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## A COMPARATIVE STUDY OF VARIOUS SAMPLE PREPARATION PROCEDURES FOR CHARACTERIZATION OF ORGANIC COMPOUNDS IN BRANDY

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### Abstract

The composition of volatile compounds in 19 different wine distillates was studied by gas chromatography (GC) coupled with flame ionization (FID) or mass-spectrometric (MS) detector. The studied samples were divided into two groups depending on the way of their production and geographical region. The effect of various sample treatment procedures on final composition of volatiles was investigated in details. The effectiveness of direct injection, headspace, solid phase extraction (SPE), solid phase microextraction (SPME) and liquid-liquid extraction (LLE) was compared. Moreover, the effect of experimental conditions of preconcentration methods such as type of sorbent, temperature, time or solvent removal procedure was studied in details. The repeatability of particular sample preparation procedure was evaluated by comparison of peak areas for randomly selected compounds obtained from 4 parallel measurements. It was shown that the most suitable sample treatment procedure in terms of repeatability is SPE followed by direct injection and headspace. LLE and SPME provide higher variability of peak areas, thus utilisation of internal standard for quantification is recommended. On the contrary, the most suitable sample treatment procedure in terms of the number of different type of compounds is liquid-liquid extraction into  $\text{CH}_2\text{Cl}_2$ . By this method, more than 240 compounds have been extracted from wine distillates produced by classical technology. Furthermore, SPME has shown different selectivity which allows one to determine compounds that could not be extracted by other studied sample preparation methods.

**Keywords:** sample preparation, headspace, LLE, SPME, wine distillates, brandy.

### Introduction

Brandy is an alcoholic beverage that can be produced by distillation of fermented grapes, or in general, from any fruit juices. It originates from the Dutch word *brandewijn* (burning wine) [1]. The most famous wine distillates originates from Cognac or Armagnac regions in France from specific vine varieties and are produced by double distillation. Moreover, the quality of final wine distillate depends on many other factors, e.g. grape cultivars, harvesting time, quality of grape cider, activity of yeasts, fermentation, used distillation technology, quality and type of wooden barrels, etc. These factors influence not only the taste, but also the aroma, which means qualitative and quantitative composition of volatile organic compounds. VOCs present in wine distillates can be divided into four groups depending on the stage when they form.

Primary aromatic compounds, such as nitrogen- and sulfur-containing compounds or terpenes, originate from fruits, thus aroma appears exactly as in the fruit during ripening [2, 3]. The secondary aromatic components are formed during the alcoholic fermentation process, and among these, the most important are linear and branched

alcohols or carboxylic acids and their esters [4, 5]. There are many papers dealing with presence of carboxylic acids and their esters in alcoholic beverages, sample preparation procedures for their selective extraction as well as quantification methods [6, 7]. Distillation is responsible for tertiary group formation of aromatic compounds in wine distillates. The amount of volatile compounds in the final product is strongly influenced by type of distillation and suitable working conditions. Finally, quaternary aromatic compounds are formed during the maturation in wooden barrels [8, 9]. The VOC formed during this stage can be successfully used to evaluate the age of brandy [10]. Aroma responsible compounds in alcoholic beverages are present in a small percentage.

Various sample preparation methods have been used to characterize volatile compounds in wine distillates. The simplest and most attractive is direct injection because no sample treatment procedure is required [6]. However, this method is suitable mostly for analysis of major components, thus determination of aroma responsible compounds requires utilisation of either large volume injection (LVI) [11] or preconcentration method. The most frequently used preconcentration methods for VOC analysis are preparative GC or high-performance liquid chromatography (HPLC) [12] that have allowed the identification of more than 330 volatile compounds, of which 162 can be considered as trace compounds in Cognac and Calvados. LLE [9, 13], SPME [10, 14], supercritical fluid extraction [15], simultaneous distillation-solvent extraction [11] as sample treatment procedures have also been used for isolation and characterization of volatiles from alcoholic beverages.

According to EU–Slovak Republic accession treaty, there are 6 types of alcoholic beverages: wine distillates, wine, herb spirits, vodka, plum and juniper brandy included in claim on Protected Denomination of Origin, Protected Geographical Indication or Traditional Specialty Guaranteed [16]. Among these products, there are three wine distillates: Karpatske brandy special which can be produced only in the Little Carpathian wine region; Urpignac and Bystricke brandy special which is produced only in surroundings of the town of Banská Bystrica. In order to successfully protect these alcoholic beverages against possible adulteration, it is important to have detailed knowledge about their chemical composition.

The aim of this work was therefore to find the most suitable sample preparation procedure for isolation and characterization of volatile compounds in wine distillates produced in Slovakia. A major goal of this work is identification of organic compounds presented in Slovakian brandies produced by various technologies and in different geographical regions.

## 1. Experimental

**1.1. Instruments.** Capillary GC was performed using two independent Agilent Technologies 6890 gas chromatographs. The first instrument was equipped with split/splitless and headspace sampler G1888 and Agilent Technologies 5973 inert mass selective spectrometer. The second instrument was equipped with split/splitless injector and flame ionization detector. Helium with a flow rate of 1.5 mL/min was used as carrier gas in all analyses. Both liquid and gaseous samples have been injected into a 30 m DB-FFAP (nitroterephthalic acid modified PEG) capillary column with 0.25 mm I.D. and 0.25  $\mu$ m film thickness (J&W Scientific) via split/splitless injector heated at 250 °C. Splitless mode was used in all experiments. The temperature program was

tuned for each sample preparation procedure depending on their requirements and expected composition of injected sample. FID temperature was kept at 280 °C.

The Mass Spectrometry conditions were: EI ionisation, SCAN mode with a scan frequency of 1.2 scan/s and a scan range of 29–350 amu in all experiments. Data handling was performed by means of Agilent Chemstation software. Identification of compounds was performed by comparison of obtained MS spectra with NIST 05 MS library. The compound was considered as identified if a quality match of more than 80% was reached.

**1.2. Sample preparation procedures.** *Direct injection (DI)*: 1 µL of raw sample has been injected directly into GC.

*Headspace (HS)*: 10 mL of sample was inserted into 25 mL headspace vial and heated at 70 °C for 15 min; 500 µL of vapour sample was injected into gas chromatograph.

*Solid phase microextraction (SPME)*: This sample treatment procedure was performed with the SPME device for manual sampling consisted of a holder assembly and several replaceable fibers, all obtained from Supelco. SPME fibers coated with polydimethylsiloxane (PDMS) of 100 µm thickness, polydimethylsiloxane/divinylbenzene (PDMS/DVB) of 65 µm and carboxen/polydimethylsiloxane (CAR/PDMS) of 75 µm were obtained from Supelco. Prior to use, the fibers were conditioned by heating in the injection port of the chromatographic system under the conditions recommended by the manufacturer for each fiber coating. All analyses were performed in 15 mL clear glass vials and the solutions were stirred with PTFE-coated magnetic stir bars. Vials were sealed with hole-caps and PTFE/silicone septa. The temperature was controlled by a Heidolph EKT 3001 system. The adsorption of organic compound from 5 mL of sample on SPME fiber took 20 min at 45 °C. Desorption was performed in GC injector in splitless mode at 230 °C for 1 min.

