High resolution liquid chromatography and time of flight mass spectrometry in perfume analysis

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Abstract
Perfumes consist of a wide range of natural and synthetic compounds that belongs to different chemical classes. Most of these compounds are generally determined by GC. However, in this study RP-HPLC-Q-TOF-MS and HILIC-Q-TOF-MS technique was applied for the determination of ingredients of original perfumes and their imitations. Antioxidants and compounds specific to fragrances of animal origin were found in original perfume samples, whereas carrier oils components were generally determined in their imitations. Furthermore, some components of essential oils were also detected. This research confirmed the theory that results obtained in the analysis of perfume using HPLC can be complementary to those one obtained during GC analysis.

1. Introduction

Perfumes have been used for thousands of years and nowadays they are considered as an essential part of human life [1, 2]. On average, every 43 hours a new female fragrance appears, while the male one appears once every 96 hours. As a result, it is assessed that the perfume business is a billion-dollar industry [3].

Perfumes have complex matrices that consist of a wide range of natural and synthetic compounds belonging to different chemical classes. Hence, the risk of contact allergy induced by their ingredients is still being the object of scientific debate [1]. Furthermore, due to the adverse effects of some of perfumes components on human health and their potential bioaccumulation, they present a clearly growing threat to health and environment. Besides, the high prices of essential oils cause that fragrance dealers more and more often decide to falsify their products by adding cheaper materials, but still asking for the same price for the mixture. According to these reasons, the use of analytical techniques to assess allergenic properties of perfume components, environmental contamination or adulteration of perfumes is inevitable [4]. Due to the fact that the most perfume ingredients have apolar and (semi-) volatile character, gas chromatography (GC)

is the most popular technique used for perfume application. However, reversed-phase high performance liquid chromatography (RP-HPLC) is technique than can be applied for the determination of non-volatile perfumes ingredients that have low thermostability. Between the variety of detectors, mass spectrometer (MS) is superior for either GC or HPLC, because of its high sensitivity and outstanding identification possibilities. Electronic nose is another popular device used for determining the perfume ingredients [3, 4].

The aim of this study was to show the potential of HPLC-MS technique in direct analysis of original perfumes and their imitations. Two different types of liquid chromatography were presented and compared. Furthermore, the identification of several perfumes ingredients has been done.

2. Experimental

2.1 Reagents and chemicals

Acetonitrile (HPLC grade) and formic acid (>98%) were obtained from Merck (Germany). Acetonitrile (LC-MS grade) was purchased from VWR Chemicals (USA) and ultrapure water was prepared using HPL5 system from Hydrolab (Wiślina, Poland).

2.2 Instrumentation

Both HILIC-Q-TOF-MS and RP-HPLC-Q-TOF-MS analyses were performed using the Agilent 1290 LC system equipped with a binary pump, an online degasser, an autosampler and a thermostated column compartment coupled with the 6540 Q-TOF-MS with a Dual ESI ion source (Agilent Technologies, Santa Clara, USA). The ESI source was operated with positive and negative ion ionization mode. The fragmentor voltage was set at 100 V and the mass range was set at 100–1500 *m/z* in MS. Furthermore, nebulizer gas was set at 35 psig, capillary voltage was set at 3500 V, and drying gas flow rate and temperature were set at 10 L min^{-1} and 300 °C, respectively. The TOF-MS system was calibrated on a daily basis.

In case of RP-HPLC, LiChrospher 100 RP-18e (125×4 mm, 5 μm; Merck, Germany) column was used in order to separate analytes. Two different solvent mixtures were examined and applied as a mobile phase: one mixture was based on acetonitrile and water mixture with formic acid (0.05%, *v/v*) and the second one was based on acetonitrile acidified with formic acid (0.05%, *v/v*). In both case, the isocratic elution was performed (100% B). The flow rate of mobile phase was 0.8 mL min^{-1} and the injection volume was 20 μL. The column temperature throughout the separation process was kept at 25°C.

In case of hydrophilic interaction liquid chromatography (HILIC), Kinetex HILIC 100A (150×4.6 mm, 2.6 μm; Phenomenex, USA) column was used in order to separate analytes. The mixture of acetonitrile and water mixture with formic acid

