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New method for the discovery of adulterated cognacs and brandies based on solid-phase microextraction and gas chromatography - mass spectrometry

The article represents new method for discovery of adulterated cognacs and brandies based on solid-phase microextraction (SPME) in combination with gas chromatography – mass spectrometry (GC-MS). The work comprised optimization of SPME parameters (extraction temperature and time, concentration of added salt) with subsequent analysis of authentic samples and comparison of the obtained chromatograms using principal component analysis (PCA). According to the obtained results, increase of extraction temperature resulted in an increase of response of the most volatile brandy constituents. To avoid chemical transformations and/or degradation of the samples, the extraction temperature must be limited to 30C. Increase of the extraction time lead to higher total peak area, but longer extraction times (>10 min for 100 μm polydimethylsiloxane and >2 min for divinylbenzene/Carboxen/polydimethylsiloxane fibers) caused displacement of analytes. Salt addition increased total response of analytes, but caused problems with reproducibility. The developed method was successfully applied for discovery of adulterated samples of brandy, cognac, whisky and whiskey sold in Kazakhstan. The obtained data was analyzed applying principal component analysis (PCA). Five adulterated brandy and whisky samples were discovered and confirmed. The developed method is recommended for application in forensic laboratories.

Key words: gas chromatography; mass spectrometry; cognac; brandy; solid-phase microextraction; principal component analysis; adulteration

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Коньяк және брендидің жалған үлгілерін анықтаудың қатты фазалы микроэкстракция мен газды хроматография-масс-спектрометрияға негізделген жаңа әдісі

Мақалада коньяк және брендидің жалған үлгілерін анықтаудың қатты фазалы микроэкстракция (ҚФМЭ) мен газды хроматография-масс-спектрометрияға (ГХ-МС) негізделген жаңа әдісі келтірілген. Жұмыс ҚФМЭ параметрлерін оңтайландырудан, кейінгі реалды үлгілерді талдаудан және алынған

хроматограммаларды басты компоненттер әдісін қолдану арқылы салыстыру. Алынған нәтижелерге сәйкес, экстракция температурасын жоғарлату бренди құрамындағы ең ұшқыш қосылыстардың жауабының жоғарлауына әкеледі. Үлгілердің химиялық трансформациясын және/немесе деградациясын болдырмау үшін экстракция температурасы 30^{0}С шектеледі. Экстракция уақытын жоғарлату шыңның жалпы ауданының жоғарлауына әкеледі, бірақ ұзақ экстракция уақытында (полидиметилсилоксан негізіндегі талшық үшін 10 минуттан ұзақ және дивинил бензол /Карбоксен/ полидметилсилоксан негізіндегі талшық үшін 2 минуттан ұзақ) экстракциялық жабындыдағы бір заттар басқа заттардың орын басады. Тұздың қосылуы анықталатын заттардың жауабын жоғарлатады, бірақ қайталанымдылық тұрғысынан проблемаларды тудырады. Шығарылған әдіс Қазақстанда сатылатын жалған бренди, коньяк және виски үлгілерін анықтауда сәтті қолданылды. Алынған мәліметтер басты компоненттер әдісімен талданылды. Талдау нәтижелерінде бренди және вискидің бес жалған үлгілері анықталып, дәлелденді. Шығарылған әдіс соттық-криминалистикалық зертханаларда қолдануға ұсынылуы мүмкін.

Түйін сөздер: газдық хромотография, масс-спектрометрия, коньяк, бренди, қатты фазалық микроэкстракция, басты компоненттер әдісі, жалған үлгі.

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Новый метод выявления поддельных коньяков и бренди на основе твердофазной микроэкстракции и газовой хроматографии – масс-спектрометрии

В статье представлен новый метод выявления поддельных коньяков и бренди, основанный на твердофазной микроэкстракции (ТФМЭ) и газовой хроматографии – масс-спектрометрии (ГХ-МС). Работа включала оптимизацию параметров ТФМЭ (температура и время экстракции, концентрация соли) с последующим анализом реальных образцов и сравнением полученных хроматограмм с использованием метода главных компонент. Согласно полученным результатам, увеличение температуры экстракции приводит к увеличению отклика наиболее летучих соединений, содержащихся в бренди. Для предотвращения химической трансформации и/или деградации образцов температура экстракции должна быть ограничена 30^{0}С. Увеличение времени экстракции приводит к увеличению общей площади пика, но более длительная экстракция (>10 мин для волокна на основе полидиметилсилоксана и >2 мин для волокна на основе дивинилбензола/Карбоксена/полидметилсилоксана) приводит к вытеснению из экстракционного покрытия одних аналитов другими. Добавка соли позволяет увеличить отклик аналитов, но вызывает проблемы с воспроизводимостью. Разработанный метод был успешно применен для выявления поддельных образцов бренди, коньяка и виски, реализуемых в Казахстане. Полученные данные анализировали методом главных компонент. По результатам анализа были выявлены и подтверждены пять поддельных образцов бренди и виски. Разработанный метод может быть рекомендован для применения в судебно-криминалистических лабораториях.

Ключевые слова: газовая хроматография; масс-спектрометрия; коньяк; бренди; твердофазная микроэкстракция; метод главных компонент; подделка.

Introduction

The problem of adulteration of alcoholic beverages is widespread in Kazakhstan and other former USSR countries. However, hundreds of cases of adulteration are also reported by mass media all around the world. Thus, in May of 2013, Czech police confiscated more than million liters of illegal alcohol [1].

Adulteration of cognac, whisky and brandy is very popular due to the relative ease of component substitution, high prices of famous brands and popularity among customers. Cognac, as any alcoholic beverage can be adulterated by partial or complete replacement of cognac spirit (distilled from fermented grapes) with food alcohol or technical (commercial) alcohol, as well as dilution or full replacement with colored water solutions. The special means and methods of falsification include replacement with a drink with a short retention (aging in barrels) period, replacement with an alcoholic extract of tea to simulate, colorization

of cognac spirit, replacement by an alcohol extract from vegetable raw materials having a high content of tanning agents (oak chips, walnut shells, etc.). Most often, adulteration of cognac, whisky and brandy is done by dilution with water, tea or alcohol-based liquids: vodka, starka (rye vodka), chacha (grape vodka), wine, etc.

