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ORIGINAL PAPER

Comparison of "herbal highs" composition

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Abstract Popularity of new psychoactive substances, known as legal highs or herbal highs, is continuously growing. These products are typically sold via internet and in so-called head shops. The aim of this study was to identify active ingredients of herbal highs and to compare their chemical composition. Twenty-nine various products seized by the police in one of the "head shops" were analysed. Herbal mixtures (0.2 g) were prepared by ultrasonic-assisted extraction with 2.0 ml of ethanol for 2 h. The extracts were analysed by gas chromatography coupled to mass spectrometry (GC/MS). The main active compounds of the herbal mixtures were synthetic cannabinoids: JWH-018, JWH-073 and cannabicyclohexanol (CP-47,497-C8-homolog). Their content differed between the products; some contained only one cannabinoid whereas the others contained two or more. Cluster analysis and principal component analysis revealed that chemical composition of many products was very similar. The similarity was connected with their flavour and not the common name. This statement was true for the synthetic cannabinoids, other potential agonists of cannabinoid receptors (amides of fatty acids) and ingredients of natural origin and confirms that herbal highs are a threat to human health because the purchaser has no information on their real composition.

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Introduction

The term "legal highs" includes a wide range of products, from synthetic or designer drugs to herbal mixtures. They are usually advertised in different ways, claimed to be room odours, herbal incenses or even bath salts. They are claimed to be used only as animal feed or plant fertilizer, being sold as "collectibles", absolutely not intended for human consumption. Most of them have not been tested on humans or animals and it seems that their producers tend to create new version of the drugs as soon as they are declared illegal. Because of that, tracking and legislating of these substances poses real challenges to law enforcement agencies. According to EMCDDA [1] herbal products marketed on the Internet and in some specialised shops under the name "Spice" have been available since at least 2006. Since 2008 "Spice" products, as well as various other "spice-like" herbal mixes, can be purchased from online shops and were available in "head" or "smart" shops selling "legal highs". The effect of smoking is described by some users as similar to those of *Cannabis*. Packing information on "spice" products indicates that they are composed of ingredients of plant origin. A number of plants are often listed on the packaging, but examination shows that many are not present [2]. Although many products available in head shops are advertised as "natural" or "herbal highs", large number are not purely herbal and contain synthetic elements that can threaten human health. Most of them are synthetic cannabinoids [3–5]. Synthetic cannabinoids are functionally similar to Δ9-tetrahydrocannabinol (THC), being the active principle of *Cannabis*. Similarly to THC, they act by binding to the same cannabinoid receptors

in the brain and other organs as the endogenous ligand anandamide. Some of them, including JWH-018, JWH-073 or CP-47,497, posses equal or higher affinity to the CB1 and CB2 receptor than THC [6]. Although they are often referred to simply as synthetic cannabinoids, many of these substances are not structurally related to so-called "classical" cannabinoids, like THC, based on dibenzopyran [7, 8]. Other cannabinoid receptor agonists include substances such as oleamide (an endogenous substance that is also used in plastics manufacture), which is structurally related to anandamide. It is believed to be a potential agonist of cannabinoid receptors; however, its actual status is not completely known [9, 10]. Pharmacology and toxicology of the plants' materials purportedly contained in "spice" products are almost completely unknown. Thus, potential health risks or possible psychoactive effect of these products cannot be definitely stated. It is to note that synthetic ingredients are not mentioned in the product information. It seems that they have been added surreptitiously, as packaging information on "spice" products mentions only herbal ingredients [1]. Few formal human studies have been published regarding pharmacology and toxicology of the synthetic cannabinoids [2]. Possibly, besides high potency, some cannabinoids could have particularly long half-lives potentially leading to prolonged psychoactive effects. Additionally, there could be considerable inter- and intra-batch variability in smoking mixtures, both in terms of substances present and their quantity. Thus, there is a higher potential for overdose than with *Cannabis*. Negative effects often include paranoia and memory loss. Prolonged use can be harmful to people with underlying mental illness including schizophrenia. Lack of consistency in the contents of "legal highs" can mean that people do not know the strength of the product they are consuming.

The cannabinoids may be easily resolved using gas chromatography, but their identification and quantitative analysis is limited because pure reference samples are hard to obtain. Because of relatively low content of psychoactive substances in herbal products (from ca. 0.1% to 1% of weight) and the presence of concealing additions (such as glycerine or amides of fatty acids), fast and unanimous identification may be performed with complex techniques such as gas chromatography coupled to mass spectrometry (GC–MS) and liquid chromatography coupled to mass spectrometry (LC–MS) [2]. Additional hindrance may introduce natural compounds of herbal matrix on which the synthetic cannabinoids solution is probably sprinkled. Few quantitative studies have been carried out to determine the amount of synthetic cannabinoids present in smoking mixtures [11, 12]. The aim of the study was identification of active components of the herbal highs and comparison of their chemical composition in order to verify if there were any similarities between the analysed samples.

