doi:10.20944/preprints201908.0145.v1

The first application of ^{1}H NMR spectroscopy for the assessment of the authenticity of perfumes

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Keywords authentication of perfume, flavours, nuclear magnetic resonance, PCA

Abbreviations

GC/MS gas chromatography coupled with mass spectrometry, EOS electronic olfactory system, DOSY diffusion ordered spectroscopy, PCA principal component analysis, TMS tetramethylsilane

ABSTRACT

The industry of the counterfeit goods is one of the largest underground business in the world and it is rapidly growing. Counterfeits can lead not only to loss of profit for honest producers but also have a negative impact on consumers who receive poor quality goods at an excessive price and may be exposed to health damages and safety issues. Perfume industry is constantly exposed to the problem of counterfeits with the fast developing parallel market of inspired perfumes being an important issue. It prompts for the identification of methods that classify the quality of this type of products. In this paper the application of proton nuclear magnetic resonance spectroscopy is employed for the authentication of perfumery products for the first time. Molecular composition of several types of authentic brand fragrances for women were compared with their cheaper inspired equivalents and fake products. Our approach offers the prospect of a fast and simple method for discrimination and counterfeit detection of perfumes using ^{1}H NMR spectroscopy.

1. Introduction

The increasing global trade and prices of products with recognized market position such as beverages, medicines, food and perfumes encourage dishonest manufacturers to counterfeit branded products. The fake goods purchased through various internet shops and websites not only cost less than the original products but are also of inferior quality [1,2]. Moreover, the counterfeit goods may be dangerous because they do not undergo the same rigorous tests that are applied by honest manufacturers to ensure safety of their products. The consequences of the adulteration of products affects not only companies by damaging the original brand's reputation but also consumers who are unaware that fake products do not comply safety standards and could be potentially dangerous for health. In majority, the fake products are dermatologically not-tested and may contain dangerous chemicals, which can cause skin reactions such as contact allergies or dermatitis [3,4].

Nowadays, fragrances are found in almost all cosmetics and personal care products such as perfumes, deodorants, aftershave or skin lotions, hair cosmetics, laundry products, face cleansing products, sunscreens as well as cleaning products. However, a given fragrance is typically a chemical mixture of several dozen to several hundred chemicals which may be responsible for the allergic contact dermatitis [5-9]. The last data indicated that more than 3000 chemical compounds are used in fragrance mixtures but only 82 substances (54 single chemicals, 28 natural extracts) were classified as established contact allergens in humans by The Scientific Committee on Consumer Safety [2]. Perfumes usually contain from 30 to 50 (sometimes even 200) ingredients responsible for their fragrance properties. These ingredients originate from natural sources or chemical synthesis. The composition and scent of the essential oils depend not only from the origin of the plant and the conditions of its collection, storage, transport, drying, but also on the process of oil production. It is particularly important to monitor contaminants that may be present in oils obtained from plants, as they may contain traces of pesticides that are toxic and carcinogenic [7,8]. Due to all mentioned properties of essential oils, the high quality natural oils are very expensive. Their replacement by synthetic chemical compounds allows to decrease the costs of perfume production, which mainly depend on the quality of perfume oil used [6]. Owing to the high prices of essential oils, manufacturers are trying to adulterate the product while maintaining the high price of it. Two techniques of adulteration based either on adding lower cost material or on dilution with water or other solvents are frequently used.

The most commonly counterfeited scents are branded perfumes and toilet waters. Imitation perfumes do not have the same quality or scent as the authentic one [2,3]. Customers at first glance are not able to distinguish the original perfume from the counterfeit, because its external features strongly resemble the proper product, but it does not correspond to it in terms of molecular composition and properties. However, in some situations consumers can avoid buying a counterfeit products simply by staying focused. Counterfeiters often use the spelling mistakes to avoid criminal liability for theft of the trademark and then on the packaging one can find Boos instead of *Boss* or J'ader instead of *J'adore*.