Adulteration of alcoholic drinks has negative economic effects as well as potentially being damaging to human health. In addition to direct profit losses, manufacturers of original products lose potential customers due to quality concerns caused by consumption of adulterated alcohols. Human health consequences can vary from chronic to acute toxic action by chemicals present in the adulterated samples. According to the data, the most fatal cases are caused by consumption of adulterated vodkas due to methanol poisoning. For example, in 2012, 26 people died in Czech Republic due to the poisoning by alcohol [2]. Quality control on the "garage" manufactures is in most cases non-existing. Such manufacturers do not care about the quality and safety of their products as they sell them under different brands.

In most countries, adulteration of alcohols is considered as a serious crime. However, this problem is difficult to avoid completely. It is well known that the market of adulterated alcohols grows if the price of original alcohol increases due to the higher demand and potential profits. Hence, the problem is particularly widespread in developing countries where people cannot afford original products.

The key instruments to fight adulteration are law enforcement agencies, e.g. police and forensic laboratories, and courts. Their work includes:

• screening of the market of alcohols for adulterated samples;

• collection of evidence to prove the fact of adulteration;

• discovering and convicting individuals or companies responsible for adulteration.

Appropriate legislation can be efficient to minimize alcohol adulteration. However, it requires scientific data and background and requires time to implement.

The efficiency of law enforcement against adulteration depends on chemical analysis that allows collection of data on chemical composition and further investigations. Without chemical analyses, it is very difficult to prove adulteration. Such analyses are typically performed in specialized forensic or general analytical laboratories. For acceptance of data by the court, such laboratories may need to be certified or accredited.

When the goal is to confirm adulteration, there are two main approaches utilized both requiring the involvement of skilled analytical chemical expertise: 1. detection of known chemical markers of adulteration and 2. determination of the concentrations of individual compounds and/or their ratios. Statistical methods (e.g., principal component or cluster analyses) are in this respect efficient tools when dealing with large number of target compounds.

Efficient methods for analysis of alcoholic beverages are based on the physical chemical methods such as gas chromatography (GC) [3-4], liquid chromatography (LC) [5-6], spectroscopic methods like ultraviolet (UV), infrared (IR) [7-8] or nuclear magnetic resonance (NMR), and mass spectrometry [9]. The most efficient methods are based on hyphenated techniques like chromatography (GC or LC) - mass spectrometry (MS), because they provide separation efficiency, selectivity as well as possibility for identification. Sensor-based devices are very good alternative for quick and on-site discovery of adulterated alcoholic samples, but their results should be confirmed by more specific analytical methods before reaching a court.

In Kazakhstan, for quality control of alcoholic beverages, most laboratories use standard GOST methods. These methods are based on determination of the main physical and organoleptic parameters, and analysis of the content of some chemicals, e.g., ethanol, alcohols, aldehydes and esters. In the forensic practice, two methods based on GC-FID [3-4] are the most often used. However, this method is based on direct sample injection and has poor sensitivity because injection of water is undesirable for GC based analyses.

Savchuk et al. [10] conducted a study to identify the compounds in brandy to confirm adulteration. Using headspace GC-MS and LC, authors has found that the presence of excessive concentrations of 2-butanol, acetic acid, ethyl acetate, acetaldehyde, ethyl lactate, methane acid, fatty acids ethyl esters, diacetals, acetoin and acetals may be the evidence of adulteration.

Despite the large number of methods available in the literature, application of most of these methods for disclosing adulteration has not been reported. Another problem is caused by the fact that providing sufficient evidence for adulteration is typically not possible applying only a single method.

According to the publications, highest number of analytes in alcoholic beverages can be detected by GC-MS in combination with headspace (HS) solid-

phase microextraction (SPME) [11]. HS SPME is very popular sampling and sample preparation technique based on the extraction of the target analytes from gaseous phase above the sample onto a thin polymer coating. Its main advantages include low cost of analysis, simplicity of use and ease of automation.

SPME was used for fingerprinting of brandies [12], wines [11], rums [13], whiskies [14] and cachaca [15]. As reported by Rodriguez et al. [16], fiber coatings based on Carboxen (CAR) / polydimethylsiloxane (PDMS) and divinylbenzene (DVB)/CAR/PDMS appeared as the most efficient for extraction taking into account total peak areas of analytes. Camara et al. [14] reported that fiber based on Carbowax(CW)/DVB provided the highest extraction efficiency of all volatile organic compounds (VOCs) excluding the most abundant esters. Rodriguez et al. [16] demonstrated that, for wine and beer samples, the maximum total peak area was observed at extraction temperature 30^0C. For whisky samples, optimal temperature was 40^0C [16]. Maximum response of analytes was normally observed at extraction time >60 min [14;16].

It was shown that ethanol concentration significantly affects SPME efficiency. Thus, the highest response was observed at ethanol concentration of 12% [13]. For some analytes, it was in the range of 10-20% [17]. Addition of sodium chloride was always used to promote analyte transfer from sample to headspace [11-17]. During method optimizations, authors mostly focused on selectivity and sensitivity rather than other very important specifications such as reproducibility, response linearity and reliability. These specifications appear of high importance in order to differentiate between adulterated and original samples as well as finding the origin of the product.

The objective of the present study was to optimize the SPME-GC-MS method for detection of VOCs in Kazakh brandies and disclosing possible adulterated brandy samples. The work comprised optimization of SPME parameters and application of the method for analysis of the authentic samples. Optimization was focused on obtaining reproducible and reliable results at sufficient sensitivity and simplicity.