Materials and methods

Chemicals and reagents

The standards of synthetic cannabinoids, including JWH-018, JWH-073 and cannabicyclohexanol, were purchased from LGC Standards (UK). Absolute ethanol of analytical grade was obtained from Merck KGaA (Germany).

Samples

The subject of study was herbal mixtures seized by the police in 2009 in a head shop. Twenty-nine products contained dried plants. The names of preparations were as follows: Tai fun blackberry, Tai fun vanilla, Tai fun orange, Exclusive original, Exclusive mint, Exclusive cherry, Natures organic jagoda [blackberry], Natures organic wiśnia [cherry], Natures organic truskawka [strawberry], Chill zone cherry, Chill zone mint, Chill zone original, Chill out cherry, Chill out mint, Chill out original, Sensation vanilla, Sensation orange, Sensation blackberry, Sensation vanilla 2, Sensation blackberry 2, Chaos mint, Chaos original, Chaos cherry, Smoke, Clover spring, Aztec thunder, Red Merkury, Zen and Zen ultra.

Samples preparation

The herbal mixtures were homogenized in a mortar. The homogenized sample (0.2 g) was subjected to ultrasonic-assisted extraction with 2.0 mL of ethanol for 2 h. The extracts were transferred into the 2.0 mL vials and analysed by GC/MS. Standard solutions of the synthetic cannabinoids were prepared at concentration 0.1 mg/mL in ethanol.

GC–MS conditions

The ethanolic extracts were analysed by GC–MS. The study was conducted using an HP 6890N GC system gas chromatograph coupled to a 5973 Network Mass Selective Detector manufactured by Agilent (United States), which is a quadruple mass analyzer. The extracts were injected automatically in splitless mode. Chromatographic separation was performed on an HP-5MS capillary column (30 m×0.25 mm×0.25 μm) in a temperature gradient consisting of three segments. Initial column temperature (50 °C) was maintained for 1 min, then increased linearly at the rate of 10 °C/min to 280 °C, and finally maintained for 10 min. Total analysis time was 42 min. Helium at a constant flow rate of 1 mL/min was used as a carrier gas. The spectrometer was operated in electron ionization mode and the electron beam energy was 70 eV. Positive

ions were analysed. Acquisition was carried out in scan mode, and the entire mass range from 29–600 amu was collected.

Chemometrical analysis

Chemometrical analysis was performed using Statistica 8.0 (Statsoft, Inc.).

Results and discussion

Ingredients identification

An example chromatogram of herbal mixture is presented in Fig. 1. As typical for analysis of herbal material, many peaks are observed on the chromatogram. Main ingredients of the analysed sample were marked. Usually, tens ingredients were contained in the samples, and their identification was very difficult. Preliminary identification was performed by searching the NIST98 MS spectra database. The results were carefully (manually) verified by experienced MS analyst, who compared both the MS spectra and the retention times. Other MS databases including G1039C Pfleger Drug Library and ENFSI10 were also applied at this stage. After verification, 69 peaks were selected for further analysis. The intensity of these peaks allowed their distinguishing from the background (signal to noise ratio at least 3:1). From the selected list of peaks, 43 were identified by the authors based on high-degree match of their MS spectra to those from the libraries.

The list of the identified substances covered:

- Synthetic cannabinoids such as JWH-018, JWH-073, cannabicyclohexanol (often called CP-47,497-C8-homolog) and its trans isomer
- Amides of fatty acids, including oleamide, palmitamide and stearamide, which could also act similarly to natural cannabinoids; their origin is difficult to determine because they could be natural components of plants, impurity (e.g. from plastics) or artificial additive
- Fatty acids and their esters, including ethyl linoleate, linoleic acid, palmitic acid and methyl palmitate; these substances are present in natural products
- Other substances potentially originating from plants, such as eugenol, beta-sisterole, phytol, eucalyptol, tocopherol, alpha-amyrine, phytosterole, hexacosane, squalene, phytol, selina-6en-4ol, persicol, nerolidol, delta-cadinene, eugenol, thymol, carvone, hydrochinone, dihydroxyacetone and 2-furanmethanol
- Others, including ethyl vanillin, acetyl vanillin, benzaldehyde, benzyl benzoate, benzophenone, hydroxybenzoic acid, cyclopentadiene and clorophene; Their origin is not clearly known. Some of them are possibly added in order to hide the psychoactive compounds by hindering the analysis. They could be flavours, preservatives or contaminants.

