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Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

IDENTIFICATION AND CLASSIFICATION OF WHISKEY ALCOHOLIC DRINKS USING MASS SPECTROMETRY AND CHEMOMETRIC TOOLS

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ABSTRACT. Adulteration of alcoholic drinks is often common, due to the products high prices and large commercialization. Using Mass Spectrometry and a Multi-Variate Statistical Package soft with PCA analysis application, whiskey from different sources was identified and compared in order to create a successfully method for identification possible adulterants. For PCA analysis the m/z abundance values (obtained from fingerprints mass spectra) were used. The results showed that the complex spectrum is a good chemical fingerprint of whiskey sample, indicating its origin and type and facilitate a good differentiation of different samples. By classification the models obtained for all whiskey samples, a method for adulterants detection was created.

Keywords: whiskey*, GC-MS, PCA analysis, spectrum fingerprint.*

INTRODUCTION

Whiskey is a high quality alcoholic beverage obtained by the distillation of spirits from various cereals. Scotch wihskey is the one produced from barley malt in Scotland and matured for three years in oak casks[1] and American whiskey is made from a corn steep, rye and some malt barley and aged in new oak barrels burned inside for at least two years. There are also blended whiskey which is obtained by a mixture of several varieties of whiskey, often more than 30, to get the same uniform taste always which has moderate quality[2]. The flavours and the signiatures of different types whiskey are the product of manufacturing process, period of fermentation processes, distillation

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and aging[3]. The natural process of aging for at least two years in oak barrels, will change the odor, flavor and color of whiskey. The chemical profile of whiskey is a complex range of flavor compounds of which the most important are carbonyl compounds, alcohols, carboxylic acids and their esters, sulfur and nitrogen compounds (pyridine, pyrazine), polyphenolic compounds, terpenes, and fatty esters.[4,5] Phenolic acids are extracted in oak whiskey, and their concentration can be influenced by the time that was in contact with wood[6].

Determination of authenticity of food products and also identification of the origin of food products is an important aspect in food quality control. Adulteration of alcoholic drinks is often common, due to the products high prices and large commercialization. Being an expensive alcoholic liquor, whiskey is often exposed to adulteration. The certification of authencity of whiskies is the main interest of producers, dealears and consumers. To prove the authencity and origin of whiskey different methods have been reported. Some of them are using gas chromatography (GC) for determination of the mixture of flavouring compunds or for analysis of isotopic *ratio*[1] of ^{13}C and ^{12}C and furfural and 5-hydroxymethyl-2- furaldehyde *ratio* as whisky markers[7]. Analysis of the tracing metals is also considered an indicator of whiskey's authencity.[8] Several GC-MS methods were used to analyze the volatiles profile in whiskey.[9,10,11,12] ESI-MS methods with direct injection of samples coupled with chemometric analysis have been shown as an efficient technique for determination of authencity of whiskies.[13,14] Whiskey has a complex variety of compounds and is a good matrix to use multivariate techniques, such as Principal Component Analysis (PCA). Different programs used in chemometric analysis were used together with analytical methods to group or to identify adulteration of whiskey sample[15].

In this study whiskey from different sources was classified and compared using a new Gas-Cromatography-Mass Spectrometry method coupled with a Multi-Variate Statistical Package soft with PCA analysis application, in order to create a rapid and efficient method for identification of possible adulterants by mixing alcoholic beverages.

RESULTS AND DISCUSSION

Four types of authentic samples of whiskey commercialized in Romania: (Jack Daniel's, (whi5), Ballantine's (whi6), Jim Beam (whi7) and Johnnie Walker (whi8)) were liquid-liquid extracted and analyzed. Another two samples of blended whiskey obtained by mixing Jack Daniel's and Ballantine's (1:1, v/v), (whi 5.6) and Jack Daniel's, Ballantine's, Jim Beam (2:1:1, v/v/v), (whi5,6,7) were extracted and analyzed**.** The whiskey extracts were analyzed by GC-MS to obtain the reconstituted chromatograms. Using the soft provided by the MS instrument, a fingerprint mass spectrum for the all analyzed samples was obtained by summing up the mass spectra taken

