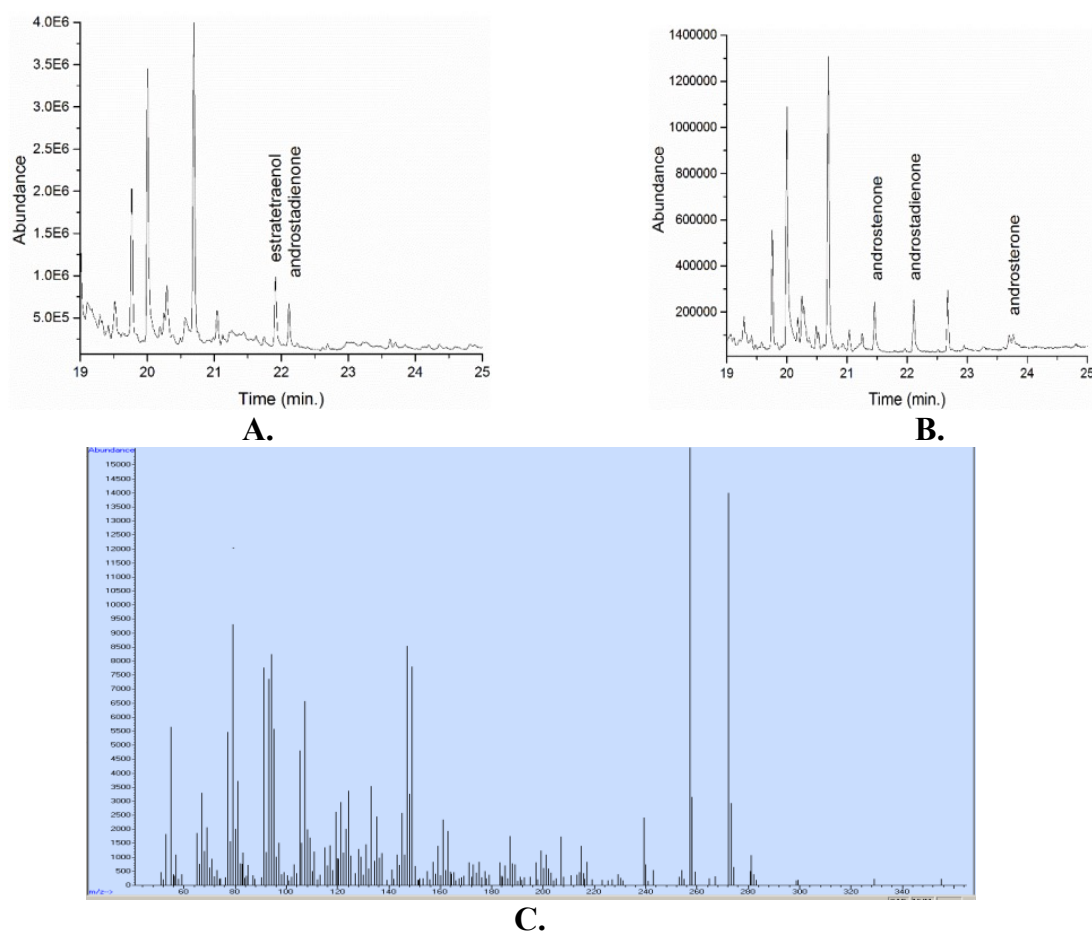


Figure 1.

Total ion chromatograms of commercially available pheromone containing perfumes: (A.) Sample 1w (women's perfume); (B.) Sample 1m (men's perfume). Electron ionization mass spectra of androstenone (C.)

Table I

Human synthetic pheromones identified in the analysed perfumes with the corresponding retention times (R. t.) and similarity indexes (% Sim)

	AOL		ANN		ANR		AND		ANND		ASA		EST	
CAS No.	1153-51-1		18339-16-7		53-41-8		4075-07-4		846-46-8		1755-32-4		1150-90-9	
Perfume No.	R. t. min	% Sim	R. t. min	% Sim	R. t. min	% Sim	R. t. min	% Sim	R. t. min	% Sim	R. t. min	% Sim	R. t. min	% Sim
1w*	-	-	-	-	-	-	22.1	99	-	-	-	-	21.9	93
1m**	-	-	21.4	99	23.8	91	22.1	99	-	-	-	-	-	-
2w	-	-	-	-	-	-	-	-	-	-	19.6	78	-	-
3w	21.2	99	21.4	99	-	-	-	-	24.2	93	-	-	-	-
3m	21.1	99	21.5	99	-	-	-	-	24.2	97	-	-	-	-
4w	21.2	99	21.5	99	-	-	-	-	-	-	-	-	-	-
5m	21.2	99	21.5	99	-	-	-	-	24.2	93	-	-	-	-

*w - women's perfume; **m - men's perfume; pheromones: AOL - Androstenol, ANN - Androstenone, ANR - Androsterone, AND - Androstadienone, ANND - Androstanedione, ASA - Androstanone, EST - Estratetraenol

The identified human synthetic pheromones have been quantified using an external standard method. For the quantitative analysis of the identified pheromones (Table II) an external standard calibration method was used:

$$Y = 2365 \cdot 10^6 X - 4 \cdot 10^6,$$

$R^2 = 0.9960$, Y – peak area, X – concentration, mg/mL.