If a substance was not identified, it was indicated as NNxxyy, where xx and yy mean its retention time in minutes and seconds, respectively (e.g. NN0326 is a substance, which is eluted from the column at 3.26 min). Information on the substances, i.e. their names (codes), retention times and mass

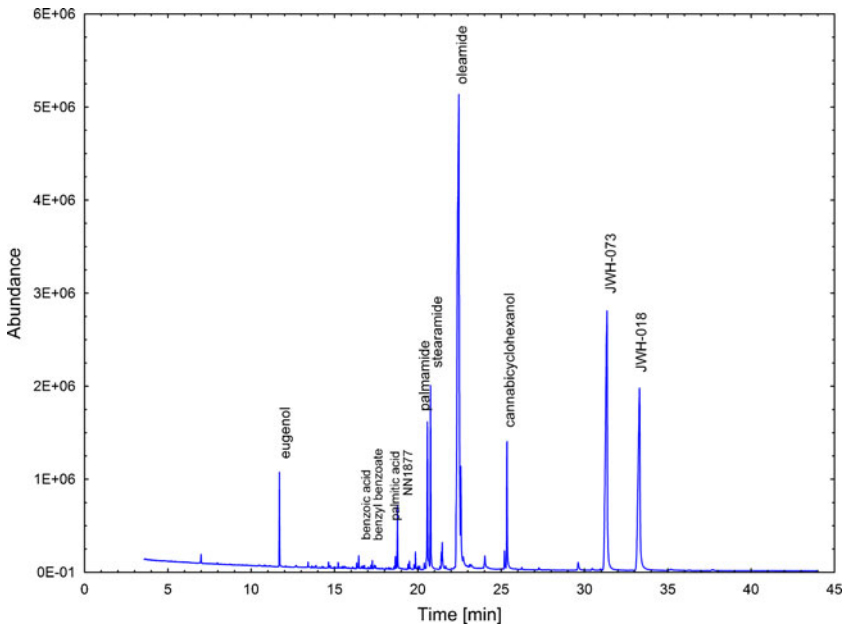

Fig. 1 GC–MS chromatogram (TIC) of an ethanolic "herbal highs" extract

spectra were inserted in the AMDIS®, and further analysis was supported by this software.

Chemometric comparison of herbal highs composition

Cluster analysis (CA) was used in order to establish if there are any similarities between the analysed preparations. This classification technique belongs to so-called unsupervised methods, when no a priori information about connections between the samples is taken into account [13]. Peak areas of the herbal highs ingredients were recorded and fourth square root were calculated. This pre-treatment technique was applied in order to reduce the influence of large peaks on the results of calculations (it is a standard procedure if peak areas in a sample differ to a large extent, alternatively a logarithm can be calculated). Finally, a database composed of 29 rows (samples, cases) and 69 columns (substances, variables) was formed. Moreover, in order to eliminate problems in calculations, all "zeros" (no substance in a sample or its concentration below limit of detection) were replaced by "ones".

The dendrogram of herbal highs is presented in Fig. 2. Based on earlier experiences in chemometrical treatment of data from drugs analysis, $1-r$ Pearson distance and Ward agglomeration method were applied for CA.

CA revealed that there are similarities in the composition of different preparations. It is seen in Fig. 2 that two main clusters were formed. The lower one seems to be more consistent. Excluding "Zen ultra", it contained three groups of products with "Mint", "Cherry" and "Original" in their names. The second (upper) cluster is composed of several sub-clusters.

Principal component analysis (PCA) belongs to projection methods, which are aimed at projecting original data set from a high-dimensional space onto a line, a plane or a 3D-coordinate system [13]. PCA was used in order to establish which substances are responsible for sample differentiation. All variables (peaks) were taken into account, and the PCA was performed based on a covariance matrix. The projection of variables on the PC1–PC2 plane is presented in Fig. 3.