every minute from 5 minutes up to 30 minutes. The obtained complex mass spectrum will be named fingerprint and the corresponding abundances m/z will be variables that will differentiate the samples. In Figure 1 the reconstructed chromatogram are shown for the Jack Daniel's whiskey extract, beside the fingerprint mass spectrum. This is a very complex chromatogram, more than 100 components being detected. Similarities between the samples are observed also in fingerprints. All whiskies fingerprints display 67 common ions. The most important are 53, 55, 57, 61, 83, 84, 85, 88, 91, 97, 101, 127, 129 and 143 m/z, with variable intensities, which can be considered diagnostic ions for whiskey. Fingerprints of different whiskey sources display also some individual variation. For example, Jack Daniel's ion of m/z 88 is the most intense and some others are unique ions (m/z 149, 165, 191, 208, 298); Ballantine's most intense ion is m/z 55, while the ion of m/z 88 appearing in fingerprint has a weak intensity. Ballentine's fingerprint has also some characteristic ions of m/z like 138, 162, 274; the intense ion of Jim Beam whiskey is m/z 55, but also ion of m/z 88 has a high intensity. Johnnie Walker whiskey fingerprint has ion of m/z 55 of highest intensity and some unique ions of m/z like 242 and 267. All authentic samples fingerprints possessed many common ions which are valuable for stabilize a common chemical label of whiskey. The used method demonstrates that is possible to identify common ions of samples and also different variables in samples.

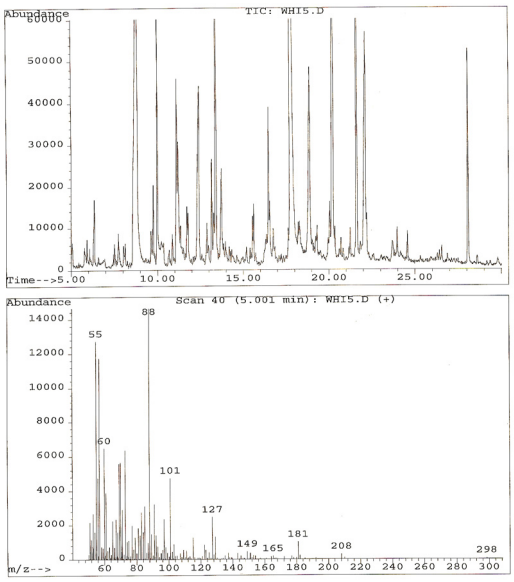

Figure 1. Reconstructed chromatograms for extract of Jack Daniel's whiskey beside the fingerprint mass spectrum

Validation of analytical method

For validation of the analytical method three samples from the same Jack Daniel's whiskey extract were analysed: whi5, whi5.1 and whi5.2 by GC-MS. For each mass spectrum fingerprint obtained, abundance fragments m/z were collected. To calculate the reproducibility, the fingerprint spectra abundances were compared for all three injections. Calculating standard deviation (SD) and relative standard deviation (RSD) in each of the three injections of the same extract (whi5), an average standard deviation (0.82) and an average relative standard deviation (9.09) were obtained. These values indicate a medium reproducibility, but given the large number of variables can be considered as acceptable. Further, the collected data were statistically interpreted.

Statistical analysis of data

The correlation of multiple mass spectra is simplified by using multivariate statistical techniques and is essential due to the large number of variables. The principal component analysis subtracted the extension of data set of highly correlated variables, so the number of variables will be reduced by constructing a new set of coordinates[15].

