A new comprehensive GC-MS method for identification of the aroma profile of traditional Greek distillate ouzo

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Abstract

A chromatographic method was developed for analysis of the aromatic substances of Greek traditional distillate called ouzo. The method was based on the use of a new very long (100 m) capillary chromatographic column which was adjusted on the Agilent GC-MS apparatus and the suitable chromatographic parameters where established after intensive trials and optimization. Thirteen samples of ouzo were analyzed in triplicate by using the developed analytical protocol and the aromatic profile of them was recorded qualitatively and quantitatively for the main aromatic compounds existent in the distillate. A large variety of over fifty aromatic compounds were identified by using the NIST MS-library and most of these compounds found to have 5C and 6C aromatic rings. Furthermore, the main aromatic constituent was found to be trans - anethole while the toxic substances of methanol and cis - anethole were absent. These findings are considered very positive for the quality of the distillate as this appears to have a strong aromatic character without the presence of substance that can harm consumer health. This work claims, for the first time, a comprehensive qualitative analysis for the ouzo distillate and the development of a prototype and general use GC-MS method for determining aromatic substances in alcohol distillates.

Key words: Ouzo, GC-MS analysis, aromatics, distillate

Introduction

Ouzo is a distilled alcoholic spirit which is vastly produced all over Greece and its consumption is part of the people's culture [1]. A series of aromatic materials had been used to improve the flavor of the spirit like anise seed, star anise seed, cinnamon, salvia, mastic etc. In most cases the alcohol content of ouzo varies in the range of 40-43 °v/v. Its production is based on the co-distillation of a 50% v/v water/alcohol solution after addition of aromatic materials in it at a percentage of about 3-5% w/v.

However, and despite the importance of its consumption a comprehensive qualitative and quantitative analysis of the spirit concerning the content of either aromatic or potentially hazardous material is lacking from the international scientific literature. Some trial to contribute on this subject were recorded [1,2,3,4,5] but because either a limited number of ouzo samples were analyzed in them or the use of GC methods with confined capacity to provide a deep insight of the qualitative aromatic profile (mainly due to the use of short length GC columns) prohibited the illustration of the full picture of the profile of ouzo volatiles reducing thus the potential for the data to be effectively used by medical teams in order to disclose the presence potentially hazardous substances in the spirit.

In the present work a new approach was used in order to better analyze the qualitative volatile profile of ouzo. Particularly, more samples were analyzed qualitatively by using GC-MS analysis employing a large (100 m in length) capillary column and a quantitative determination was performed using suitable GC-grade standards of the pure volatiles to produce the calibration equations for the main aromatics included in ouzo spirit. This method can be used without any modification for the determination of the volatile profile of a series of distillates like the Turkish raki or the Syrian arak assisting in achieving a better qualitative identification of minor aroma constituents that might have a negative effect on human health despite their low concentration in the above mentioned spirits.

Materials and methods

Chromatographic hardware

An Agilent GC-MS system type was used which was equipped with a 100 m long capillary GC-column type J&W HP88 112-88 A7 100m x250μm x 0.25μm, silica type.

Chromatographic method

The developed chromatographic method was based on the following parameters:
Injection mode : Split with ratio: 100:1
Injection size : 2.5 μl Sample Dilution : none
Oven Program : 50 degrees C foe 5 min, then at 5 °C/min to 130 °C and finally at 3 °C/min up to 250 °C
Carrier gas: helium-GC-grade
Heater Temperature : 300 °C
MS parameters : LowMass:: 50.0 High Mass: 450.0 MS-Source temp: 230 °C MS quad temp.: 150 °C.

Ouzo Samples & Chromatographic standards of methanol, estragole & trans-anethole

A set of 13 samples of ouzo obtained from different suppliers around Greece was used for the GC-MS analysis. Each one of these samples was analyzed in triplicate and the average figures were used for quantitative determinations of the main aromatics: methanol, estragole, trans-anethole. The calibration curves used for the quantitative determination of the three main aromatics were constructed by using dilutions of chromatography grade standards obtained by RIGAS LABS S.A-Chemical Supplier. – Thessaloniki Greece.

Results and discussion

a) The qualitative profile of ouzo

A total of 45 volatile substances were identified by using the developed GC-MS analytical method. The total number substances result a significant improvement compared with the ones obtained in previous works. These substances are presented in the Table 1 which follows along with their proposed chemical structures. Most of them are volatile chemicals with 5 C and 6 C rings and most of them well known chemical.