*SPE procedure*: 5 mL of sample has been pipetted into 50 mL volumetric flask and vigorously shaken for 5 min. Immediately, 5 mL of vapour phase has been taken by syringe through glass microcolumn filled with TENAX TA (60–80 mesh) sorbent (Chrompack) with a flow rate of 5 mL/min. The distance between the microcolumn and the liquid surface was about 1 cm. After finishing of sorption step, glass tube has been disconnected from syringe and inserted into modified splitless injector at a carrier gas pressure of 10 kPa and heated at 225 °C for 2 min [17, 18].

*Liquid-liquid extraction with rotovap preconcentration (LLE-VD)*: 50 mL of sample was extracted with four 12.5 mL portions of dichloromethane and NaCl in separated funnel. Collected extracts were preconcentrated to 1 mL using rotovap at 35 °C.

*Liquid-liquid extraction with Kuderna–Danish preconcentration (LLE-KD)*: the same procedure as for LLE-VD was carried out with the expectation of preconcentration step: in this case a Kuderna–Danish apparatus was used with a water bath constantly kept at 85 °C.

The repeatability of particular sample preparation procedure was determined from data obtained by 4 independent analyses of the sample A03 that was treated by the same procedure under optimal condition. For each assay, the average peak area and the relative standard deviation (RSD) were calculated based on the peak areas found for base ion of selected compound.

Table 1

The list of samples under study

Label	Sample	Producer	Year of production	Ethanol [%]	water	added ethanol	wine distillate	sugar/sweeteners	brandy aroma	E150a	E151
A01	Karpatske brandy special	Vitis Pezinok	2004	40	x		x	x			
A02	Karpatske brandy special (I)	Vitis Pezinok	2005	40	x		x	x			
A03	Karpatske brandy special (II)	Vitis Pezinok	2005	40	x		x	x			
A04	Vinovica	Vitis Pezinok	2004	40	x		x				
A05	Karpatske KB	Vitis Pezinok	2004	40	x	x	x	x	x	x	x
A06	Karpatske KB	Vitis Pezinok	2005	40	x	x	x	x	x	x	x
A07	Pezignac	Vitis Pezinok	2005	38	x	x	x	x	x	x	x
A08	Frucon	Frucona, Kosice	2003	40	x		x				
A09	Trencianske brandy special	Old Herold, Trencin	2003	36	x		x				
A10	Trencianske hradne	Old Herold, Trencin	2005	36	x	x	x	x	x	x	
A11	Trencianske rezane	Old Herold, Trencin	2005	37.5	x	x	x	x		x	
A12	Brandy rezane	Old Herold, Trencin	2001	38	x	x	x	x	x	x	
A13	Brandy rezane	Old Herold, Trencin	2002	38	x	x	x	x	x	x	
A14	Trencianske brandy	Old Herold, Trencin	2001	37	x	x	x	x	x	x	
A15	Trencianske brandy	Old Herold, Trencin	2002	37	x	x	x	x	x	x	
A16	Bystricke brandy	Dunajskrob, Banska Bystrica	2004	37.5	x	x	x	x	x	x	
A17	Klastorne brandy	St. Nicolaus, Liptovsky Mikulas	2005	37	x	x	x	x	x	x	
A18	Spis brand special	Gas Family, Stara Lubovna	2005	38	x	x		x	x	x	

**1.3. Samples and chemicals.** 18 different wine distillates under study have been divided into two major groups. The first group contains wine distillates which can be considered as an imitation of classical brandy. These are produced as wine distillates diluted by ethanol from other sources and are characterized by presence of food additive E150a "Plain caramel". The second group consists of wine distillates produced by classical technology that is wine distillate aged in wooden barrels for certain period of time. In this group, all studied samples are VSOP grade except for the sample A04 "Vinovica". The samples A02 and A03 are produced in the same year but differ in date of expedition which is March 17 (A02) or June 02 (A03). The samples have been obtained directly from producers. The list of used samples with some characteristic information is shown in Table 1.

Dichloromethane and NaCl were purchased from Merck (Germany). Acetaldehyde, methanol, ethanol, acetone, methyl acetate, ethyl acetate, isobutyl acetate, 2-butanol, propanol, butyl acetate, isobutanol, isopentyl acetate, butanol, pentyl acetate,

isopentanol, hexanoic acid ethyl ester, pentanol, hexanol, octanoic acid ethyl ester and phenylethanol were obtained from Fluka (Germany).

## 2. Results and discussion

The samples under investigation underwent all preparation procedures discussed above (see Section 1.2). For a better comparison of various sample treatment methods, only the chromatogram obtained for the sample A03 will be shown. This sample belongs to the second group (wine distillates produced by classical technology) and thus the presence of compounds characteristic of grapes aroma is expected to be found.

In the first step, a direct injection of neat sample into the GC was performed. After initial temperature of 35 °C for 1 min, the column was programmed at 3 °C/min to 230 °C. When FID detector was used, the chromatograms obtained for all studied samples were poor of peaks, which can be attributed to the used sample preparation procedure that allows detection of just major compounds (alcohols and esters of some carboxylic acids). With high probability, other compounds are present in amounts not detectable by using FID, making necessary the use of preconcentration techniques. Another possibility is to employ more sensitive detector, such as mass spectrometry.

The GC-MS chromatogram obtained for the sample A03 is shown in Fig. 1. It can be seen that from 21 peaks which are present in the chromatogram at relatively high concentration levels only 15 have been successfully identified. However, additional 79 peaks were present at trace level. Most from the identified peaks are linear or branched alcohols and carboxylic acids and their ethyl esters.

Table 2 shows the repeatability data for the compounds with relatively high content in the sample 03. Practically for all identified compounds, satisfactory relative standard deviation (RSD) below 5% was found. For compounds containing free carboxylic group and carbonyl group attached to linear alkyl chain, a little higher RSD value (5.4%) was observed.

In HS injection method, only volatile compounds presented in wine distillates are evaporated and injected into the GC. In order to focus volatiles at the column head, a suitable initial low temperature of 35 °C was set and held for 1 min; then, at 2 °C/min the temperature increased to 100 °C, was held for 5 min, and at 10 °C/min increased to 220 °C. An operating temperature of 70 °C and equilibrium time of 20 min were found as the optimal HS conditions. Fig. 2 shows the GC-MS chromatogram obtained for the sample A03 by a static headspace injection method. It can be seen that chromatogram is cleaner and poorer on number of presented peaks compare to direct injection.