The scree plot (not presented in the paper) revealed that two first PCs allow significant reduction of the data dimension without significant loss of information. The first PC explained almost 64% of the total variance, and the second was responsible for another 12%. The highest

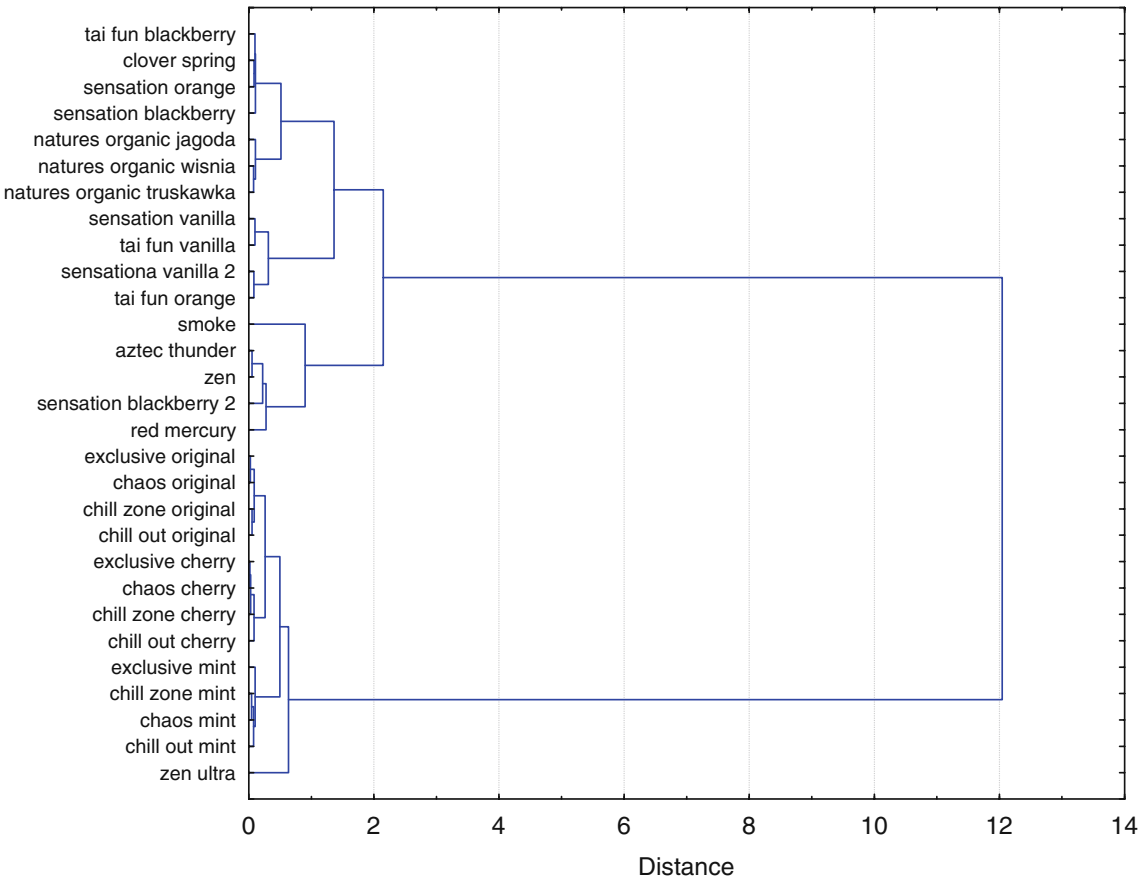

Fig. 2 The dendrogram of herbal highs. The distances between the samples were calculated using the Pearson correlation coefficient. The Ward method was used for agglomeration

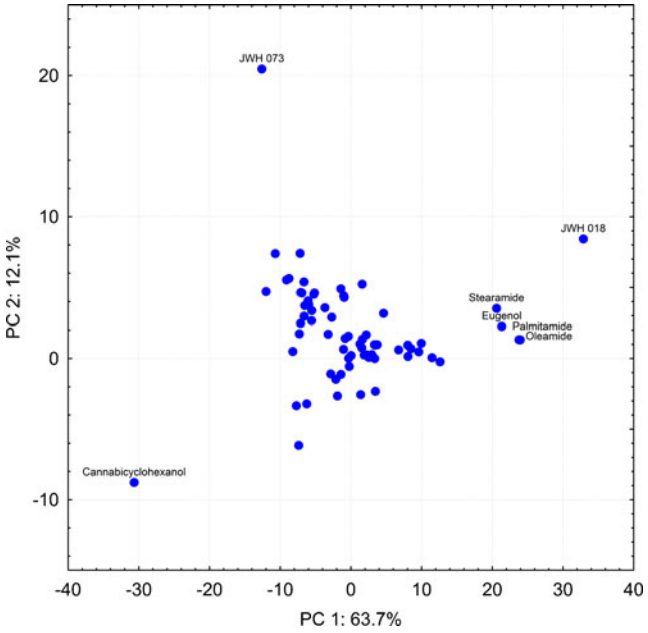

Fig. 3 The results of PCA analysis: The PC1-PC2 loadings of the herbal highs ingredients. The variables with significant size of the loading were described