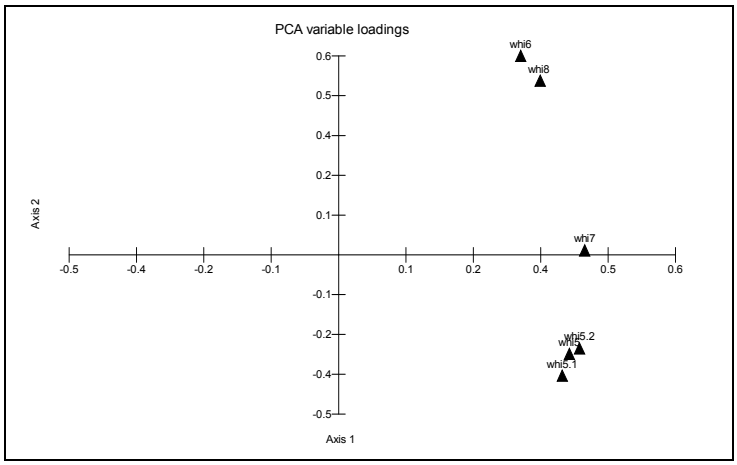

Figure 2. The graphical representation of the two main components obtained by the 67 variables (values m/z from fingerprint mass spectrum) for three samples of Jack Daniel's (whi5; whi5.1; whi5.2), Ballantine's (whi6), Jim Beam (whi7), and Johnnie Walker (whi8).

The PCA analysis variables are abundances of fragments (m/z) from mass spectrum fingerprint obtained by summing the abundances of ions from mass spectra obtained every minute. PCA method search for correlations between abundances values of m/z and extract linear combinations of the

nearest abundance (major components) which define the difference between samples. To construct the complex matrix, 67 variables (the percentage values of reports m/z spectra fingerprint) were considered. Following the interpretation of the results obtained by mass spectrometry using the MVSP soft with PCA analysis application, the resulting graphs, obtained from analysis of three injections of whiskey Jack Daniel's (whi5; whi5.1; whi5.2) layout very close to each other, which prove that the method is reproducible. Also the representation graph for all four types of whiskey shows that samples can be differentiated (Figure 3).

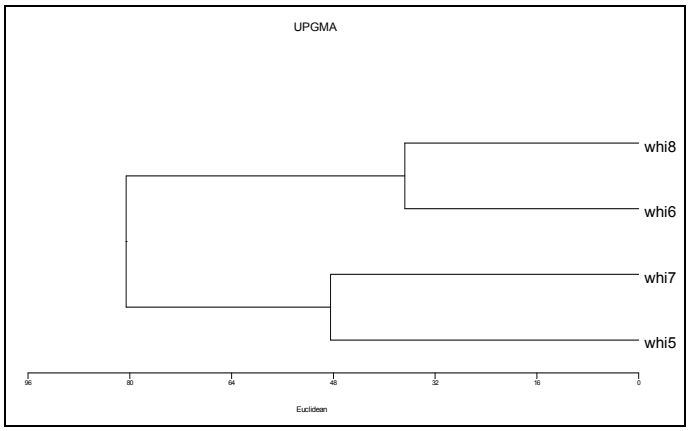

Figure 3. The dendogram obtained by measuring the Euclidean distances for the four extracts of whiskey Jack Daniel's (whi5), Ballantine's (whi6), Jim Beam (whi7), Johnnie Walker (whi8) based on the values of m/z from fingerprint mass spectra

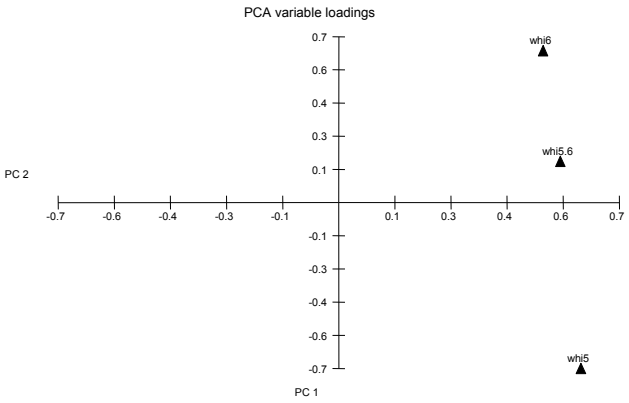

Figure 4. The graphical representation of the two main components obtained by the 67 variables (the percentage values of reports m/z from fingerprint mass spectrum) for extracts of Jack Daniel's (whi5), Ballantine's (whi6) and mixtures of the two in equal proportions (whi5.6).

The cluster analysis shows us the degree of relatedness by the m/z values from mass spectra fingerprint. This similarity of samples can be attributed to their different origins: Jack Daniel's (whi5) and Jim Beam (whi7) comes from the US (Bourbon) and Ballantine's (whi6) and Johnnie Walker (whi8) come from Scotland (Scotch).