Obviously, only compounds with highest concentration, such as acetaldehyde, ethanol, ethyl acetate, ethyl esters of carboxylic acids, linear and branched alcohols appeared on chromatogram. The average numbers of compounds presented on chromatograms for the second group are 18. The obtained repeatability data are shown in Table 3. For the majority of the compounds, the RSD values vary within the range from 3 to 6 which makes this sample treatment procedure suitable for the quantification of these particular components. However, this sample treatment method provides higher variability in comparison to the RSD values obtained for the same compounds by direct injection. The most critical is change in the RSD value for acetic acid (5.4 by DI and 9.5 by HS method). The chromatograms for wine distillates from the first group have shown the presence of only two peaks (ethanol and ethylacetate). It was assumed that

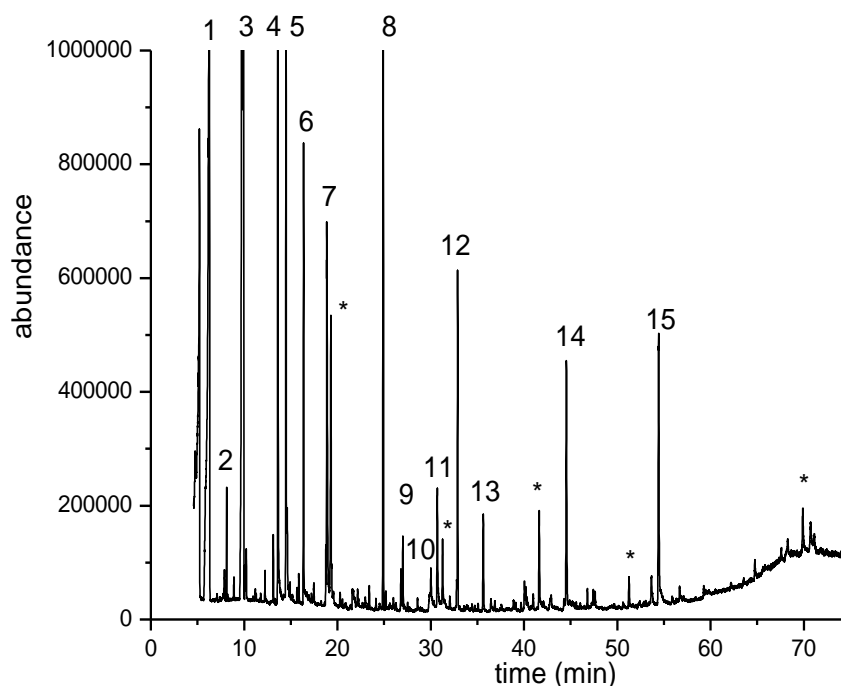


Fig. 1. The GC-MS chromatogram obtained for the sample A03 by direct injection technique. The identified compounds: (1) 2-methylpropanol, (2) 1-butanol, (3) 1-butanol-3-methyl, (4) 2-propanone-1-hydroxy, (5) 1-hexanol, (6) octanoic acid ethyl ester, (7) acetic acid, (8) decanoic acid ethyl ester, (9) butanedioic acid, diethyl ester, (10) furanone, (11) 1,2-cyclopentadione, (12) decanoic acid ethyl ester, (13) phenylethanol, (14) 2-hydroxy-gamma-butyrolactone, (15) 5-(hydroxymethyl)-2-furancarboxaldehyde. The peaks marked by \* have not provided sufficient quality match factor, thus are considered as unknown

Table 2

Repeatability data obtained for the sample A03 by the direct injection ( $n = 4$ )

Compound	Base ion	Average peak area	RSD, %
1	43	36954255	5.6
2	56	867389	3.9
3	55	48246845	4.1
4	43	61597180	5.4
5	56	7508854	3.3
6	88	5866889	4.6
7	43	12255270	5.4
8	88	13286487	2.8
9	101	1572798	2.8
10	55	1020271	4.8
11	98	4014369	4.7
12	88	4049295	4.1
13	91	3538630	2.5
14	57	8248727	3.5
15	97	7025417	2.8

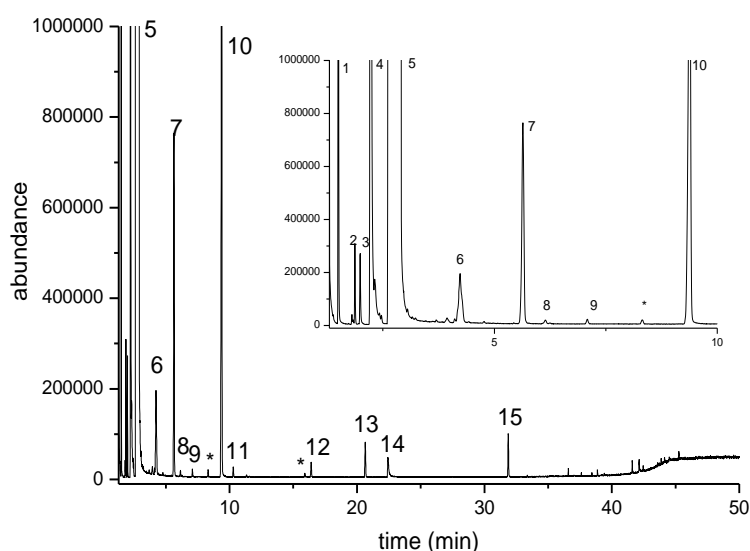


Fig. 2. The GC-MS chromatogram obtained for the sample A03 and the static headspace technique. The identified compounds: (1) acetaldehyde, (2) formic acid ethylester, (3) diethoxy methane, (4) acetic acid, ethylester, (5) ethanol, (6) propanol, (7) 2-methyl propanol, (8) 1-butanol, 3-methyl, acetate, (9) butanol, (10) 1-butanol-3-methyl, (11) hexanoic acid ethyl ester, (12) hexanol, (13) octanoic acid ethyl ester, (14) acetic acid, (15) decanoic acid ethyl ester. The peaks marked by \* have not provided sufficient quality match factor, thus are considered as unknown

Table 3

Repeatability data obtained for the sample A03 by the headspace injection ( $n = 4$ )

Compound	Base ion	Average peak area	RSD, %
1	29	9247789	3.0
2	31	852583	5.2
3	59	1072599	5.1
4	43	51078641	2.4
6	31	4211212	4.4
7	43	6197912	1.9
8	43	1126353	3.6
9	56	158665	5.6
10	55	12120180	3.7
11	88	147802	5.7
12	56	358609	6.9
13	88	692753	4.9
14	43	687530	9.5
15	88	735295	6.9

significantly high concentration of ethanol in wine distillate samples, and consequently, also in gaseous phase prevents evaporation of other volatile compounds at lower or even trace concentration levels. Thus, in the next experiment all samples have been diluted by water to reach final concentration of ethanol in range 15–18%. This modification did not show significant improvement. The chromatograms obtained for the second group of samples are practically the same. In the first group, a slight increase in peak areas has been observed and also additional compound at the very low concentration level has been found in the sample A08.