Experimental part

Samples

The method development was done using three-star "Kazakhstan" brandy ("Bacchus" OJSC, Almaty, Kazakhstan) [18] purchased at the official shop of the manufacturer. This brandy was selected because it apparently is the most popular in Kazakhstan. In Kazakhstan, this brandy is traditionally called "коньяк" (pronounced as "cognac"). However, according to the international legislation [19], only the French cognac regions has a right to produce alcoholic beverages under the name "cognac". However, the technology for production of Kazakh "коньяк" is very close to the technology for production of original cognac. Further in the paper, such samples will be denoted "Kazakh brandies" or brandies.

To decrease ethanol concentration to the optimal value around 12% [13], all experimental samples were prepared by mixing 2.00 mL of the alcoholic beverage sample with 4.00 mL of distilled water in 20 mL vials (CTC Analytics, Switzerland). After preparation, samples were located onto a 32-position tray for 10/20 mL vials of Combi Pal autosampler and analyzed by GC-MS.

Instrumentation

All experiments were conducted on a 6890N/5973N GC-MS system (Agilent, USA) equipped with two split/splitless inlets and flame-ionization detector (FID). SPME was conducted using Combi-PAL autosampler (CTC Analytics, Switzerland) installed on the GC-MS system and equipped with temperature-controlled agitator and fiber conditioning station. Desorption of the extracted compounds from the fiber coating was conducted in the GC inlet at 240^0C equipped with 0.75 mm i.d. SPME liner (Supelco, USA). The oven temperature was programmed from 40^0C (held for 2 min) to 180^0C at a ramp 2^0C/min, followed by a 10^0C/min ramp to 280^0C (held for 20 min).

Separation prior to MS detection was done on a 30 m x 0.25 mm DB-35MS (0.25 μm film) column at constant flow rate of helium (99.995% purity, Orenburg-Tehgas, Russia) equal to 1 mL/min. MS detection was performed by scanning ions in the range 34-300 amu. Temperatures of interface, ion source and quadrupole were 240, 230 and 150°C, respectively.

Separation prior to FID was performed using a DB-1MS 30 m x 0.25 mm, 0.25 μm i.d. capillary column at a constant flow rate of helium equal to 1.5 mL/min. Detection was carried out at temperature of 250^0C. Flow rates of hydrogen, air and makeup gas (helium) were set to 30, 300 and 15 mL/min, respectively. Total time of the analysis was 102 minutes.

In the study, two SPME fibers (Supelco, USA) were used: 100 μm PDMS and DVB/CAR/PDMS.

Characterization of the experimental sample used for method optimization

The sample of "Kazakhstan" brandy ("Bacchus" OJSC, Almaty, Kazakhstan) was analyzed using two different approaches:

1) extraction time 30 sec, temperature 30^{0}C, without addition of salt;

2) extraction time 30 min, temperature 50^{0}C, with addition of 1.7 g desiccated NaCl (chemically pure, LaborPharma, Kazakhstan).

Extraction was conducted using DVB/CAR/PDMS fiber coating that previously has been proven to be selective to a wide range of VOCs present in alcoholic beverages [16]. Pre-incubation time was set to 5 min. Longer extraction times lead to an excessive loading of the fiber coating and a significant overload of the MS detector causing a rapid reduction of the MS electron multiplier lifetime. To overcome this problem when analyzing the samples using a 30 min extraction time, the MS detector was switched off during the time periods of elution of the abundant peaks (esters) using a special option in the software. Identification of peaks was performed using NIST'08 and Wiley 7th edition MS libraries.

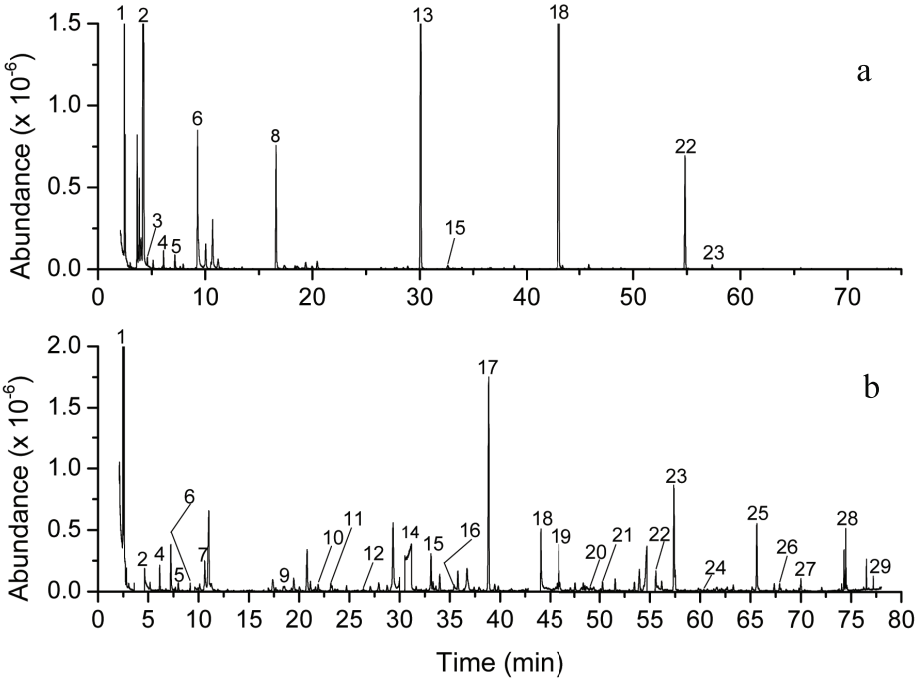

Figure 1 – Chromatograms of "Kazakhstan" brandy ("Bacchus" OJSC, Almaty, Kazakhstan) obtained by SPME-GC-MS using DVB/CAR/PDMS fiber and two different extraction condition: (a) – extraction time 30 sec, temperature 30^{0}C, without addition of salt; (b) – extraction time 30 min, temperature 50^{0}C, with addition of 1.7 g NaCl.