loadings to the first PC had JWH-018 and cannabicyclohexanol followed by oleamide, palmitamide, stearamide and eugenol. The main component of PC2 was JWH-073. The loading of cannabicyclohexanol was also significant. It is clearly seen that synthetic cannabinoids are the most important variables responding for sample differentiation. These substances were also found by other authors as the active ingredients of "herbal highs" [11, 12, 14]. All above-mentioned compounds were marked in Fig. 3. From the loading plot, information about the correlation of feature variables can be deduced. The correlation of the features is described by the cosine of the angle between the loading vectors. The smaller the angle the higher is the correlation. Uncorrelated features are orthogonal to each other. When analysing the PC1–PC2 loading plot, a strong negative correlation between JWH-018 and cannabicyclohexanol is observed (high amounts of JWH-018 in a sample correspond to low amount of no presence of cannabicyclohexanol and vice versa). The correlation was confirmed by calculation of Pearson correlation coefficient r, which was −0.87. On the other hand, there is a high correlation between JWH-018 and oleamide, palmitamide, stearamide and eugenol (r was between 0.93 and 0.95). The amount of JWH-073 was not correlated to the amount of JWH-018 (r=−0.25) or cannabicyclohexanol (r=0.28); the vectors between corresponding to JWH-073 and JWH-018 (or cannabicyclohexanol) are almost orthogonal.

The sequential plot of the above-mentioned substances in the analysed products is presented in Fig. 4. The colour depth corresponds to the peak area (fourth squares of peak areas were used in comparison in order to lower the difference between very high and low peaks).

It is to note that there were significant differences in the synthetic cannabinoids content between the preparations. Some products contain only one synthetic cannabinoid, whereas the others two or even more. Two products ("Zen ultra" and "Chill zone cherry") contained all potential synthetic cannabinoids and eugenol. Significant differences in the synthetic cannabinoids content were observed also by other authors [11, 12, 14]. The composition of "legal highs" depends strongly on a local law. If a ban is introduced, active ingredients are replaced by their derivatives or other substances affecting cannabinoid receptors [14]. The preparations analysed in the study were seized by the Polish police in the beginning of 2009, just before amendment of the Act on Counteracting Drug Addiction, which means that all identified substances were "legal". At that time, JWH-018 and cannabicyclohexanol were also main ingredients of "herbal highs" analysed by German and Japanese research groups [11, 12]. JWH-073 was identified in products acquired after the prohibition in Germany [13, 14]. Great differences in peak areas correspond to the great differences in substances concentrations in the preparations, which is very important from the toxicological point of view. It is known that synthetic cannabinoids are added to the herbal mixtures in order to evoke the psychoactive action (so far, no typical alkaloids were found by their analysis and users report mixtures without chemical additives concordantly as non-effective). Quantitative analysis of the synthetic cannabinoids in the preparations (not presented in the paper) indicated that their content is in the range from tens to hundreds of milligrammes per sample, which was consistent with the concentrations reported by other authors [11, 12]. Taking into account higher affinity of JWH-018, JWH-073 and cannabicyclohexanol to the cannabinoid receptors (CB1, CB2), it can explain why smoking of herbal highs leads to more severe effects than administration of *Cannabis* products and causes serious health problems.

The preparations in the sequential plot (Fig. 4) were sorted according to their flavours (if the flavour was declared on the label). When analysing deeply the plot, many "blocks" strictly connected with the preparation flavours could be observed. It leads to surprising conclusion that the kind of synthetic cannabinoid depended not on the basic name of preparation (e.g. "Tai fun" or "Chaos"), but on its flavour. It explains also why the clusters presented in Fig. 2 contain the samples with identical flavour (blackberry, vanilla and orange—the upper cluster; mint and cherry (and "original")—the lower cluster).

In the next step, chemometrical analysis of remaining variables was performed. As listed above, many substances identified in the samples were potentially of natural origin,