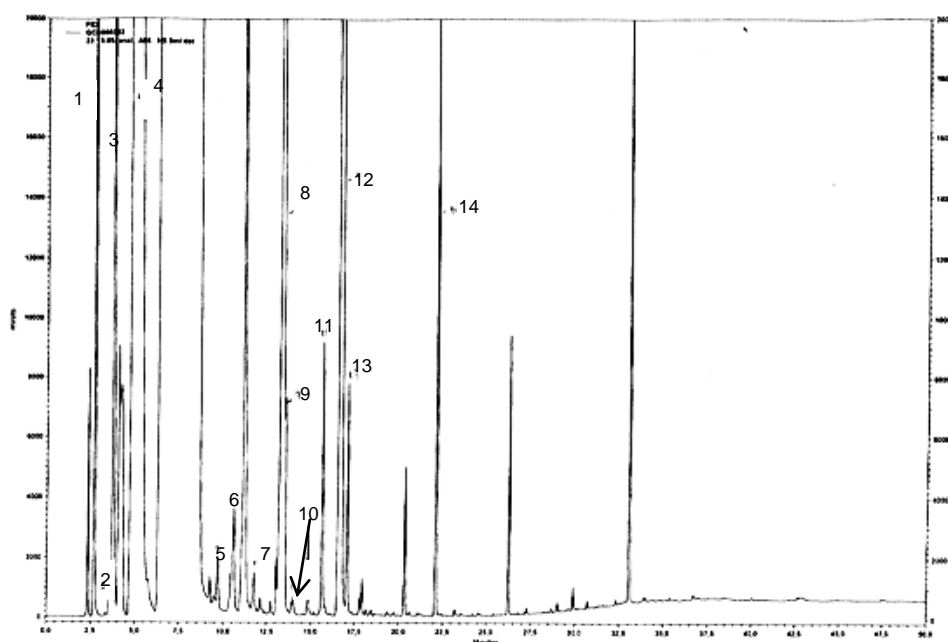


Fig. 3. The GC-FID chromatogram obtained for the sample A03 and the SPE sample treatment procedure. The identified compounds: (1) acetaldehyde, (2) acetone, (3) acetic acid, methylester, (4) acetic acid, ethylester, (5) acetic acid, 2-methyl-propylester, (6) 2-butanol, (7) acetic acid, butylester, (8) 2-methyl propanol, (9) 1-butanol, 3-methyl-, acetate, (10) 1-butanol, (11) acetic acid, pentylester, (12) 1-butanol-3-methyl, (13) hexanoic acid ethyl ester, (14) hexanol

Based on the results obtained till this point, the application of a sample preparation method in which volatile compounds in gaseous phase were trapped in a sorbent layer seemed more suitable. For this reason, a simple SPE method was used. The factors limiting the type and amount of used sorbent are compounds breakthrough volumes. The smaller amount of sorbent is preferable in analysis of high-boiling compounds while a higher amount of sorbent is preferable for volatile compounds. From various studied sorbents, Tenax TA has shown the most suitable properties due to the low affinity to water. Moreover, from most compounds studied in this paper, methanol and ethanol have the lowest breakthrough volumes on Tenax TA at 20 °C. This means that methanol and ethanol will pass through sorbent whereas other compounds could be retained only by 10 mg of sorbent. Because desorption is performed directly in split/splitless injector, oven temperature program requires longer isothermal conditions at the beginning of analysis until ethanol peak is eluted from the column. After that, temperature gradient was set up on 5 °C/min till 210 °C and held for 10 min.

Fig. 3 shows the chromatogram obtained for the sample A03 by the SPE sample treatment procedure. It is clear that on the chromatogram a large number of peaks are presented and up to 12 compounds are eluted in front of ethanol. Unfortunately, in this case, FID detector was used, thus identification of compounds was done by comparison of elution time with that of standards.

Table 4

Repeatability data obtained for the sample A03 by the SPE method ( $n = 4$ )

Compound	Avarage peak area	RSD, %
1	171792	3.5
2	3872	2.9
3	1087	4.3
4	10235120	2.6
5	11372	1.2
6	35345	1.9
7	9975	2.0
8	723928	1.6
9	37175	2.1
10	3461	3.7
11	46294	3.9
12	1518328	3.3
13	30514	6.7
14	74745	8.6

By means of this method, 19 compounds (higher linear and branched alcohols, acetone, acetals) have been successfully identified. The calculated repeatability values shown in Table 4 vary from 1.2 to 8.6. The highest precision was observed for iso-buty-lacetate. On the contrary, hexanol showed the lowest precision. This sample preparation method is characterized by the lowest RSD values for most compounds; however the range for RSD values is significantly higher compared to previous sample preparation methods.

The consistency of aromatic fraction of the samples belonging to both groups was evaluated in terms of sum peak areas of peaks eluting before and after the ethyl alcohol peak, respectively; the higher this value the richer the flavor. From Table 5, it can be easily assumed that the second group showed higher sum of peak areas, either for the part eluting in front of ethanol or for the part eluting after. In particular some observations can be made about single components of the aromatic fraction. The main components that elute in front of ethanol are acetaldehyde, methyl and ethyl acetate or ethyl formate that were determined at a higher level in comparison to the first studied group. This is in agreement with previously published data that report a significant increase of ethyl acetate or esters content during the aging process [19].

The other sorptive technique which has been used as a sample treatment procedure for isolation of volatile compounds from wine distillates is SPME. During optimization of working conditions, type of SPME fibers, sorption temperature and time have been tested in details. It was found that the best results are achieved when PDMS or PDMS/DVB fiber is inserted into gaseous phase of sample heated at 45 °C for 20 min. The used temperature program is the same like in DI methods. The chromatogram obtained for the sample A03 using the SPME fiber coated with 65 µm layer of PDMS/DVB is shown in Fig. 4. It is obvious that the chromatogram contains significantly higher number of compounds compared to previous sample treatment methods. From all 186 peaks, only 46 provided satisfactory quality match factor to be considered as identified. These compounds belong to different chemical classes such as organic acids, their various esters, linear and branched alcohols, furan and their derivatives.

Table 5

The sum of peak areas for compounds eluted in front of and after ethanol obtained by the SPE method

Sample abbr.	Sample group	Sum of peak areas for the compounds eluted in front of ethanol	Sum of peak areas for the compounds eluted after ethanol
A01	2	9874	2728
A02	2	10468	3004
A03	2	10153	2986
A04	2	5669	2970
A05	1	3598	2864
A06	1	4113	3286
A07	1	4994	3288
A08	2	12026	1906
A09	2	4075	2560
A10	1	196	191
A11	1	293	196
A12	1	240	347
A13	1	251	270
A14	1	496	400
A15	1	319	382
A16	1	161	164
A17	1	114	108
A18	1	117	495

Also terpenes, like  $\alpha$ -amorphene (25.368 min in sample 8), murrolene (26.834 min in sample 8),  $\gamma$ -cadinene (27.927 min in sample 8), cadina-3,9-diene (28.027 min in sample 8),  $\alpha$ -curcumene (28.834 min in sample 8), (-)-calamenene (30.648 min in sample 8),  $\beta$ -damascone (30.319 min in sample 8), 6-methyl  $\alpha$ -ionone (35.816 min in sample 8), and  $\beta$ -damascenone (28.313 min in samples 4 and 7) were also successfully extracted. These compounds were not present in extracts obtained by other sample treatment procedures. Thus, this method can provide complementary qualitative and quantitative information about terpenes and sesquiterpenes present in wine distillates.