The panorama of these substances can be used by medical research groups in order to identify potential hazardous materials contained in ouzo while the method can extend its application on other distilled spirits like the Turkish raki or Syrian arak. The large number of volatiles which was managed to be obtained is a proof that the method of the present work is superior of others developed in the past.

From the study of the chromatographic charts two major aromatic substance identified in the samples trans-anethole while estragole was also present. Another important finding was that toxic cis-anethole and methanol were not present in all ouzo samples and a variety of volatile chemical substances of different classes was contributing in the aromatic profile of ouzo including esters, alcohols, aldehydes, cetones, cyclic compounds, organic acids and amino acids.

b) Trans-anethole content of ouzo samples

The trans-anethole content of ouzo samples are presented expressed in ppm and in grams/100 litres of pure ethanol in Figure 1,2 respectively.

Table 1.The aromatic profile of Greek ouzo

No	Identified Substance name (NIST database)	Elution time (min)
1	Acetaldehyde, hydroxyl-	**21.040**
2	2- Propanone,1- hydroxyl-	**22.361**
3	Benzene,1- methoxy-4-(1-propenyl-)	**32.667**
4	2-Furancarboxaldehyde,5-hydroxy-	**51.047**
5	2-Hydroxy-gamma-butyrolactane	**51.372**
6	Alanine	**6.196**
7	Acentic acid,oxo-	**6.623**
8	1- Adamantanemethylamine,a	**9.301**
9	Acetic acid (aminocarbonyl-)	**24.338**
10	4-Pyridinol,acetate (ester)	**26.538**
11	Formic acid,ethenylester	**29.720**
12	Benzaldehyde,4-methoxy	**43.379**
13	4H- Cyclopropa(5,6)benz(1,2)	**56.743**
14	Methyl Alcohol	**8.666**
15	Estragole	**32.702**
16	Methanone, (3,4-diethoxyphenyl-)	**46.862**
17	2-Furanmethanol	**29.714**
18	2-Propenoic acid,ethenylester	**38.922**
19	1,6,3,4-Dianhydro-2-deoxy-a-c	**50.076**
20	Acetic acid,oxo-,methylester	**23.753**
21	Acetic acid (acetyloxy)	**24.378**
22	1-Formyl-3-methylaziridine-2-	**24.627**
23	Furfural	**26.548**
24	Propanoic acid,2-oxo-	**27.900**
25	Pyrazole-4-carboxaldehyde	**30.415**
26	4(1H)-Pyrimidinone	**30.903**
27	Benzene, (2- methoxy-2-propenyl-)	**32.722**
28	o-Methylisourea hydrogen sulphate	**24.388**
29	Methylenecyclopropanecart	**29.724**
30	Pyrazole-4-carboxaldehyde	**30.390**
31	4H-Pyran-4-one,2,3-dihydro-3	**45.599**
32	Butanenitrile,2,3-dioxo-,dioxil	**22.325**
33	Pilocarpine	**26.482**
34	Cyclopentanepropanoic acid	**51.377**
35	1-n-Butoxy-2,3-dimethyldiazin	**17.249**
36	Acetic acid,hydrazide	**22.325**
37	Pyrimidine-2,4(1H,3H)-dion,5	**27.885**
38	1H- Pyrolizine-7-methanol,2,3	**41.046**
39	'2H-Tetrazole,2-methyl-	**42.261**
40	1,3,5-Pentathionethione,1-(4-methyl)	**52.134**
41	3-Amino-2-oxazolidinone	**27.900**
42	1-(3-Methylbutyl)-2,3,4,5 tetra	**30.253**
43	Acetonitrile,hydroxyl	**32.677**
44	Propanoic acid,2-(aminooxy)	**24.739**
45	Butahedioic acid,2,3-bis(acetate)	**27.844**