Several methods have been suggested for the analysis of fragrances such as chromatography [10-12], spectroscopy [13-16], mass spectrometry [17,18] and electronic nose [19,20]. Among these methods, the gas chromatography coupled with mass spectrometry (GC/MS) are the most common techniques used in the perfume laboratories. In recent years, an artificial electronic olfactory system (EOS) has been also successfully introduced for the analysis of volatiles. In turn, the nuclear magnetic resonance spectroscopy is an excellent tool for precise structural characterization of a pure compound but in case of mixtures the overlap signals appear as the main issue. Interestingly, up to now the NMR spectroscopy was applied only to characterize the compositions of several perfumes and also for separation of the components of a given mixture on the basis of their diffusion coefficients using diffusion ordered spectroscopy (DOSY) [14-16].

Herein, we present a fast, simple and reliable method for the detection of counterfeit of brand women perfumes. Within our studies, ^{1}H NMR spectra of original perfumes were compared with their fake version and inspired equivalents. To the best of our knowledge, the use of ^{1}H NMR spectra has never been reported for this purpose before. Our results offer the perspective for a routine way of a rapid discrimination and counterfeit detection of perfumes by means of NMR spectroscopy.

2. Results and discussion

Representative ^{1}H NMR spectra of the authentic, inspired and counterfeited perfumes are shown in the Figure 1.

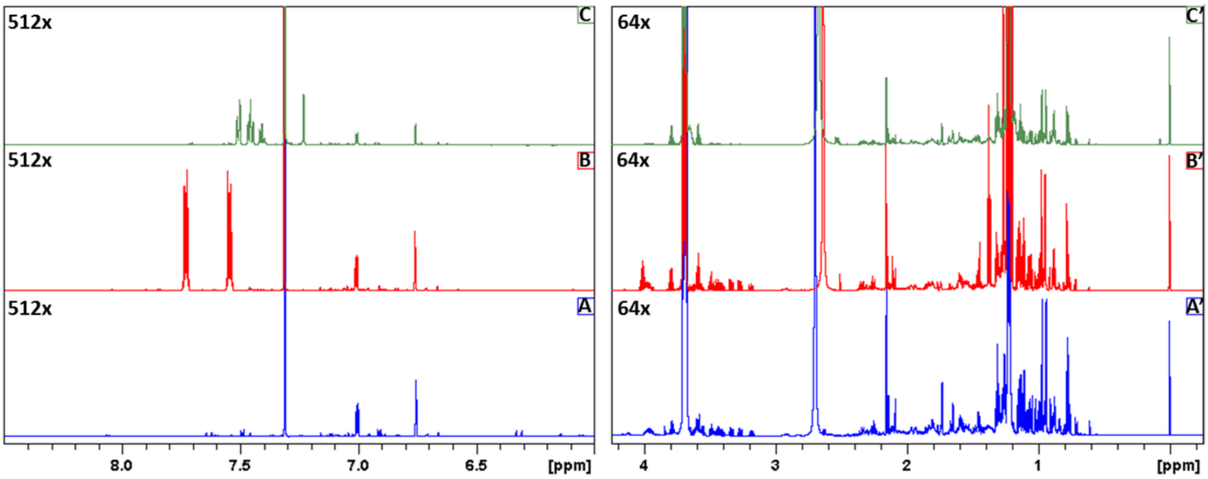

Fig. 1. Representative ^{1}H NMR spectra of authentic (A,A'), inspired (B,B') and counterfeited (C,C') samples in two spectral region from -0.25 to 4.25 ppm and from 6 to 8.5 ppm.

Due to the very large number of signals, the obtained NMR spectra were first analysed by the main components analysis (PCA) which is concerned with explaining the variance structure of a set of variables through linear combinations of the variables (principal components, PCs). PCA scores plot discriminating the authentic perfume samples, from inspired and counterfeit samples, based on selected signals of protons detected by ^{1}H NMR. For this purpose the PCA analysis was conducted for three spectral ranges of chemical shifts. The first covered the range from 0.6 to 3.0 ppm, the second from 3.0 to 6.0 ppm, and the last from 6.0 to 8.5 ppm. Interpretation of the results obtained for the first interval did not allow to distinguish between the original samples and the inspired as well as adulterated samples, because all samples in this range of chemical shifts were very similar and no statistically significant differences were observed between them.