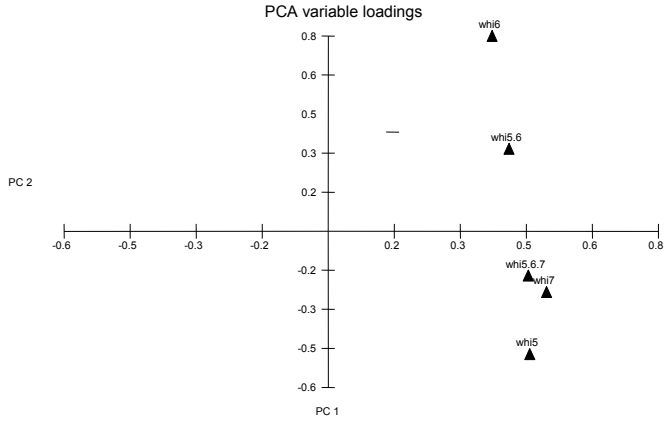

Figure 5. The graphical representation of the two main components obtained by the 67 variables (the percentage values of reports m/z from fingerprint mass spectrum) for extracts of Jack Daniel's (whi5), Ballantine's (whi6), Jim Beam (whi7) mixture Jack Daniel's 50% Ballantine's 50% + (whi5.6) and 50% mixture Jack Daniel's (whi5), 25% Ballantine's (whi6) and 25% Jim Beam (whi7): (whi5.6.7)

The analysis of the main components from mixtures obtained by combining samples of whiskey, shows that samples can be differentiated from the originals from which they are derived using this fingerprint obtained by mass spectrometry. The described developed method can be successfully used to detect a mixture or a fake made by mixing two or three original drinks. (Figure 4-5)

CONCLUSIONS

The obtained results showed that the recorded complex spectrum is a good chemical fingerprint of whiskey sample and facilitate a good diferentiation of whiskey samples in terms of origin and type. By classification models obtained for all whiskey samples, a method for adulterants detection was created. The proposed method can be applied successfully to identify the origin of alcoholic beverages and by creating a database it is possible to detect forgeries drinks.

EXPERIMENTAL SECTION

Materials and methods

Samples of whiskey

Original Jack Daniel's, Ballantine's, Jim Beam, and Johnnie Walker were purchased from a liquor store from Romanian. The blended whiskey was obtained by mixing of 50% original Jack Daniel's and 50% original Ballantine's whiskey and other blended combining 50% original Jack Daniel's, 25% original Ballantine's, 25% original Jim Beam.

Method of extraction

Whiskey samples were liquid-liquid extracted. As extraction solvent, a mixture of ethyl acetate: *n*-hexane: dichloromethane (5:1:1, v/v/v) was used. 20 mL sample of whiskey were extracted with 1 mL of solvent mixture and analyzed by GC-MC.

General experimental procedure

The GC-MS analysis were done using a GC-MS Hewlett Packard 5890 (EI mode) using Rtx-5MS capillary column, 30 m × 0.25 mm, 0.25 μm film thickness, using a temperature program from 50 ^{o}C, 2 min, 3^{o}C /min at 180^{o}C, 30^{o}C /min at 220^{o}C, then 220 ^{o}C, for 5 min. The flow rate of helium, the carrier gas was 1 mL/min. The injector, interface, ions source and quadrupole are operated at 250°C, 280°C, 200°C and 100°C. The mass spectrometer was used in electron impact mode; electron energy of 70 eV and electron emission 300μA. 2μL of each extract of whiskey (whi5,6,7 or 8) were injected into the GC-MS to yield for each of them the reconstituted one chromatogram. Using the mass spetrometer soft fingerprints mass spectra for the comparison sample were obtained by summing up the mass spectra taken every minute from 5 minutes up to 30 minutes.

Statistical analysis of data

To classify the whisky samples, the obtained mass spectra were converted in samples chemical fingerprints, which were interpreted and compared using a Multi-Variate Statistical Package (MVSP) soft with PCA analysis application. MVSP is an easy to use program that performs a number of multivariate numerical analyses. It can also perform cluster analysis, with 23 different distance and similarity measures and seven clustering strategies.[16]

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