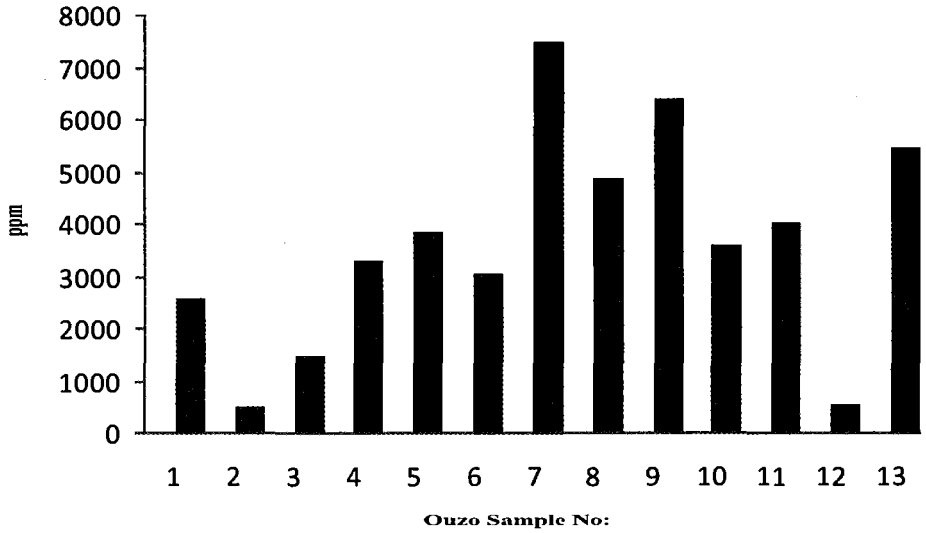

Figure 1. The concentration (ppm) of trans-anethole in various ouzo samples

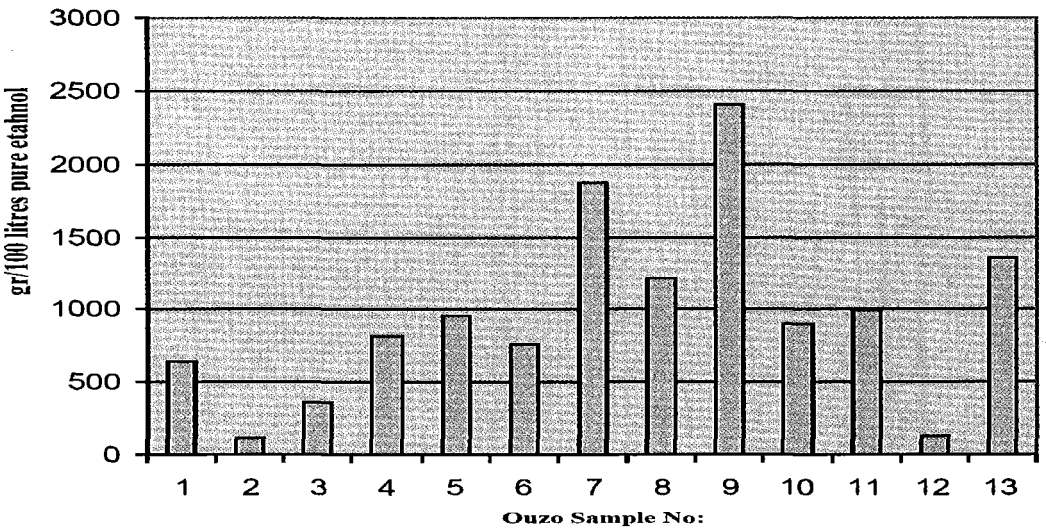

Figure 2. The concentration (grams/100 pure ethanol) of trans-anethole in various ouzo samples

The data presented in Figures 1,2 mark a substantial variation in concentration of trans-anethole between different samples. Particularly, the concentration of trans-anethole varies in the range of 100-2500 grams/100 litres pure ethanol. However the majority of the samples fall in the range 500-1500 grams/100 litres pure ethanol. This fact implies substantially different degree of aromatization by different ouzo producers.

c) Estragole content of ouzo samples

The estragole content of ouzo samples are presented expressed in ppm and in grams/100 litres of pure ethanol in Figure 3,4 respectively.