The repeatability of SPME sample treatment procedure obtained for 20 randomly selected compounds is shown in Table 6. The selected compounds belong to various classes such as organic acids and their ethyl esters, alcohols or furan derivatives and present on chromatogram at different concentration levels. As was expected, the found RSD values which vary within the range of 9.2 to 29 are significantly higher compared to previous methods. This is caused by manual operation of both sample treatment procedure as well as injection and by large number of steps that are involved during sample preparation. Thus, SPME with manual holder seems to be unsuitable for reliable quantification of VOC in wine distillates, unless proper internal standard is employed.

The SPME sample treatment method did not show any rules based on our sample classification. However, it showed classification depending on the way of production. Samples that originate from the Little Carpathian wine region contained approximately 130 compounds. The samples from Trencin contained 70 to 90 compounds and less than 60 compounds were found in other samples from the first group. On the contrary, brandies produced by classical technology contained more than 140 peaks independently on geographical region.

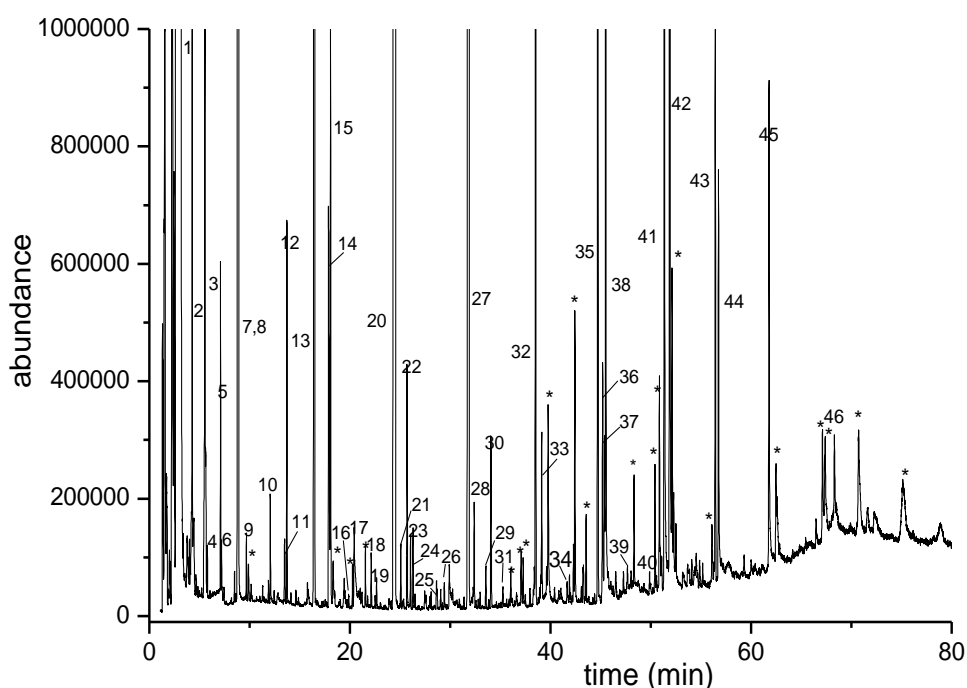


Fig. 4. The GC-MS chromatogram obtained for the sample A03 by using the SPME fibre coated with 65  $\mu\text{m}$  layer of PDMS/DVB. The identified compounds: (1) ethanol; (2) 1-propanol; (3) 1-propanol, 2-methyl-; (4) 1-butanol, 3-methyl-, acetate; (5) limonene; (6) 1-butanol; (7) 1-butanol, 2-methyl; (8) 1-butanol, 3-methyl-; (9) benzene, 1-methyl-4-(1-methylethyl)-; (10) propanone, 1-hydroxy-; (11) propanoic acid, 2-hydroxy-, ethyl ester; (12) 1-hexanol; (13) octanoic acid, ethyl ester; (14) acetic acid; (15) furfural; (16) benzaldehyde; (17) formic acid; (18) 2-furancarboxaldehyde, 5-methyl-; (19) decanoic acid, methyl ester; (20) decanoic acid, ethyl ester; (21) octanoic acid, 3-methylbutyl ester; (22) 2-furanmethanol; (23) butanedioic acid, diethyl ester; (24) ethyl 9-decenoate; (25) 1,2-cyclopentanedione; (26) *n*-capric acid, isobutyl ester; (27) dodecanoic acid, ethyl ester; (28) pentadecanoic acid, 3-methylbutyl ester; (29) butanoic acid, 1,1-dimethyl-2-phenylethyl ester; (30) phenylethyl alcohol; (31) 2(3H)-furanone, 5-butyldihydro-4-methyl-; (32) tetradecanoic acid, ethyl ester; (33) octanoic acid; (34) pentadecanoic acid, ethyl ester; (35) hexadecanoic acid, ethyl ester; (36) 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; (37) ethyl 9-hexadecenoate; (38) *n*-decanoic acid; (39) 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-; (40) benzoic acid; (41) dodecanoic acid; (42) 2-furancarboxaldehyde, 5-(hydroxymethyl)-; (43) dibutyl phthalate; (44) tetradecanoic acid; (45) *n*-hexadecanoic acid; (46) 9,12-octadecadienoic acid, (Z, Z). The peaks marked by \* have not provided sufficient quality match factor, thus are considered as unknown

Table 6

Repeatability data obtained for the sample A03 by the SPME sample treatment procedure ( $n = 4$ )

Compound	Base ion	Avarage peak area	RSD, %
5	68	2113728	24
6	56	567855	29
8	55	72514673	21
9	119	2300422	25
10	43	2607540	29
11	45	2859371	15
12	56	5087076	15

13	88	143143837	24
18	110	968414	13
19	74	643409	28
20	88	507990329	29
22	98	2693994	12
25	98	527988	9.2
27	88	257139550	27
32	88	24009108	28
35	88	25461152	26
41	73	17843662	26
42	97	30388641	15
44	73	72514673	21
45	73	3060812	26