From the obtained chromatograms (Figure 1), 49 compounds were identified (Table 1). These compounds mainly included aliphatic and aromatic alcohols, esters, aldehydes and ketones. Most of the detected compounds are known constituents of cognac [10], brandy [10], whisky [14], rum [13], wine [11] and other alcoholic beverages [16-17].

For discovery of adulterated samples, the ratio between these compounds may be important. Some samples may contain chemical markers of origin, e.g., beta-damascenone. If these compounds are volatile and have sufficient concentration, they will be detected by SPME-GC-MS method.

The chemical composition of Kazakh brandy appears to be rather complex. Some of the detected compounds are unstable under certain conditions. To provide the reliability and reproducibility of the developed method, the chemical composition of the samples should not be affected during the sample preparation procedure. Hence, for the method optimization, peak areas of the most intensive peaks were considered: ethyl acetate, isoamyl alcohol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl hexadecanoate and furfural.

Optimization of extraction temperature

Prepared brandy samples were extracted at 30, 45, 60 and 70^{0}C, respectively, using DVB/CAR/PDMS fiber. To avoid MS detector overload at high temperature, extraction time was set to 15 seconds. Pre-

incubation time was 15 min. Selection of the minimum extraction temperature was limited by the minimum adjustable temperature in the agitator of the autosampler. The choice of the maximum extraction temperature was due to the fact that for T>70^{0}C, vapor pressure of ethanol rapidly increases. Ethanol may thus occupy all adsorption sites at the coating whereby the extraction of other compounds may be significantly slowed down.

Table 1 – Compounds reliably identified in the studied sample of "Kazakhstan" brandy ("Bacchus" OJSC, Almaty, Kazakhstan)

#	Compound	RT (min)	CAS No.	Significant ions, m/z (relative abundance, %)
1	Acetic acid	2.37	64-19-7	60 (85)
2	Ethyl acetate	2.4	141-78-6	88 (7); 61 (18)
3	2-Methyl-1-propanol	2.5	78-83-1	41 (56); 74 (12)
4	Diethoxymethane	2.8	462-95-3	59 (100); 103 (68)
5	2-Methyl butanal	3.1	96-17-3	86 (17)
6	Ethyl propanoate	3.7	105-37-3	102 (16)
7	Isoamyl alcohol	4.23	123-51-3	55 (100); 70 (77)
8	Ethyl isobutyrate	4.5	97-62-1	116 (22); 88 (18)
9	1-Butanol-3-methyl formate	5.6	110-45-2	55 (100); 70 (60); 41 (33)
10	Ethyl butanoate	6.1	105-54-4	88 (61); 60 (21)
11	Ethyl 2-hydroxypropanoate	7.3	97-64-3	45 (100); 75 (11)
12	2-Methyl ethylbutanoate	7.6	7452-79-1	102 (95); 74 (26)
13	Ethyl 3-methylbutyrate	7.9	108-64-5	88 (100); 60 (40)
14	1-Hexanol	9.1	111-27-3	56 (100); 55 (49)
15	3-Methylbutyl acetate	9.3	123-92-2	70 (78); 87 (18)
16	Furfural	10.6	98-01-1	96 (100); 39 (31)
17	Ethyl hexanoate	16.6	123-66-0	88 (100); 99 (58)
18	Benzaldehyde	18.5	100-52-7	105 (100); 106 (84)
19	Ethyl 2-hydroxy-4-methylpentanoate	21.1	10348-47-7	69 (100); 87 (67)
20	1-Octanol	21.6	111-87-5	55 (100); 41 (94); 56 (89)
21	Isoamyl lactate	21.9	19329-89-6	45 (100); 70 (54)
22	2-Nonanol	23.1	628-99-9	45 (100); 69 (25)
23	Acetophenone	26.4	98-86-2	105 (100); 77 (73); 120 (27)
24	Phenylethyl alcohol	29.3	60-12-8	91 (100); 92 (56); 122 (28)
25	Ethyl octanoate	30.1	106-32-1	88 (100); 101 (38); 127 (30)
26	Octanoic acid	31.3	124-07-2	60 (100)
27	3-Ethyl benzaldehyde	32.6	34246-54-3	105 (100); 122 (62); 134 (60)
28	Ethyl succinate	33.1	123-25-1	101 (100); 129 (67)
29	Methyl salicilate	33.9	119-36-8	120 (100); 92 (48)
30	Decanol	35.4	112-30-1	55 (70); 70 (67)
31	2-Phenyl acetate	38.8	103-45-7	104 (100); 91 (16)
32	Ethyl decanoate	43.05	110-38-3	88 (100); 101 (42)
33	Ethyl 9-decenoate	43.4	67233-91-4	70 (63); 55 (58)
34	5-Ethyl-2-methyloctane	44.9	62016-18-6	57 (100)
35	Octanoic acid, 3-methylbutyl ester	45.8	2035-99-6	70 (100); 127 (30)
36	1-(2,5-Dimethylphenyl) ethanone	48.9	2142-73-6	133 (100); 148 (23)
37	Isobutyl decanoate	51.5	30673-38-2	155 (100); 56 (81)
38	4-Hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid	53.4	39212-21-0	126 (100); 98 (24)
39	D-Nerolidol	53.9	142-50-7	69 (100); 93 (69)
40	Ethyl dodecanoate	54.8	106-33-2	88 (100); 101 (47)
41	3-Methyl butyl decanoate	57.3	2306-91-4	70 (100); 155 (22)
42	3, 7, 11-Trimethyl-6,10-dodecadien-1-ol	61.6	20576-54-9	69 (100); 81 (65)
43	Ethyl tetradecanoate	65.6	124-06-1	88 (100); 101 (55)
44	3,7,11,15-Tetramethyl-6,10,14-hexadecatrien-1-ol	67.3	36237-66-8	81 (100); 69 (98)
45	Isoamyl laurate	67.8	6309-51-9	70 (100); 55 (15)

Continuation of table 1

#	Compound	RT (min)	CAS No.	Significant ions, m/z (relative abundance, %)
46	Farnesol acetate	70.01	4128-17-0	69 (100); 93 (38)
47	Diisobuthyl phthalate	74.0	84-69-5	149 (100); 150 (0.91)
48	Ethyl 9-hexadecenoate	74.3	54546-22-4	55 (100); 69 (83)
49	Ethyl hexadecanoate	74.5	628-97-7	88 (100); 101 (58)

Optimization of extraction time

Prepared brandy samples were extracted at 30^{0}C for 0.5; 1.0; 2.0; 5.0; 10.0; 20.0; 30.0; 60.0 and 100 min. Pre-incubation time was 5 min. The experiment was conducted using two different fibers: 100 μm PDMS and DVB/CAR/PDMS.