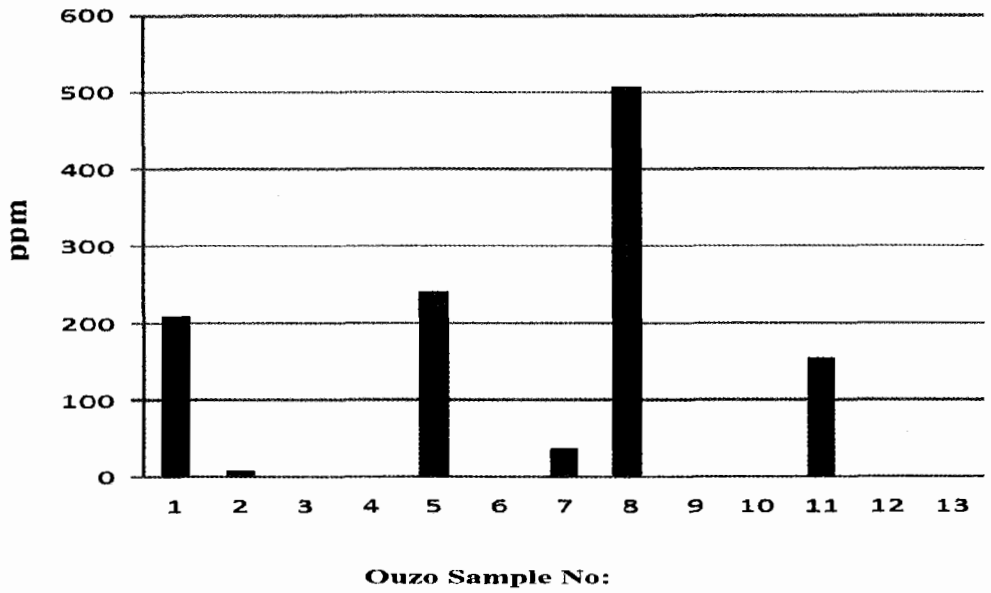

Figure 3. The concentration (ppm) of estragole in various ouzo samples

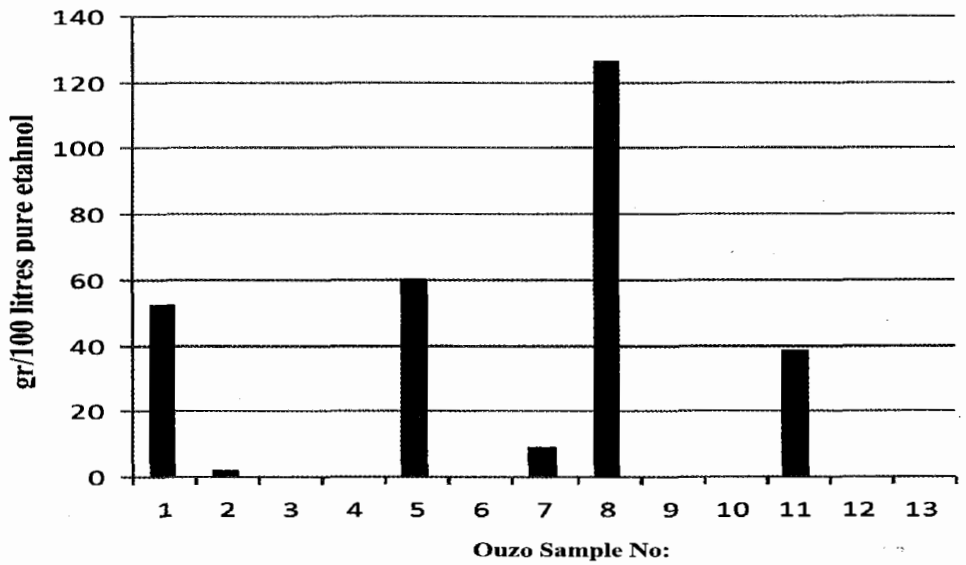

Figure 4. The concentration (grams/100 litres pure ethanol) of estragole in various ouzo samples

From the data presented in Figures 3,4 it is concluded that there is a significant variation in estragole concentration among different ouzo samples with some samples having even zero concentration. The range of the estragole concentration found to be 0-500 ppm or equivalently 0-125 grams/100 litres pure ethanol.

Conclusions

A new improved method GC-MS method was used for analysis of traditional Greek ouzo distillate samples which employed a very long (100m) capillary GC-column achieving better separation. By using

this method the presence of 45 volatile substances was confirmed in the analyzed ouzo samples. According the analysis data methanol was found to be virtually absent in the samples (it was present only in one sample and in negligible concentration) as well as the toxic cis-anethole. However, trans-anethole confirmed as the main ouzo aromatic and found to vary significantly among samples. Similar results were obtained with estragole which also varied in concentration among different ouzo samples and in some of them was absent. The developed analytical GC-MS protocol can be generally used to analyze similar to ouzo distillates like raki etc.

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