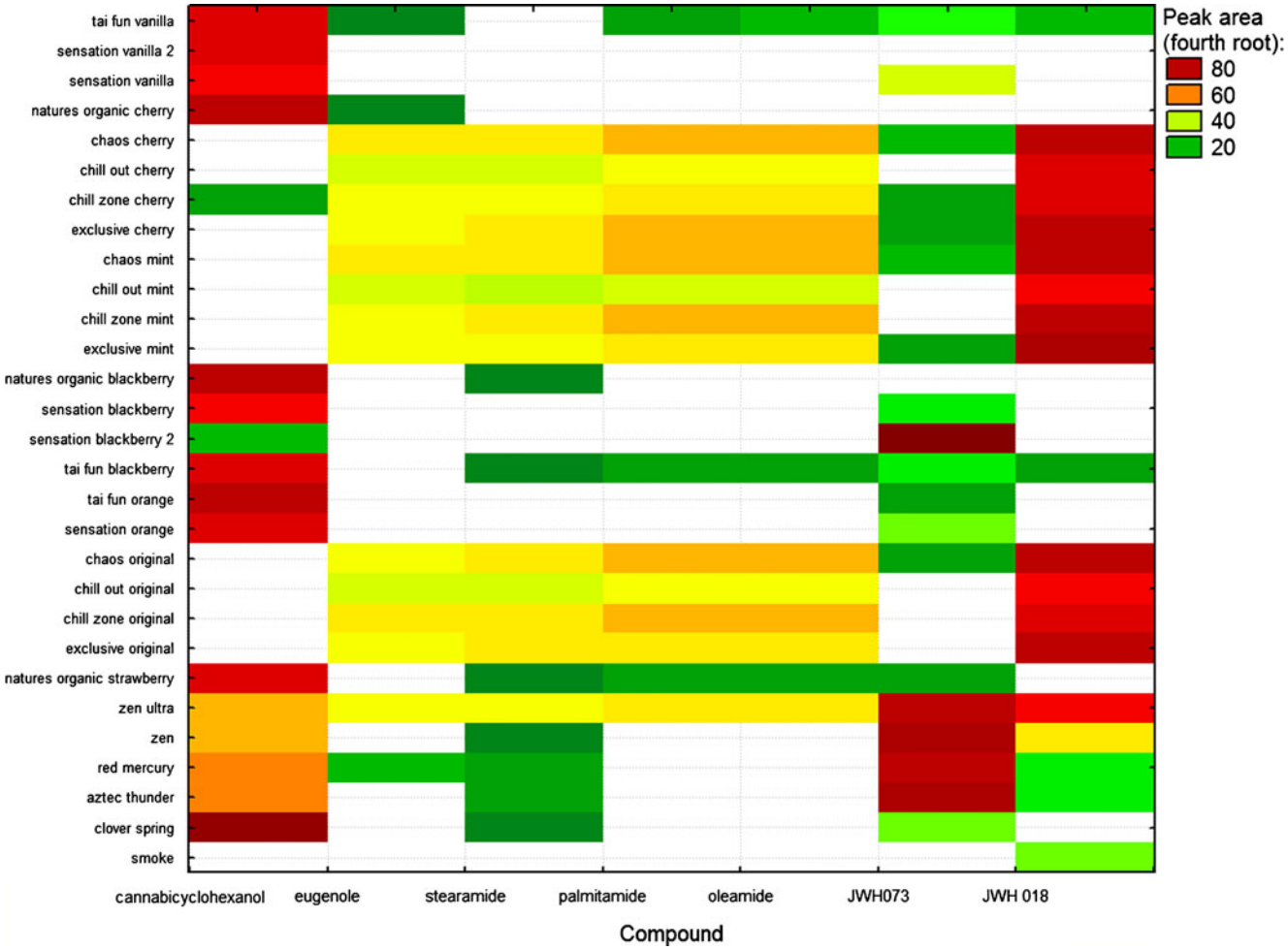

Fig. 4 The sequential plot of JWH-018, JWH-073, cannabicyclohexanol, oleamide, palmitamide, stearamide and eugenol in the analysed products

whereas the others are artificial additives. According to the declaration of manufacturers on the labels, herbal highs are mixtures of psychoactive plants originating from South America, Africa or Asia. Therefore, the authors decided to check if there were any correlations between natural components of the analysed preparations or the samples could be clustered according to their origin. The synthetic cannabinoids and the substances potentially being artificial additives were omitted in the analysis. The chemometric analysis started once again from cluster analysis of the preparations. It turned out that the results were similar to those presented in Fig. 2; two clusters were formed, one of them contained the samples with "mint", "cherry" and "original" in their names (this clusters covered also "zen ultra", as in Fig. 2), whereas the second included the other preparations. PCA was performed to analyse the correlation between the variables (the ingredients) and their impact on the samples differentiation. The first two PC explained about 80% of the total variance. The highest loadings to the PC1 had oleamide, palmitamide, eugenol and stearamide. Fatty acids and their esters had also significant impact on PC1. The most important ingredients of the PC2 were hydrochinone, eucalyptol, alpha-amyrin and betasitisterole. The most interesting finding of this part was that even if synthetic cannabinoids and potential artificial additives were omitted in the analysis, the samples were clustered according to their flavours and not their names.

Therefore, the authors decide to limit the further chemometric analysis to the samples with define flavour (plus the preparations with "original" in their names). Only substances of potential natural origin were taken into account (amides of fatty acids were excluded in this part of analysis). In this step, PCA was applied both for assessment of ingredients correlation and the sample grouping. The loading plot is presented in Fig. 5, and the score plot is shown in Fig. 6.