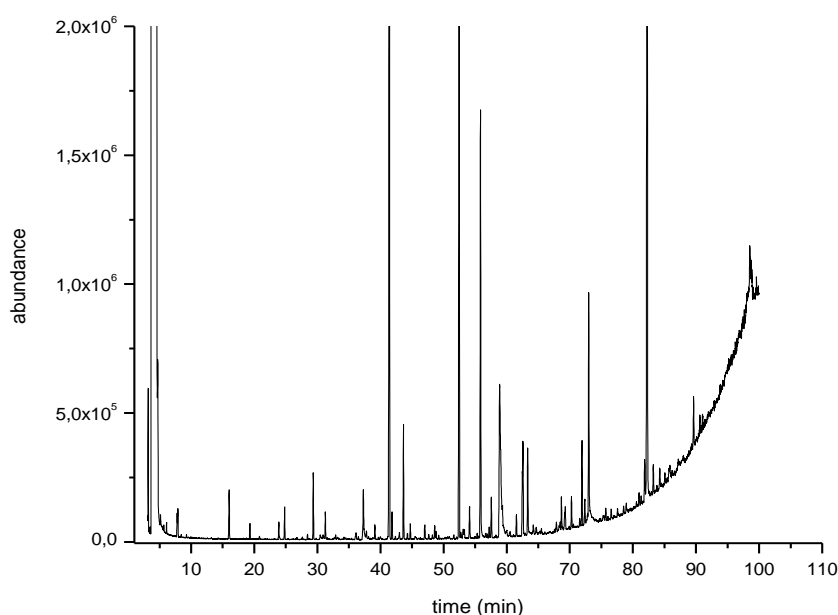


Fig. 5. The GC-MS chromatogram obtained for the sample A03 and LLE to  $\text{CH}_2\text{Cl}_2$  followed by the rotovap preconcentration

The last studied sample treatment procedure, LLE, allows one to determine organic compounds which can be extracted by organic solvents. Various mixtures of organic solvents for the extraction of volatiles from wine distillates have been described in literature. However, the most frequently used solvent is dichloromethane. 50 mL of sample was extracted with 12.5 mL of dichloromethane four times and the final extract was preconcentrated into 1 mL using rotovap at 35 °C. By this sample treatment procedure, also organic compounds with the higher boiling point are present in the final extract. Therefore, a temperature program with slow gradient in full temperature range was used: 35 °C, held for 1 min, at 2 °C/min increased to 230 °C, held for 10 min. The chromatogram (Fig. 5) shows that VOCs are more concentrated from 40 min (the highest peak at around 40 min is ethyl decanoate) while the opposite behavior, more or less, happens to be when using other sample preparation methods. It is likely that these VOCs missing in the first region of chromatogram are lost during rotovap evaporation.

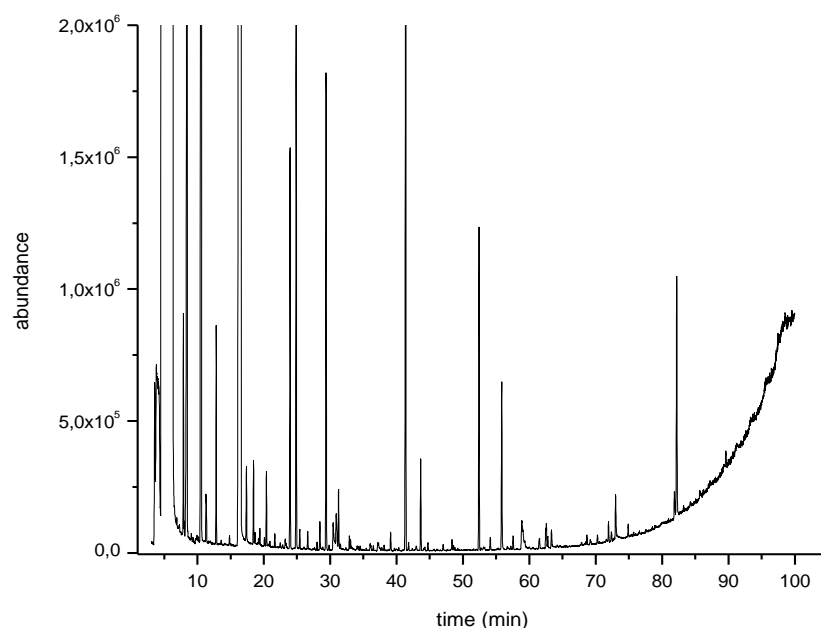


Fig. 6. The GC-MS chromatogram obtained by using LLE to  $\text{CH}_2\text{Cl}_2$  followed by the Kuderna–Danish distillation

Therefore, the utilisation of a softer method for solvent removal such as Kuderna–Danish distillation is preferred. The temperature of water bath during distillation process was kept at 85 °C. As a consequence, the final sample volume varies from sample to sample and depends on its composition. Moreover, a fast comparison of samples based on peak areas or their heights is not as straightforward as when evaporation to constant volume is used.

Fig. 6 shows the chromatogram obtained for the sample A03 using LLE followed by Kuderna–Danish distillation. From comparison of Fig. 5 and Fig. 6, it is obvious that solvent removal by Kuderna–Danish distillation has a positive impact on the composition of the final extract: an increase of the number of compounds, as well as peak areas has been observed for volatile compounds eluting up to 40 min.

On the contrary, peaks eluting after 40 min show a decrease in peak areas which is caused by different final sample volumes. The final volume of the sample 03 treated by LLE-KD was 2.8 times higher than the final volume obtained by LLE-VD. This is in agreement with observed peak areas for LLE-VD and LLE-KD. For better comparison of studied sample preparation methods, samples have also been analysed under the same chromatographic conditions as were used in DI and SPME experiments.

The chromatogram is shown in Fig. 7 and repeatability data obtained for 19 randomly selected compounds by LLE-KD are shown in Table 7. Again, the same strategy as in SPME was used in order to select compounds for evaluation of repeatability of the sample treatment procedure.

LLE is characterized by RSD values within the range of 4.0–19.2. The repeatability data obtained from peak areas are satisfactory for its quantification. However because the majority of selected compounds showed higher variability (11 compounds showed  $\text{RSD} > 10\%$  and 8 compounds showed  $\text{RSD} > 14\%$ ), the use of internal standard is recommended.