In this experiment, flame ionization detector was used instead of mass selective detector due to its better linearity at higher analyte concentrations. Utilization of MS detector in the preliminary experiment led to a strong detector overload. Obtained chromatograms were integrated by calculating the sum of areas of all peaks having RT>8 min, and areas of the most abundant peaks.

Optimization of salt addition

The mass of NaCl varied during the experiment to 0.25, 0.75, 1.25 and 1.75 g. The highest value corresponded to NaCl saturation in 12% ethanol solution. Extraction was done by DVB/CAR/PDMS fiber at 30^{0}C during 30 sec.

Analysis of the real samples using the optimized method

Thirty-two samples of brandy, cognac, whisky and whiskey were analyzed (Table 2). The samples were collected in the period October, 2012 to February, 2013 from local shops in Almaty and Almaty oblast. According to available information (color, packaging, smell, taste), part of the samples represented adulterated alcohol products.

SPME was carried out for 0.50 min at 30^{0}C. All samples were separately analyzed using 100 μm PDMS and DVB/CAR/PDMS fibers. To obtain the required sensitivity at low extraction time, mass spectrometric detection was performed in selected ion monitoring (SIM) mode according to the program which included 29 groups of ions (Table 3). The SIM program included all the compounds previously identified in all the studied samples of alcoholic beverages. Identification of peaks on the collected SIM chromatograms was conducted by retention times using confirmation ions. For each compound, the peak area was determined on extracted ion chromatogram by the most abundant ion. The data was then entered into MS Excel sheet and imported into SPSS Statistics (IBM) ver. 20 software for statistical processing of the results. The statistical processing was conducted by principal component analysis (PCA) using covariance matrices and Bartlett variables. Two most important variables were extracted and plotted.

Table 2 – Samples of alcoholic beveragesanalyzed by the developed method

Sample ode	Name (manufacturer) [percentage of ethanol]	Type	Country
12	Kazakhstan (Bacchus OJSC) [40%]	Brandy	Kazakhstan
25	Gold [42%]	Brandy	Kazakhstan
27	LE Graff (“BN” Liquor-vodka factory) [40%]	Brandy	Kazakhstan
33	Hot Irishman [40%]	Whiskey	Ireland
37	Remy Martin VS Super Rieur [40%]	Cognac	France
40	Marsel (Akros) [40%]	Brandy	Kazakhstan
42	Kazakhstan (Aliya LTD) [40%]	Brandy	Kazakhstan
48	Al-Farabi (WIMPEX) [42%]	Brandy	Kazakhstan
51	Scottish Collie [40%]	Whisky	Scotland
52	Seitek, 5 stars	Brandy	Kazakhstan
55	Chinese [38%]	Brandy	China
56	Kazakhstan (Wine Master LTD) [40%]	Brandy	Kazakhstan
59	Gosudarev Zakaz (“BN” Liquor-vodka factory) [40%]	Brandy	Kazakhstan
63	Kazakhstan (Bacchus LLP) [40%]	Brandy	Kazakhstan
64	Chivas Regal (Pernod Ricard) [40%] *	Whisky	Scotland
65	Chivas Regal (Pernod Ricard) [40%]	Whisky	Scotland

Continuation of table 2

Sample ode	Name (manufacturer) [percentage of ethanol]	Type	Country
66	Black Label (Johnnie Walker) [43%]	Whisky	Scotland
67-72	Black Label (Johnnie Walker) [43%] *	Whisky	Scotland
73	Black Label (Johnnie Walker) [43%]	Whisky	Scotland
74	Black Label (Johnnie Walker) [37.5%] *	Whisky	Scotland
75	Jack Daniels [40%]	Whisky	USA
76	Jack Daniels [40%]	Whisky	USA
77	Gold Label (Johnnie Walker) [43%]	Whisky	Scotland
78	Hennessy V.S.O.P. Privilege Cognac [40%]	Cognac	France
79	Asanali (Bacchus OJSC) [40%]	Brandy	Kazakhstan
80	Meiram [40%]	Brandy	Kazakhstan
81	Kazakhstan Very Special Old V.S.O.P. Exclusive (“BN” liquor-vodka factory) [40%]	Brandy	Kazakhstan
Note: * - potentially adulterated samples			

Table 3 – Selected ion monitoring program for the analysis of samples of alcoholic beverages by SPME-GC-MS