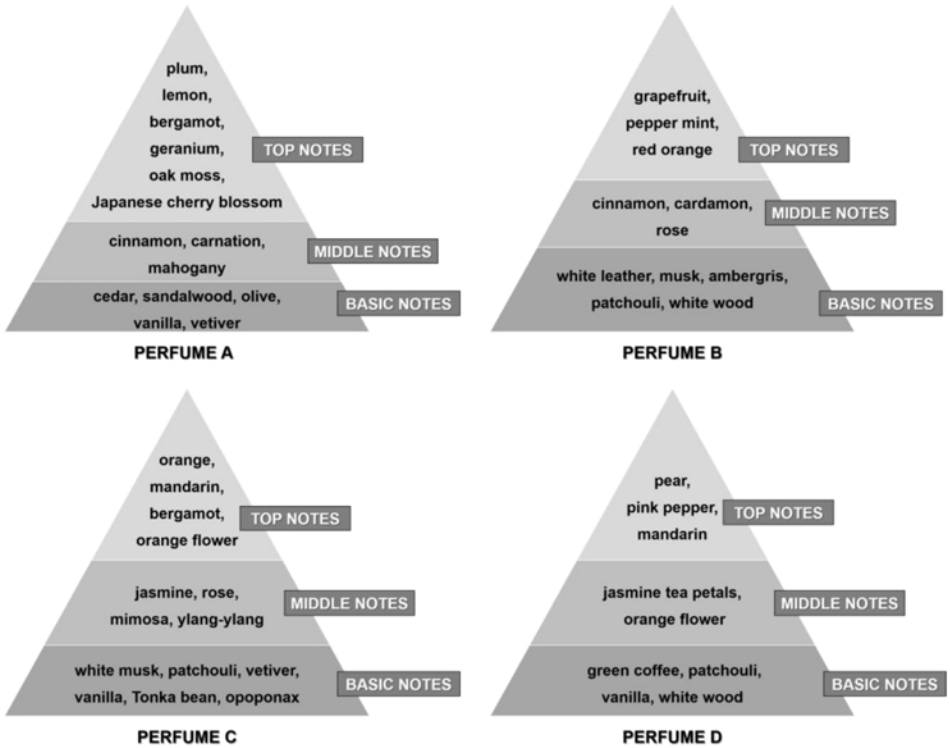

Fig. 1 Fragrance pyramids of original perfumes analyzed by HPLC-MS technique.

(0.05%, *v/v*) was used as a mobile phase. The other parameters have been set as above.

2.3 Sample and sample preparation

In case of this research, two samples of perfumes for men (A, B), two samples of perfumes for women (C, D) and four samples of perfume imitations were analyzed. The original ones were bought in popular perfumery in Gdańsk, whereas their cheaper versions were bought in Chinese shop. The scent compositions of original perfumes are shown at Fig. 1.

Both original and cheaper perfume samples (250 µL) were diluted in 250 µL of acetonitrile containing 3% of water. Such prepared samples were injected (20 µL) directly into the HPLC-Q-TOF-MS system.

3. Results and discussion

In this presented study, different chromatographic system has been used in order to determine perfumes components. The samples of original perfumes and their imitations were analyzed by SCAN mode. Each obtained chromatogram LC-HRMS

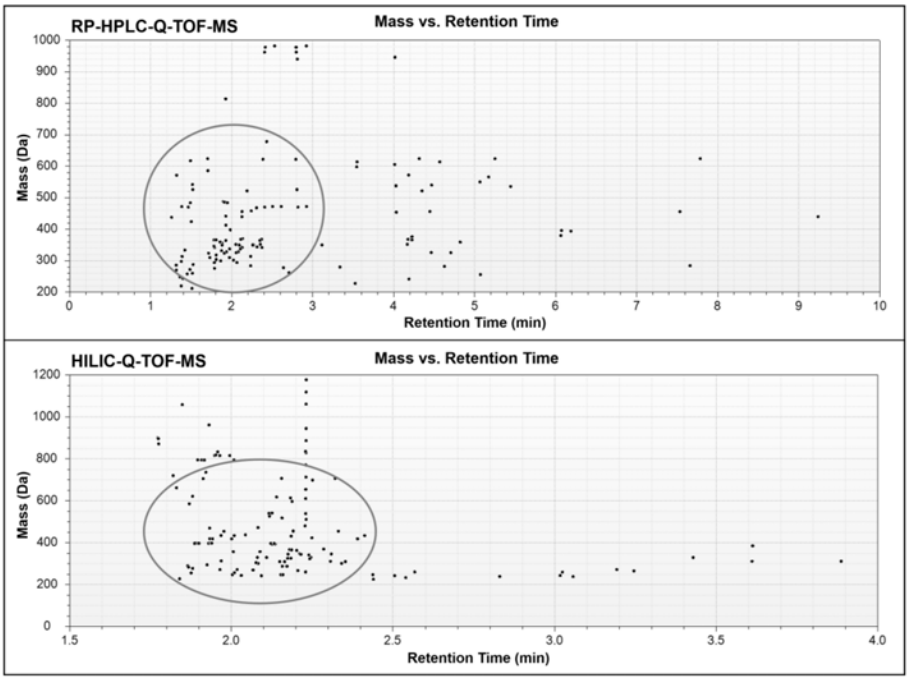

Fig. 2 The relationship between the mass of chemical species detected in perfumes samples and the retention time.

were processed with Molecular Feature Extraction (MFE) mode. The results achieved for sample A (both ESI modes) are shown in Fig. 2. In case of RP-HPLC-Q-TOF-MS, low molecular compounds (200–500 Da) and medium molecular weight compounds (500 Da <) were generally detected. In case of HILIC-Q-TOF-MS, the same situation was observed. However, the use of HILIC enabled to determine more compounds with mass higher than 700 Da.