Table II

Quantitative estimation of synthetic human pheromones in perfume samples

Perfume No.	AOL (mg/mL)	ANN (mg/mL)	ANR (mg/mL)	AND (mg/mL)	ANND (mg/mL)	ASA (mg/mL)	EST (mg/mL)
1w*				0.12			0.19
1m**		0.07	0.04	0.08			
2w						0.02	
3w	0.12	0.29			0.09		
3m	0.12	0.27			0.09		
4w	1.14	0.10					
5m	0.04	0.16			0.03		

*w - women's perfume; **m - men's perfume; pheromones: AOL - Androstenol, ANN - Androstenone, ANR - Androsterone, AND - Androstadienone, ANND - Androstanedione, ASA - Androstanone, EST - Estratetraenol

Experiments on human behaviour of the synthetic pheromones under controlled conditions have been performed usually at concentrations at which the individuals could easily detect the pheromone's scent by olfaction. This information is important, especially in case of the most frequently occurring substance in the studied samples, namely androstenone. Studies have shown that this pheromone has a positive impact only on those who finds its odour pleasant and has a negative impact on interpersonal relationships of people who described its odour as unpleasant, resembling to urine and sweat [5]. Manufacturers tend to solve this problem using various perfume flavours and fragrances able to mask the unpleasant smell of androstenone.

It is also worth mentioning that samples 3w and 3m originating from the same manufacturer showed no difference in terms of the number, type and the concentration of contained pheromones (Table II). The highest concentration of synthetic molecule recorded in the analysed samples is androstenol (1.14 mg/mL), being found in a perfume for women. Experiments performed on women

exposed to androstenol rated the interactions with men as being positive [6].

Androstadienone, one of the most investigated pheromones in terms of its effects on human behaviour was only found in two of the studied fragrances. It was present in a higher concentration in sample 1w for women compared to sample 1m for men commercialized by the same manufacturer. The unbalanced use of this pheromone considering the target consumer's gender might be correlated with previous experimental findings, which accounted for the so called "beneficial effects" mostly in case of women, such as increase in heart rate, reduction of respiratory rate and a positive influence on their mood [12]. Moreover, in women exposed to vapours of androstadienone, an activation of hypothalamus is observed, which in case of males could not be recorded [8-10]. Usually, in controlled studies investigating the effects of androstadienone on human behaviour, hormone concentrations of 250 μ M (~ 68 μ g/mL) is employed, concentration at which the exposed subjects may already consciously detect its odour [3, 4, 6, 11]. Obviously, the

concentrations found in the analysed perfume samples exceed this value, and the potential negative effects due to their “unpleasant” odour tends to be masked by stronger perfume fragrances added in the composition of the perfumes.

Estratetraenol, a female sex hormone, has only been found in the composition of a single perfume sample for women. Studies have been shown that estratetraenol influences positively men’s mood [7] and activates the olfactory regions in women and hypothalamus in men [9, 10]. Once again the found concentration of estratetraenol in the perfume sample (0.19 mg/mL) was much higher than the one required for conscious olfactory detection of this hormone [4].

Considering the numerous knowledge gaps regarding the effects of synthetic pheromones on human behaviour, potential gender-based effects and the absence of any quantitative study correlating hormone exposure and observed effect, the perfume manufacturers do not seem to follow any scientific protocol in the design and fabrication of these products.

Conclusions

The current study is the first to show the type and concentrations of synthetic human pheromones in perfumes. Only in seven out of twelve perfumes synthetic human pheromones have been identified. Androstene is the most frequently occurring pheromone in the analysed samples (both for both women and men). In some cases no difference is observed in the nature and concentration of pheromones used in function of the gender of the target consumers. The unpleasant odour of pheromones tends to be masked by stronger perfume fragrances added in the composition of the perfumes, which eventually makes the difference between products intended for men and women.

Considering the rising number of perfumes with pheromones, being sold mostly over the internet, without any control by national or international authorities or regulating bodies, there is an increasing demand in promoting analytical methods for their quality assessment. These initiatives could eventually assure a compliance with the consumer product regulations and endorse a safe use of these products. Most of these perfumes benefit of an aggressive advertising, exposing the consumers to

potential frauds and more importantly, to health associated risks.

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