#	Group start time, min	Ions (Dwell)		Detected compounds
		Quantification ions (Dwell 50 ms)	Confirmation ions (Dwell 10 ms)	
1	2.2	41; 59; 60; 86; 88	61; 74; 103	acetic acid, ethyl acetate, 2-methyl-1-propanol, diethoxymethane, 2-methyl butanal
2	3.4	55; 102; 116	70; 88	ethyl propanoate, isoamyl alcohol, ethyl isobutyrate
3	5.2	55	41; 70	1-butanol 3-methyl formate
4	5.6	88	60	ethyl butanoate
5	6.6	45; 88; 102	60; 74; 75	ethyl 2-hydroxypropanoate, 2-methyl ethylbutanoate, ethyl-3-methylbutyrate
6	8.6	56; 70	55; 87	1-hexanol, 3-methylbutyl acetate
7	10.1	96	39	furfural
8	15.9	88	99	ethyl hexanoate
9	17.8	105	106	benzaldehyde
10	20.3	45; 55; 69	41; 56; 70; 87	ethyl 2-hydroxy-4-methylpentanoate, 1-octanol, isoamyl lactate
11	22.3	45	69	2-nonanol
12	25.6	105	77; 120	acetophenone
13	28.4	88; 91	92; 101; 122; 127	phenylethyl alcohol, ethyl octanoate
14	30.4	60		octanoic acid
15	31.6	101; 105; 120	92; 122; 129; 134	3-ethyl benzaldehyde, butanedioic acid ethyl ester, methyl 2-hydroxy-benzoate
16	34.4	55	70	decanol
17	37.7	104	91	2-phenyl acetate
18	41.9	70; 88	55; 101	ethyl decanoate, ethyl 9-decenoate
19	43.8	57; 70	127	5-ethyl-2-methyloctane, 3-methylbutyl octanoate
20	47.7	133	148	1-(2,5-dimethylphenyl)ethanone
21	50.3	155	56	isobutyl decanoate
22	52.1	69; 88; 126	93; 98; 101	ethyl dodecanoate
23	56.0	70	155	3-methylbutyl decanoate
24	60.2	69	81	3,7,11- trimethyl-6,10-dodecadien-1-ol
25	64.1	88	101	ethyl tetradecanoate
26	65.8	70; 81	55; 69	isoamyl laurate
27	68.5	69	93	farnesol acetate
28	72.4	55; 88; 149	69; 101; 150; 284	diisobuthyl phthalate, ethyl hexadecanoate
29	75.5	104	105	nonyl 2-phenylethyl oxalate

Data processing

The data were processed using the SPSS Statistics (IBM) ver. 20 software. Principal component analysis (PCA) using covariance matrices and Bartlett variables were retrieved. Two first two component were extracted and plotted.

Results and discussion

Optimization of extraction temperature

Increase of extraction temperature from 30 to 70^0C lead to the 3-fold increase of the total peak area (Figure 2a) as the peak areas of the majority of the individual analytes due to the increase of their vapor pressures (Figure 2b). An increase of the extraction temperature from 30 to 70^0C resulted in a 5-fold increase of the response of ethyl decanoate. On the other hand, a decrease in response was observed for some compounds as, e.g., ethyl acetate (Figure 2b), which can be explained by a decreasing distribution constant between the polymer fiber coating and the headspace.

Despite the observed increase of the analytes response, the results are hampered by higher signal relative standard deviations (RSDs) and several new non-reproducible peaks appearing in the chromatograms obtained at extraction temperatures over 40^0C. Such changes may be caused by unwanted side reactions running in the vials. Such reactions may occur between brandy constituents or with oxygen present in the headspace. Such fluctuations in the results obviously affect the reliability of the method in a negative way. A further problem associated with extraction at high temperatures is caused by a higher water response, especially when using DVB/CAR/PDMS fiber. This may negatively affect the lifetime of the MS detector filament and thus the overall detector sensitivity.

To be safe, the extraction temperature 30^0C was selected as optimal. The selected temperature is close enough to room temperature to do extraction without autosampler. It significantly expands the applicability of the method in the practice of forensic examination.

Optimization of extraction time

The total peak area increases with increasing extraction time reaching a plateau after approx. 60 min (Figure 3). These results correspond to earlier findings [17]. However, the equilibrium in the system is not established even after 100 min of extraction (Figures 3-4). Thus, the responses of the heaviest compounds (ethyl tetradecanoate and ethyl hexadecanoate) increase linearly during the studied time period, which can be explained by their higher affinity to the coating and a lower volatility compared to other compounds. The response of the lightest of the studied compounds (ethyl hexanoate) increases during the first 10 min of extraction process followed by a gradual decrease (Figures 4-5).

It appears that the obtained data may be rationalized in terms of a displacement from the SPME fiber of the more volatile compounds by heavier compounds due to a limited capacity of the coating and higher adsorption affinity of the heavier compounds. Displacement of analytes from the fiber can potentially lead to problems with linearity of calibration plots and thus quantification, two factors that for forensic purposes appear crucial. For DVB/CAR/PDMS fiber, extraction time before displacement apparently is limited to 2 min, whereas for PDMS fiber, this time range appears much wider (10 min). This fact can be explained by its absorptive nature (versus adsorptive nature of DVB/CAR/PDMS) and higher analyte capacity. During SPME by absorptive coatings, their whole volume is involved while adsorptive fibers provide only their surface area.

When selecting an optimal extraction time, the linear range of the detector plays a crucial role. In the present experimental set-up, linearity of the applied Agilent 5973N mass spectrometric detector under the optimized conditions is limited to the extraction time of 1 min. For samples having higher concentrations of VOCs, linear range may be even narrower (less than 30 sec). Safe work at higher extraction times requires detector/filament switch off during elution of the most abundant peaks (esters and isoamyl alcohol).

Comparison of the data collected using the two different fiber coatings at different extraction times (Figures 4-5) disclosed that the DVB/CAR/PDMS fiber provides higher responses of less abundant peaks, which obviously may be crucial for their detection. There is no problem with the detection of most abundant peaks because they provide a high response. Despite better selectivity of the DVB/CAR/PDMS fiber [16], higher sensitivity without unwanted displacement may be achieved using 100 μm PDMS fiber. However, further studies are required eventually to select the optimal fiber coating.

Optimization of salt addition

An increase of salt concentration in the range of 0 - 1.25 g leads to an increase of total peak area (Figure 6a). The responses of the more polar compounds such as isoamyl alcohol continuously increase in the whole studied region (Figure 6b)

whereas the peak areas of the most abundant compounds (ethyl octanoate and ethyl decanoate) increase with increasing NaCl mass from 0.75 to 1.25 g followed by a slight decrease. This decrease may be caused by a competition with an increased vapor pressure of ethanol, which is caused by an increased salt concentrations. As discussed above, such competition processes are not desirable. Thus, it is concluded that salt addition should be avoided.