The analysis of data presented in Fig. 6 indicated that even if only limited number of variables was taken into account, the majority of preparation was divided according to their declared flavour. The clusters containing "blackberry", "mint", "vanilla" and "cherry + original" preparations are clearly visible. Some preparations were classified incorrectly, but the preparations can be separated to form

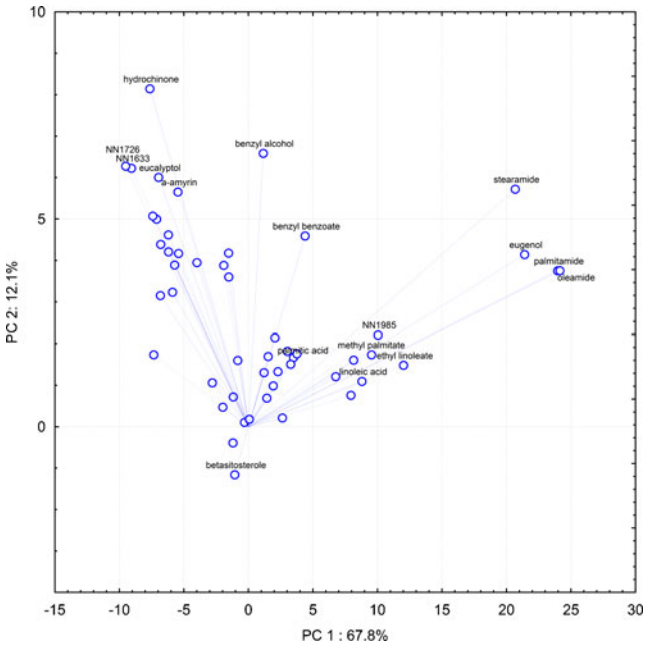

Fig. 5 The PC1 and PC2 loadings of natural components of herbal highs

even more homogenous groups using more than two PCs (data not presented), e.g. the PC4 scores for "natures organic cherry" and "natures organic strawberry" were around 20, whereas the scores for "blackberry" preparations were −20. Data presented in Fig. 5 shows that the ingredients with the highest loadings to the PC1 were eugenol, fatty acids and their esters; whereas to the PC2—hydrochinone and eucalyptol. Betasitosterole was the only one substance with negative loading to the PC2.

Conclusions

The main active ingredients of herbal highs were synthetic cannabinoids: JWH-018, JWH-073 and cannabicyclohexanol, and this confirms that narcotic effects observed after their smoking result from artificial additives and not from psychoactive action of natural constituents. Statistical and chemometrical analysis led to discovering that the composition of the examined samples of legal highs was correlated, and the herbal matrixes were similar accordingly. The kind of the synthetic cannabinoid depended strongly on the declared flavour of the preparation and not its common name. The content of other substances was also correlated with the preparation flavour. It could be confusing for the potential user because it is expected that the products with the same name contain the same (active) ingredients. This additional threat could be taken into account when toxicity of herbal highs is discussed.