On the other hand, the analysis of the scores of PC1 versus PC2 versus PC3 (describing 98.5 % of total variation) conducted for the range from 3.0 to 5.0 ppm led to two distinctive groups. The first included original products, the second contained inspired equivalents and falsified products. The signals that were responsible for the differentiation of these two groups were those from water (4.82 ppm) and isopropyl myristate (4.00 ppm), which were observed in the case of non-original products. This was very characteristic for falsified and lower quality products, because manufacturers in order to reduce the cost of the perfume production often use more water or other solvents such as isopropyl myristate to prepare the final product. As a consequence, consumers buy poor quality goods at an excessive price. A PCA score plot based on the proton spectra of 22 samples is shown in the Figure 2.

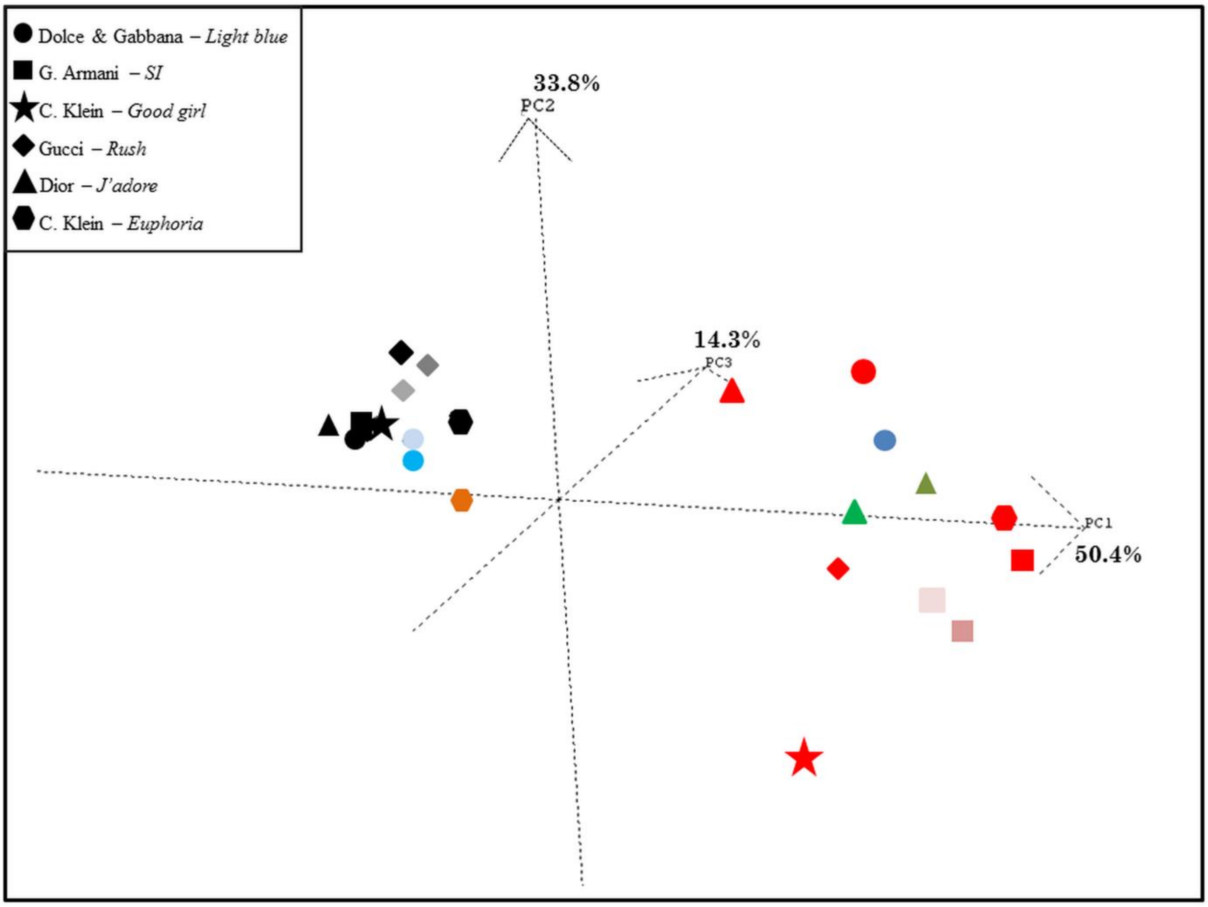

Fig. 2. Scores plot of PC1 vs PC2 vs PC3 from PCA of proton spectra from the authentic perfume (black color, different shapes marked various scents) and counterfeited (red color, different shapes marked various scents) and inspired samples (colors, different shapes marked various scents) for the range of chemical shifts from 3 to 6 ppm.