Analysis of the real samples

The attempt to differentiate tested samples using principal component analysis (PCA) of the areas of all the peaks on the chromatogram as data set was made (Figure 7). It is immediately seen that samples 25 and 27 differ substantially from the other samples. The most intense peaks characteristic to the samples of cognac and brandy were missing in the chromatograms of these samples.

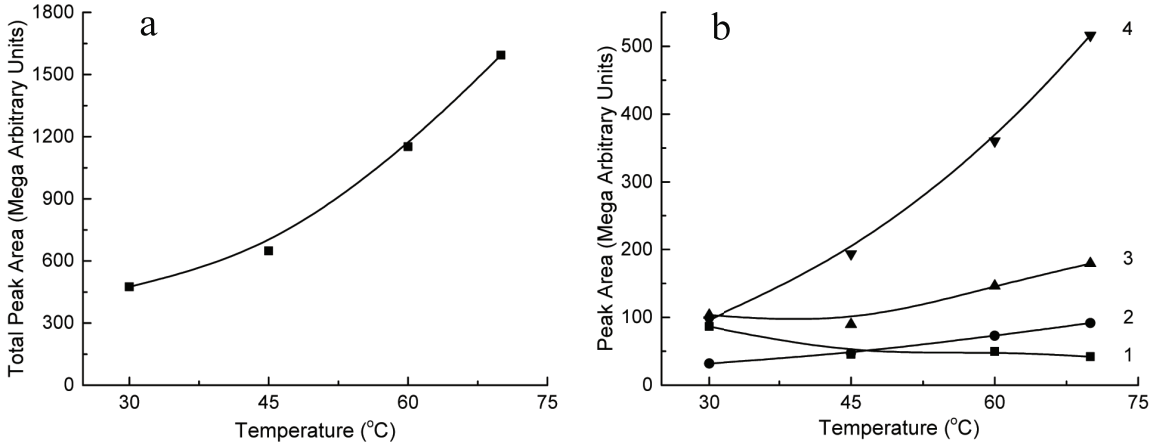

Figure 2 – Effect of the extraction temperature on the total peak area (a) and peak areas of individual brandy VOCs (b).
Note: 1 –ethyl acetate 2 – ethyl hexanoate; 3 – isoamyl alcohol; 4 – ethyl decanoate

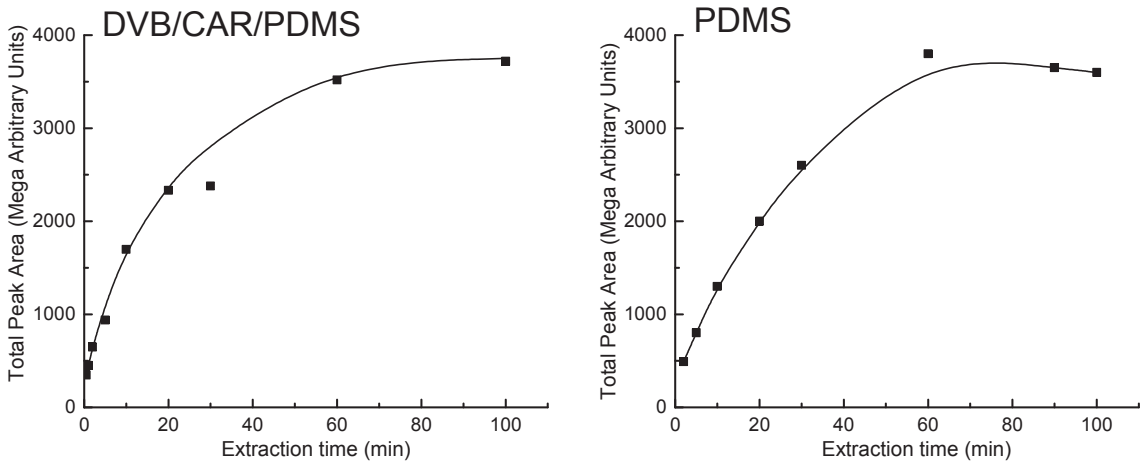

Figure 3 – Effect of extraction time on the total peak area of brandy VOCs by SPME-GC-MS using DVB/CAR/PDMS and 100 um PDMS fiber coatings

The resulting diagrams allowed differentiating samples of cognac and whisky - cognac samples were mostly on the left side of the diagram while whisky samples were on the right. Potentially adulterated whisky samples (#64 and #67-72) were well differentiated from the original products (#65 and #66) in the PCA plots. Whiskey sample #33 was very close to the other adulterated samples on the both PCA plots. This sample can be classified as potentially adulterated. However, it should be

noted that whereas the original whisky samples (#65 and #66) are of Scotch original sample #33 is an Irish whiskey. Differentiation of Scotch and Irish products will require access to further samples. The potentially expected adulterated whisky sample (#74) is very far from the original one (#73) in the PCA map, it is much closer to the group of adulterated samples. It is in this connection also worthwhile to mention that the two samples of American whiskey (#75 and #76), which apparently should be identical are located far away from each other in the PCA map (Figure 6a), which could suggest that these samples should be subjected to further investigation. Thus, the final conclusion can be done by a detailed comparison to the proven original samples.

The PCA plots obtained based on the data collected using two different fibers appear relatively similar. However, DVB/CAR/PDMS provided slightly higher differentiation efficiency of adulterated and original whisky samples. In most cases, DVB/CAR/PDMS fiber allowed detection of a higher number of target compounds compared to the PDMS fiber (Figure 8). Extraction selectivity of the DVB/CAR/PDMS coating to higher numbers of compounds can be explained by its multicomponent chemical composition and higher selectivity to polar compounds mostly present in alcoholic samples.