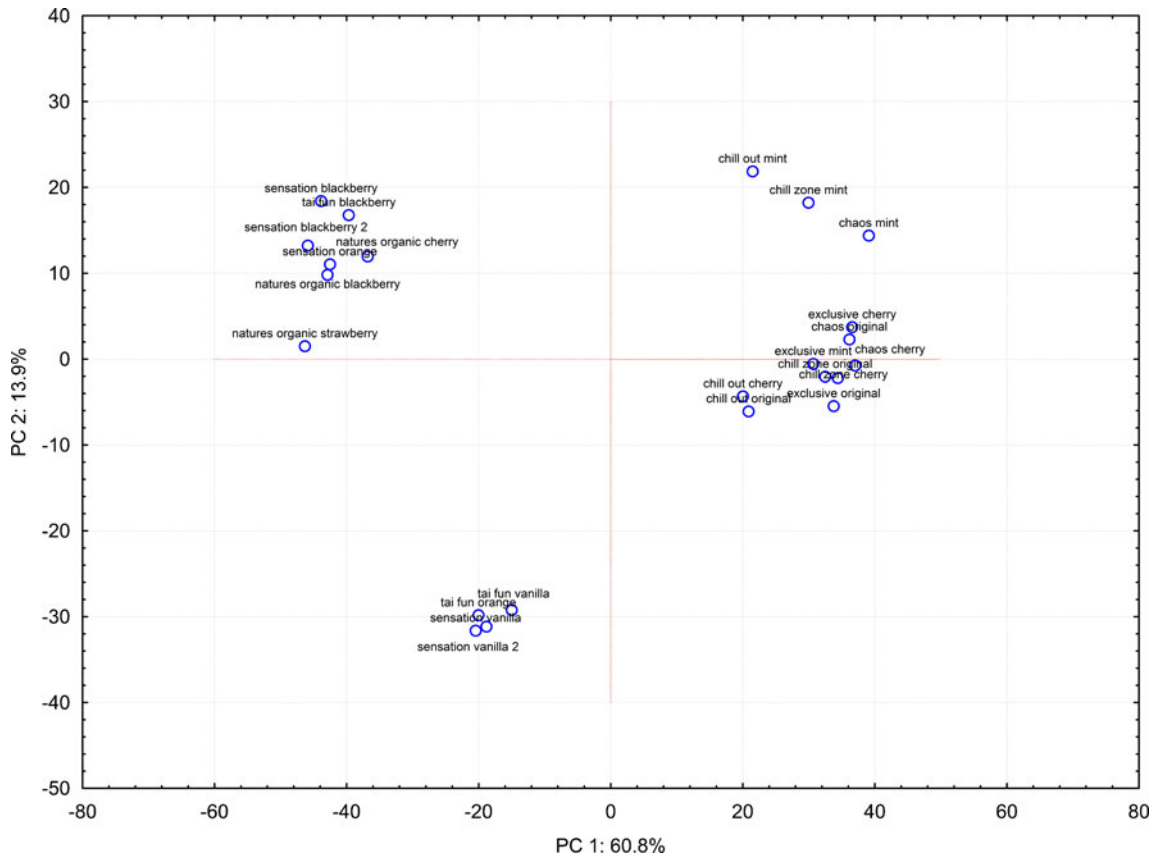

Fig. 6 The PC1–PC2 scores of herbal highs preparations based on natural components presented in Fig. 5

References

1. A report from an EMCDDA expert meeting, Lisbon (Portugal) (2009) EMCDDA Action on new drugs briefing paper: Understanding the "Spice" phenomenon. www.tubim.gov.tr/Dosyalar/Spice.pdf, accessed on 15 March 2010
2. Szukalski B, Błachut D (2010) Zmodyfikowane kannabinoidy—nowe groźne narkotyki [in Polish]. Probl Krym 268:14–25
3. Auwarter V, Dresen S, Weinman W, Müller M, Puetz M (2009) Spice and other herbal blends: harmless incense or cannabinoid designer drugs? J Mass Spectrom 44:832–837
4. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Goda Y (2009) Identification ofa cannabimimetic indole as a designer drug in a herbal product. Forensic Toxicol 27:61–66
5. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Haishima Y, Goda Y (2009) Identification of a cannabinoid analog as a new type of designer drug in a herbal product. Chem Pharm Bull 57:439–441
6. Aung MM, Griffin G, Huffman JW, Ming-Jung W, Keel C, Yang B, Showalter VM, Abood ME, Martin BR (2000) Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB1 and CB2 receptor binding. Drug Alcohol Depend 60:133–140
7. Huffman JH, Mabon R, Mu MJ, Lu J, Hart R, Hurst DP, Reggio PH, Wiley JL, Martin BR (2003) 3-Indolyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interactions with the CB1 cannabinoid receptor. Bioorg Med Chem 11:539–549
8. Huffman JH, Thompson ALS, Wiley JL, Martin BR (2008) Synthesis and pharmacology of 1-deoxy analogs of CP-47, 497 and CP-55, 949. Bioorg Med Chem 16:322–335
9. Lambert D, Di Marzo V (1999) The palmitoylethanolamide and oleamide enigmas: are these two fatty acids amides cannabimimetic? Curr Med Chem 6:757–773
10. Leggett JD, Aspley S, Beckett SRG, D'Antona AM, Kendall DA (2004) Oleamide isa selective endogenous agonist of rat and human CB1 cannabinoid receptors. Br J Pharmacol 141:253–262
11. Lindigkeit R, Boehme A, Eiserloh I, Luebbecke M, Wiggermann M, Ernst L, Beuerle T (2009) Spice: a never ending story? Forensic Sci Int 191:58–63
12. Uchiyama N, Kikura-Hanajiri R, Ogata J, Goda Y (2010) Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. Forensic Sci Int 198:31–38
13. Otto M (2007) Chemometrics. Statistics and Computer Application in Analytical Chemistry, Wiley, Weinheim
14. Dresen S, Ferreiros N, Putz M, Westphal F, Zimmermann R, Aufwarter V (2010) J Mass Spectrom 45:1186–1194