As shown on the scores plot in the Figure 2, the authentic perfumes were not clearly separated from each other. Therefore, PCA plot obtained for the range of chemical shifts from 6.0 to 8.5 ppm proved to be the most important because it allowed not only to distinguish branded samples from non-original ones, but also enabled to differentiate original fragrance compositions between each other. In that way, all perfume samples were well-grouped and clearly separated. The total variation described by the scores of PC1 versus PC2 versus PC3 was 79.7%. This plot shows that the counterfeits differed a lot from the original perfume and also occupied a different region in the plot in comparison to all the inspired samples. This proves that the adulterated perfume have completely different aroma and thus the chemical composition. It was particularly evident in the case of three fragrances: *Light Blue, Rush* and *Good Girl*. Unlike counterfeit perfumes, the inspired samples showed considerable similarities with the authentic that can be observed for fragrances like *Rush* and *Si*. This results are shown in the Figure 3.

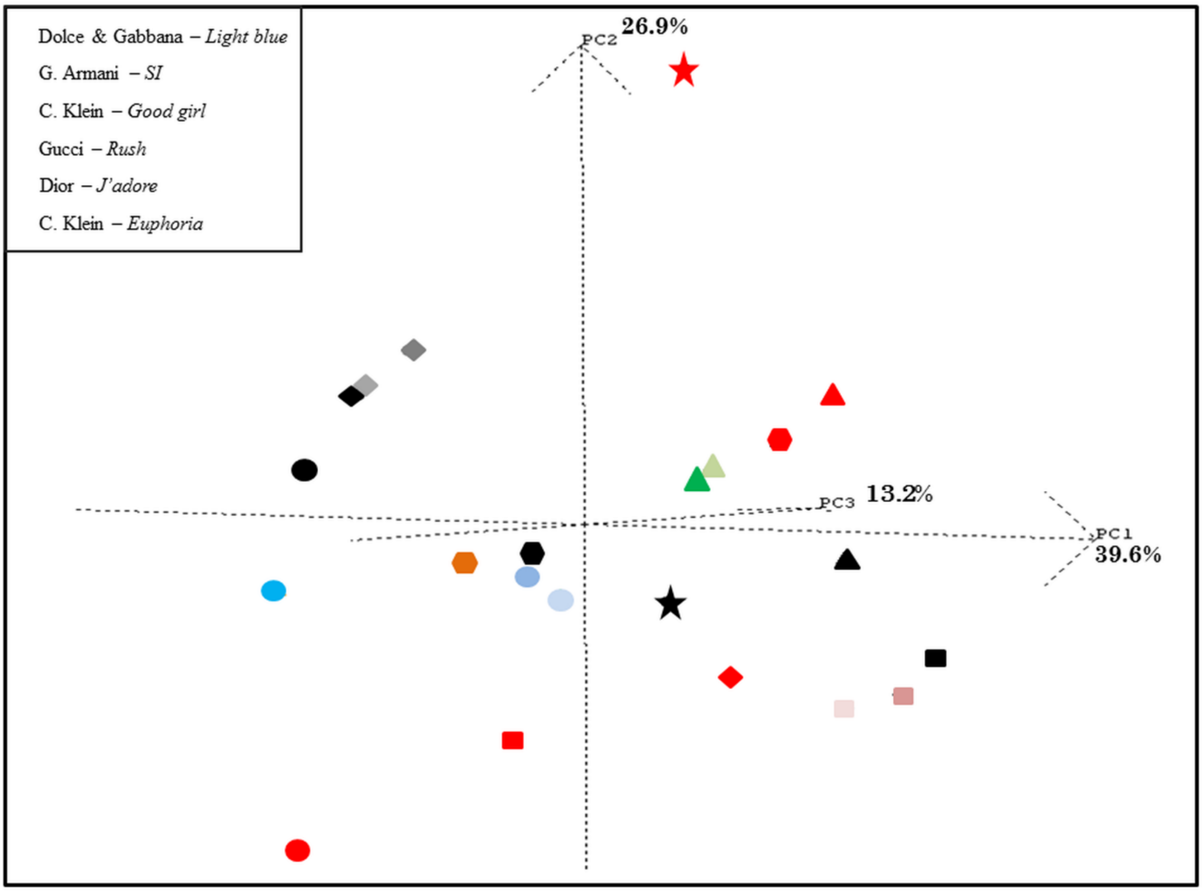

Fig. 3. Scores plot of PC1 vs PC2 vs PC3 from PCA of proton spectra from the authentic perfume (black color, different shapes marked various scents) and counterfeited (red color, different shapes marked various scents) and inspired samples (colors, different shapes marked various scents) for the range of chemical shifts from 6 to 8.5 ppm.

In the next part of our studies the authenticity of samples was checked by the comparison of each NMR spectrum of a unknown sample with original reference sample and analyzed for possible differences. To check the chemical similarity, all signals in both spectra were separately integrated and linear regression analysis was conducted to compare the integration values for both spectra. For each comparison the coefficient of determination (R^2) of the fit was calculated. Results for one selected fragrance have been presented in the Figure 4 (for complete results see Supporting Information). As expected the highest value of R^2 parameter was obtained when two authentic samples were compared (R^2=0.98, Figure 4a). The dramatic difference in the chemical composition was observed for comparison between the authentic and counterfeit samples, where the R^2 value was 0.05. This result indicated that the counterfeit samples are not produced by dilution of the original one. Furthermore, they contained different compounds which may be responsible not only for consumers health problems but also constitutes a direct indication of its illegality. In turn, for inspired samples the coefficient of determination were in the range from 0.3 to 0.8 – in the case of comparison between the authentic and inspired samples presented in Figure 4b, its value equaled 0.32.