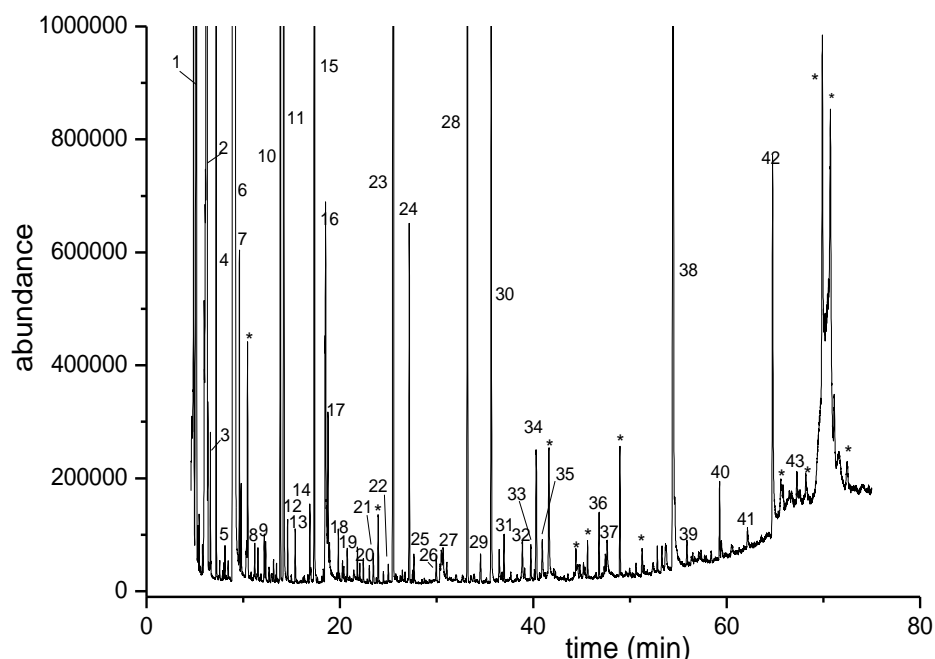


Fig. 7. The GC-MS chromatogram obtained by LLE to  $\text{CH}_2\text{Cl}_2$  followed by the Kuderna–Danish distillation under the same temperature program as was used for DI and SPME. The identified compounds: (1) 1-propanol; (2) 1-propanol, 2-methyl-; (3) 2-propen-1-ol; (4) 1-butanol; (5) cyclopentanone; (6) 1-butanol, 3-methyl-; (7) hexanoic acid, ethyl ester; (8) propanoic acid, 2-oxo-, ethyl ester; (9) 2-propanone, 1-hydroxy-; (10) propanoic acid, 2-hydroxy-, ethyl ester; (11) 1-hexanol; (12) 3-hexen-1-ol, (E)-; (13) 3-hexen-1-ol, (Z)-; (14) acetic acid, hydroxy-, ethyl ester; (15) octanoic acid, ethyl ester; (16) acetic acid; (17) furfural; (18) acetic acid, diethoxy-, ethyl ester; (19) benzaldehyde; (20) 1-octanol; (21) propanedioic acid, diethyl ester; (22) 2-furancarboxylic acid, ethyl ester; (23) decanoic acid, ethyl ester; (24) butanedioic acid, diethyl ester; (25) p-menth-1-en-8-ol; (26) 2(5H)-furanone; (27) 1,2-cyclopentanedione; (28) dodecanoic acid, ethyl ester; (29) benzyl alcohol; (30) phenylethyl alcohol; (31) cis 3-methyl-4-octanolide; (32) 2-furancarboxylic acid, hydrazide; (33) phenol, 4-ethyl-2-methoxy-; (34) butanedioic acid, hydroxy-, diethyl ester, (+/-); (35) octanoic acid; (36) hexadecanoic acid, ethyl ester; (37) *n*-decanoic acid; (38) 2-furancarboxaldehyde, 5-(hydroxymethyl)-; (39) vanillin; (40) dibutyl phthalate; (41) pentadecanoic acid; (42) *n*-hexadecanoic acid; (43) heptadecanoic acid. The peaks marked by \* have not provided sufficient quality match factor, thus are considered as unknown

The chromatograms obtained by LLE-KD showed that wine distillates in the first group could be roughly subdivided into three groups. The most organic compounds (cca 190) were found in wine distillates produced in Pezinok. Approximately 130 organic compounds have presented in wine distillates produced in Trencin. The other studied samples showed presence of 70–90 organic compounds. Generally, 19–45 compounds are presented in relatively high concentration depending on the type of sample. Other compounds are usually presented at trace level. On the contrary, more than 240 organic compounds have been found in wine distillates in the second group. Slight lower number of compounds (198) was found in the sample A04 which was not aged in wooden barrels. The identified organic compounds belong to different organic classes, i.e. ethyl esters of carboxylic acids, linear and branched alcohols, aldehydes, carboxylic acids, furans and their derivatives or phenolic compounds. The selectivity of particular sample treatment procedure towards identified compounds found in the sample A03 is shown in Table 8.

Table 7

Repeatability data obtained for the sample A03 by the LLE-KD sample treatment procedure ( $n = 4$ )

Compound	Base ion	Average peak area	RSD, %
4	56	11431057	6.9
7	88	3037954	16
10	45	55892376	5.4
15	88	27748908	19
12	41	10978275	7.7
17	96	3483229	4.8
23	88	65644964	9.7
28	88	22750367	19
30	91	22342951	17
24	101	7707290	14
22	95	365444	14
25	59	287856	13
19	106	338176	4.0
26	55	656856	8.6
31	99	1138741	11
33	137	1009659	17
34	117	1960696	13
38	97	41397965	7.7
39	151	705948	15

Table 8

The identified compounds found in the sample A03 by the sample treatment procedures under study

Compound name	Sample treatment procedure				
	DI	LLE	SPME	HS	SPE*
1,4-benzenediol, 2-methyl-	x				
1,4-butanediol	x	x			
1.2-cyclopentanedione	x	x	x		
1.6-octadien-3-ol, 3,7-dimethyl-		x			
1-butanol	x	x	x	x	x
1-butanol, 2-methyl-	x	x	x		
1-butanol, 3-methyl-	x	x	x	x	x
1-butanol, 3-methyl-, acetate		x	x	x	x
1-heptanol		x			
1-hexanol	x	x	x	x	x
1-hydroxy-2-butanone	x				
1-octanol		x			
1-pentanol	x	x			
1-pentanol, 3-methyl-		x			
1-penten-3-ol		x			
1-propanol	x	x	x	x	
1-propanol, 2-methyl-	x	x	x	x	x
1-propanol, 3-ethoxy-		x			
2(3H)-furanone, 5-acetyldihydro-	x				

2(3H)-furanone, 5-butyldihydro-4-methyl-	x	x	x		
2(5H)-furanone	x	x			
2(5H)-furanone, 5-methyl-	x				
2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-			x		
2-butanol		x	x		x
2-butanone, 3-hydroxy-	x	x			
2-cyclopenten-1-one, 2-hydroxy-3-methyl-3-methyl-	x				
2-cyclopentene-1,4-dione	x	x			
2-furancarboxaldehyde, 5-(hydroxymethyl)-	x	x	x		
2-furancarboxaldehyde, 5-methyl-	x	x	x		
2-furancarboxylic acid, ethyl ester		x			
2-furancarboxylic acid, hydrazide		x			
2-furanmethanol	x	x	x		
2-hydroxy- $\gamma$ -butyrolactone	x				
2-methoxy-4-vinylphenol		x			
2-propanone, 1-hydroxy-	x	x			
2-propen-1-ol	x	x			
3-butene-1,2-diol	x				
3-hexen-1-ol (E)	x	x			
3-hexen-1-ol (Z)		x			
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-			x		
5-acetoxymethyl-2-furaldehyde		x			
5-hydroxymethyldihydrofuran-2-one	x				
9,12-octadecadienoic acid, (Z, Z)-			x		
acetaldehyde				x	x
acetic acid	x	x	x	x	
acetic acid, butylester					x
acetic acid, ethylester				x	x
acetic acid, 2-methyl-propyl ester					x
acetic acid, 2-methyl ester					x
acetic acid, pentylester					x
acetic acid, diethoxy-, ethyl ester		x			
acetone					x
acetic acid, hydroxy-, ethyl ester	x	x			
Benzaldehyde	x	x	x		
benzene, 1-methyl-4-(1-methylethyl)-			x		
benzoic acid			x		
benzoic acid, ethyl ester		x			
benzyl alcohol		x			
butane, 1,1-diethoxy-3-methyl-		x			
butanedioic acid, diethyl ester	x	x	x		
butanedioic acid, hydroxy-, diethyl ester, (+/-)	x	x			
butanoic acid, 1,1-dimethyl-2-phenylethyl ester			x		
butanoic acid, 2-hydroxy-3-methyl-, ethyl ester		x			
butanoic acid, 3-methyl-, ethyl ester		x			
Butyrolactone	x				
cis 3-methyl-4-octanolide		x			