The next step was to identify the perfumes ingredients. Perfumes contain various chemicals that can be classified in six categories: solvents, essential oils, dyes, modifiers, blenders, and fixatives [5]. It was decided that the identification would be based on information about perfumes composition, which is partially available, and the list of compounds used in perfumery published by International Fragrance Association (IFRA). Some compounds detected in the perfume samples were presented in Table 1. The results for two types of perfumes are shown in Table 2.

Compounds belong to fatty acids were detected in all samples. They are the components of carrier oils that are used to dilute essential oils and absolutes. Compounds specific to fragrances of animal origin (musk, ambergris) were found in both original perfume samples. These substances are not only used as base notes in perfumery, but also as fixatives. Due to the limited amount of natural musk and ambergris available on the market, they are very expensive. Furthermore, antioxidants (avobenzone, diethylamino hydroxybenzoyl hexyl benzoate)

Table 1
Basic information about compounds detected in perfumes samples.

Compound	Molecular formula	Monoisotopic mass/ Da	Ionization mode	Theoretical *m/z*
ambroxide	$C_{16}H_{28}O_2$	236.2140	Positive	237.2213
atranol	$C_8H_8O_3$	152.0473	Negative	151.0401
avobenzone	$C_{20}H_{22}O_3$	310.1569	Positive	311.1642
dimethyl benzyl carbinyl butyrate	$C_{14}H_{20}O_2$	220.1463	Positive	221.1536
diethylamino hydroxy-benzoyl hexyl benzoate	$C_{24}H_{31}NO_4$	397.2253	Positive	398.2326
mintlactone	$C_{10}H_{14}O_2$	166.0994	Positive	167.1066
muscone	$C_{16}H_{30}O$	238.2296	Positive	239.2369
oleic acid	$C_{18}H_{34}O_2$	282.2559	negative	281.2486
palmitic acid	$C_{16}H_{32}O_2$	256.2402	Negative	255.2329
pentadecanoic acid	$C_{15}H_{30}O_2$	242.2246	Negative	241.2173
stearic acid	$C_{18}H_{36}O_2$	284.2715	Negative	283.2642
vanillin	$C_8H_8O_3$	152.0473	Positive	153.0546

that are added to perfumes to protect their color and scent composition were detected in original perfumes. In case of perfume A, atranol, dimethyl benzyl carbinyl butyrate and vanillin were detected. These compounds are characteristic for some essential oils. The first one can be determined in oak moss essential oils, the second one in plum essential oils, whereas the third one is specific to vanilla essential oils. In case of its imitation, atranol was only detected. This compound has been identified as the allergen, so its concentration in perfumes should be regulated. Mintlactone was identified in both samples C and C'. It is a fragrance compound that can be found in Tonka bean oils. Coumarin is the second compound that can be detected in this essential oil.

4. Conclusions

The price of perfumes is affected by the cost of their production. Because of the usage of small availability components, some of the perfumes are expensive and still very desirable. For this reason, many perfume imitations are reaching the markets. Most of compounds that vary perfumes and their imitations are commonly detected during GC analyses. However, the determination of essential oil components, musk and other fixatives confirmed that the LC-MS can be used as a complementary technique to GC or GC-MS. Two different chromatographic systems were applied for perfume analysis. In both cases low molecular weight compounds (from 200 Da to 500 Da) were generally detected. Nonetheless, most identified perfumes ingredients were only determined in samples analyzed with RP-HPLC-Q-TOF-MS system.

Table 2
Compounds detected in original perfume samples (A, C) and their imitations (A', C') using two different chromatographic system (+ = detected under used conditions, – = not detected under used conditions).

Sample	Compound	Experimental *m/z*	Mass accuracy /ppm	RP-HPLC	HILIC
A	ambroxide	237.2217	1.81	+	–
	atranol	151.0410	–5.96	+	+
	dimethyl benzyl carbinyl butyrate	221.1537	0.45	+	+
	diethylamino hydroxy-benzoyl hexyl benzoate	398.2331	1.26	+	+
	oleic acid	281.2480	–2.13	+	–
	palmitic acid	255.2332	1.76	+	+
	pentadecanoic acid	241.2178	–2.07	+	+
	stearic acid	283.2639	–1.06	+	+
	vanilin	153.0539	–4.57	+	–
A'	atranol	151.0406	3.31	+	–
	palmitic acid	255.2339	3.92	+	–
C	ambroxide	237.2214	–0.42	+	–
	avobenzone	311.1644	0.64	+	–
	mintlactone	167.1067	0.56	+	–
	muscone	239.2372	–1.25	+	–
	oleic acid	281.2494	2.84	+	–
	palmitic acid	255.2336	2.74	+	+
	stearic acid	283.2647	1.77	+	+
C'	coumarin	147.0441	0.00	+	–
	mintlactone	167.1064	–1.20	+	–
	oleic acid	281.2491	1.78	+	–
	palmitic acid	255.2338	3.53	+	+
	pentadecanoic acid	241.2180	2.90	+	+
	stearic acid	283.2648	2.12	+	+

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