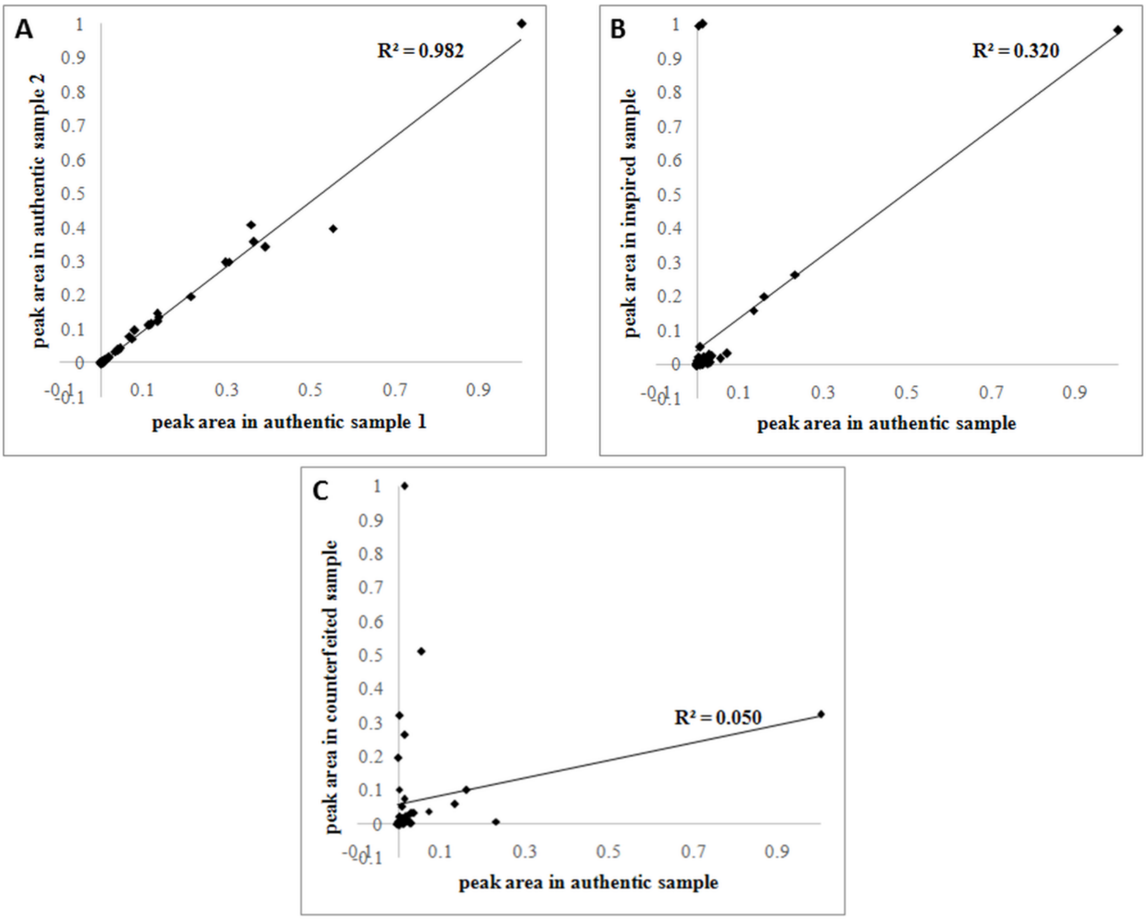

Fig. 4. The comparison of the peak area from NMR spectra for a) two authentic samples, b) the authentic and inspired one, and c) the authentic and counterfeit sample.

Given the obtained data the use of ^{1}H NMR fingerprints for fraud detection seems inevitable. Our results indicate that combination of ^{1}H NMR spectroscopy with principal component analysis offers not only the possibility for simultaneous identification of counterfeits samples but also allows to determine the molecular composition of the analysed mixture.

3. Materials and methods

3.1 Samples preparation

In the present work, the seven authentic samples of perfumes (Dolce&Gabbana *Light Blue*, G. Armani *Si*, C. Klein *Good girl*, Gucci *Rush*, Dior *J'adore* and C. Klein *Euphoria*), six counterfeits and ten inspired perfumes were analyzed. The counterfeit and inspired samples were purchased over the internet. All samples for NMR measurements were prepared in the same way. A volume of 50 μL of analysed perfumery product was dissolved in 600 μL of deuterated chloroform ($CDCl_3$) with the addition of 2% tetramethylsilane (TMS) as an internal standard.

3.2 NMR measurements

All ^1H NMR spectra were recorded using a Bruker Avance II Plus 16.4 T spectrometer (Bruker BioSpin, Germany) operating at the ^1H frequency 700.08 MHz The instrument was equipped with a 5 mm broadband BBI probe. ^1H NMR spectra were acquired with a calibrated 90° pulse for 32 scans collecting 64 K data points over a spectral width of 14,097 Hz. The repetition time of 8.27 s, including a relaxation delay of 6 s, was calculated as $7T_1$ of the longest relaxation time to ensure complete magnetization recovery. An exponential line broadening of 0.05 Hz was applied to raw data prior to Fourier transformation. All samples were run at 300 K. The spectra were calibrated at 0 ppm from the TMS peak, which was used as a chemical shift standard. All spectral regions were individually corrected using a fifth–order baseline function.