Cyclopentanone		x			
decanoic acid, ethyl ester	x	x	x	x	
decanoic acid, methyl ester		x	x		
dibutyl phthalate	x	x	x		
dodecanoic acid			x		
dodecanoic acid, ethyl ester	x	x	x		
ethyl 9-decenoate		x	x		
ethyl 9-hexadecenoate			x		
formic acid	x		x		
formic acid, ethyl ester				x	
Furfural	x	x	x		
heptadecanoic acid	x	x			
hexadecanoic acid, ethyl ester	x	x	x		
hexanoic acid, ethyl ester	x	x		x	x
limonene		x	x		
methane, diethoxy				x	
methyl 2-furoate	x				
<i>n</i> -capric acid, isobutyl ester			x		
<i>n</i> -decanoic acid	x	x	x		
<i>n</i> -hexadecanoic acid	x	x	x		
octadecanoic acid	x				
octanoic acid		x	x		
octanoic acid, 3-methylbutyl ester		x	x		
octanoic acid, ethyl ester	x	x	x	x	
oleic acid	x				
pentadecanoic acid		x			
pentadecanoic acid, 3-methylbutyl ester			x		
pentadecanoic acid, ethyl ester			x		
Phenol	x	x			
phenol, 2,6-dimethoxy-	x	x			
phenol, 2-methoxy-	x	x			
phenol, 4-ethyl-2-methoxy-	x	x			
phenylethyl alcohol	x	x	x		
p-menth-1-en-8-ol	x	x			
propane, 1,1,3-triethoxy-	x	x			
propanedioic acid, diethyl ester		x			
propanoic acid	x				
propanoic acid, 2-hydroxy-, ethyl ester		x	x		
propanoic acid, 2-oxo-, ethyl ester		x			
propanoic acid, 3-ethoxy-, ethyl ester		x			
propanone, 1-hydroxy-			x		
tetradecanoic acid	x	x	x		
tetradecanoic acid, ethyl ester	x	x	x		
vanillin	x	x			

\* Compounds were identified based on the comparison of retention time with standards.

### Conclusions

A comparison of various sample treatment procedures for the isolation of volatiles from wine distillates was performed. As expected, DI and HS method provide sufficient repeatability in the narrow range from 3% to 6% for the most compounds and relatively clean chromatograms. These simple sample preparation methods are especially suitable for the determination of major constituents of wine distillates such as acetates, linear and branched alcohols or ethyl esters of carboxylic acids. Sample preparation methods based on extraction by solid phase SPE and SPME have showed better selectivity toward volatile compounds. Surprisingly, the SPE method showed the lowest RSD values for all identified compounds. However, these small RSD values are in agreement with previously published data obtained for extraction of halogenated volatile organic compounds from water samples [18].

On the contrary, the SPME method has provided the worst repeatability in comparison with other studied sample treatment methods. Indeed, RSD values higher than 20% were observed for the majority of selected compounds. This is caused by manual operation and large number of steps that are involved during sample preparation. Thus, it is recommended to use proper internal standard in order to get reliable concentration data. However, these methods except of the previously mentioned compound also allowed extraction of terpenes, furans and their derivatives, furfural and its derivatives and other compounds which could not be extracted by other studied sample treatment methods. The most suitable sample treatment procedure seems to be liquid-liquid extraction to dichloromethane followed by Kuderna–Danish solvent removal. The repeatability of LLE-KD varies within the range of 10–20% which makes this sample treatment method suitable for quantification purposes. Furthermore, repeatability can be improved by employing of proper internal standard. Moreover, more than 240 organic compounds have been found in wine distillates produced by classical technology. The identified organic compounds belong to different organic classes, e.g. ethyl esters of carboxylic acids, linear and branched alcohols, aldehydes, carboxylic acids, furans and their derivatives, phenolic compounds. LLE in combination with SPME provide possibility to identify wide number of compounds belonging to various organic classes.

The number of organic compounds presented in wine distillate will allows differentiating between wine distillates produced by classical technology and those produced by mixing of wine distillate with ethanol from other sources.

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## СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ РАЗЛИЧНЫХ СПОСОБОВ ПРОБОПОДГОТОВКИ ОБРАЗЦОВ ДЛЯ ХАРАКТЕРИСТИКИ ОРГАНИЧЕСКИХ СОЕДИНЕНИЙ В БРЕНДИ

*И. Шпанек, О. Вывиурска, К. Макишова*

### Аннотация

Изучен состав летучих соединений 19 различных винных дистиллятов методами газовой хроматографии (ГХ) с пламенно-ионизационным (ПИД) и масс-спектрометрическим (МС) детектированием. Исследуемые образцы были разделены на две группы в зависимости от способа производства и географического происхождения. Детально изучено влияние различных способов подготовки на конечный состав легколетучих соединений. Проведено сравнение эффективности прямого инжестирования, анализа равновесного пара, твердофазной экстракции (ТФЭ), твердофазной микроэкстракции и жидкость-жидкостной экстракции. Кроме того, подробно изучено влияние условий предварительного концентрирования компонентов: типа сорбента, температуры, времени и способа удаления растворителя. Воспроизводимость способа подготовки образца оценивали, сравнивая площадь пиков произвольно выбранных соединений для 4 параллельных измерений. Показано, что наиболее воспроизводимые результаты получены в случае ТФЭ с последующим прямым инжестированием или анализом равновесного пара. При жидкость-жидкостной экстракции и твердофазной микроэкстракции наблюдается большая вариабельность площадей пиков, поэтому для количественного определения следует использовать внутренний стандарт. Наиболее подходящий способ пробоподготовки образца, обеспечивающий извлечение наибольшего числа соединений различных типов, – это жидкость-жидкостная экстракция  $\text{CH}_2\text{Cl}_2$ . В этом случае из винных дистиллятов, произведенных по классической технологии, было проэкстрагировано более 240 соединений. Кроме того, ТФЭ показала различную селективность, что позволяет определять соединения, которые не могут быть извлечены при других рассмотренных способах пробоподготовки.

**Ключевые слова:** пробоподготовка, анализ равновесного пара, жидкость-жидкостная экстракция, твердофазная микроэкстракция, винные дистилляты, бренди.

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