3.3 Statistical analysis

Statistical analysis was performed using AMIX 3.9.14 (Bruker, Germany). The variation of the data was explored by principal component analysis (PCA). The spectral region from 0 to 8.5 of ^1H NMR spectra was chosen as input data for PCA analysis. Baseline offset was corrected, and the selected spectral region was mean-normalized to arrive at the total area for each sample.

4. Conclusion

The first approach for a rapid and reliable discrimination of perfume based on proton nuclear magnetic resonance spectroscopy has been demonstrated. Our method is characterized by very fast and simple sample preparation and allows for discrimination of the authentic, counterfeit and inspired perfumes on the basis of the synergic combination of ^1H NMR spectroscopy and chemometric techniques. The data obtained in our research indicated that ^1H NMR fingerprints may serve as an alternative method to identify a counterfeit products.

Author contributions

The study was conceived by B.P.-S., G.C. and Ł.A. B.P.-S. and G.C. designed and performed the experiments. B.P.-S. and G.C. analysed the data, B.P.-S. and G.C. wrote the paper, B.P.-S., G.C. and Ł.A. read and revised manuscript critically.

Acknowledgment

This work was supported by funds from Lodz University of Technology. Authors express their gratitude to Schimar Ltd. Łódź for providing authentic samples of perfumes.

Competing interests

All authors disclose no potential competing interest.

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