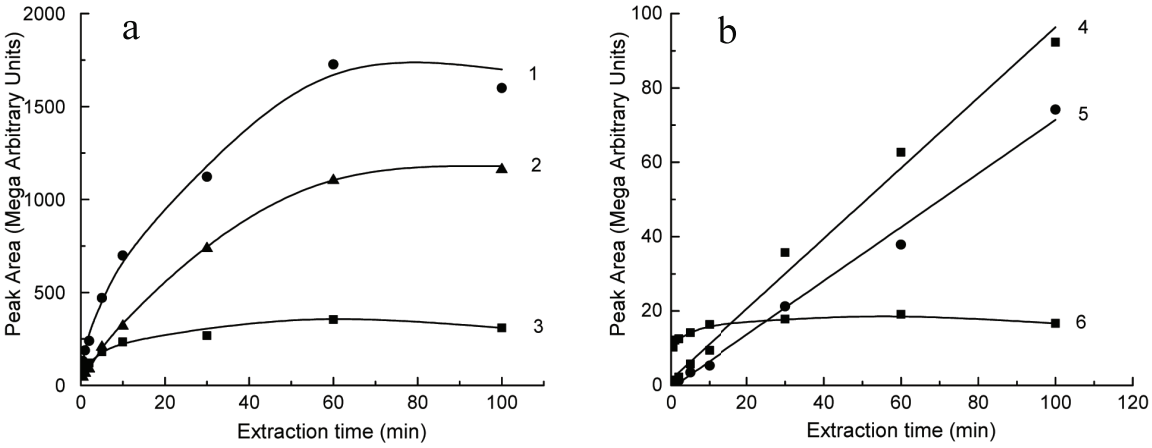

Figure 4 – Peak areas of selected compounds at different extraction times using 100 μm PDMS coating.
Note: 1 – ethyl decanoate; 2 – ethyl dodecanoate; 3 – ethyl octanoate; 4 – ethyl tetradecanoate; 5 – ethyl hexadecanoate; 6 – ethyl hexanoate

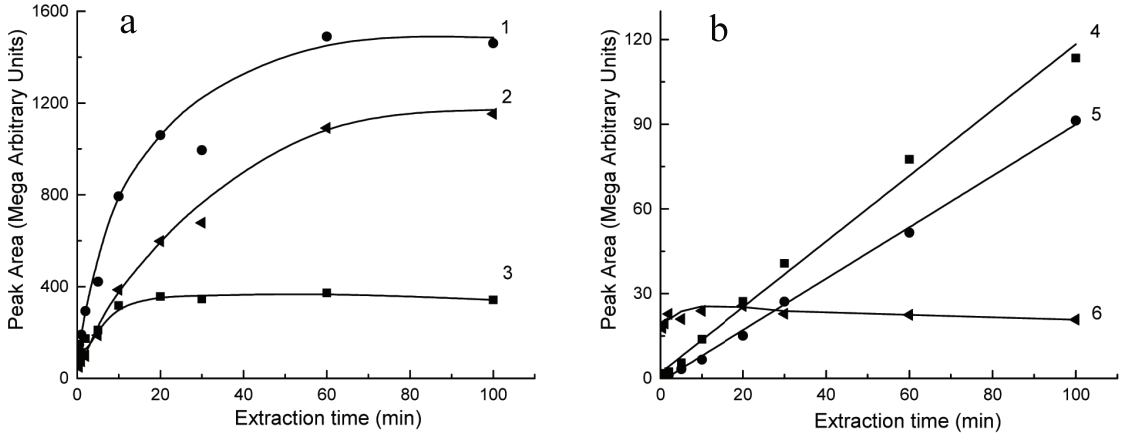

Figure 5 – Peak areas of selected compounds at different extraction times using DVB/CAR/PDMS coating.
Note: 1 – ethyl decanoate; 2 – ethyl dodecanoate; 3 – ethyl octanoate; 4 – ethyl tetradecanoate; 5 – ethyl hexadecanoate; 6 – ethyl hexanoate

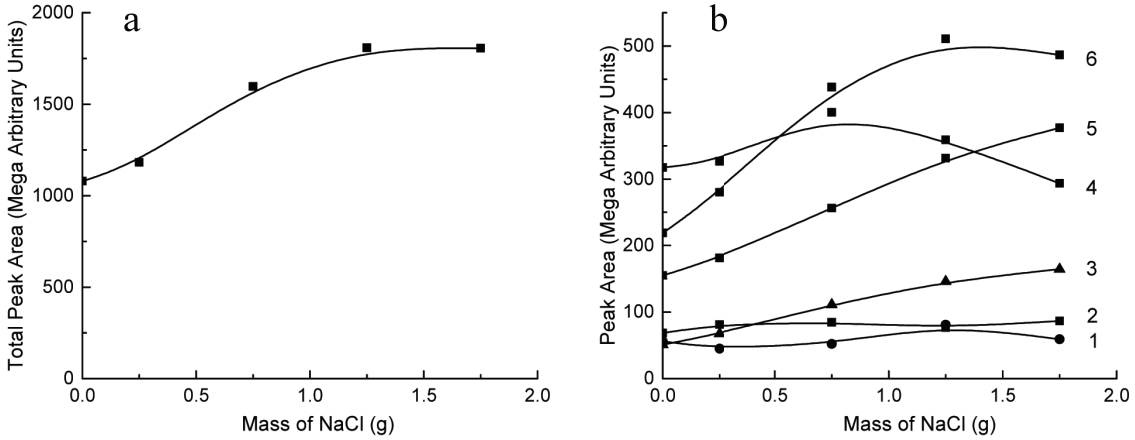

Figure 6 – Effect of the amount of salt additive to cognac sample on the extraction efficiency of certain compounds. *Note:* 1 – furfural (x10); 2 – ethyl acetate; 3 – ethyl hexanoate; 4 – ethyl decanoate; 5 – isoamyl alcohol; 6 – ethyl octanoate; x10 – peak area multiplied by 10; M a.u. – Mega arbitrary units. Fiber coating DVB/CAR/PDMS, extraction temperature 30C, extraction time 30 sec, pre-incubation time 15 min, sample volume 6 mL, vial volume 20 mL.

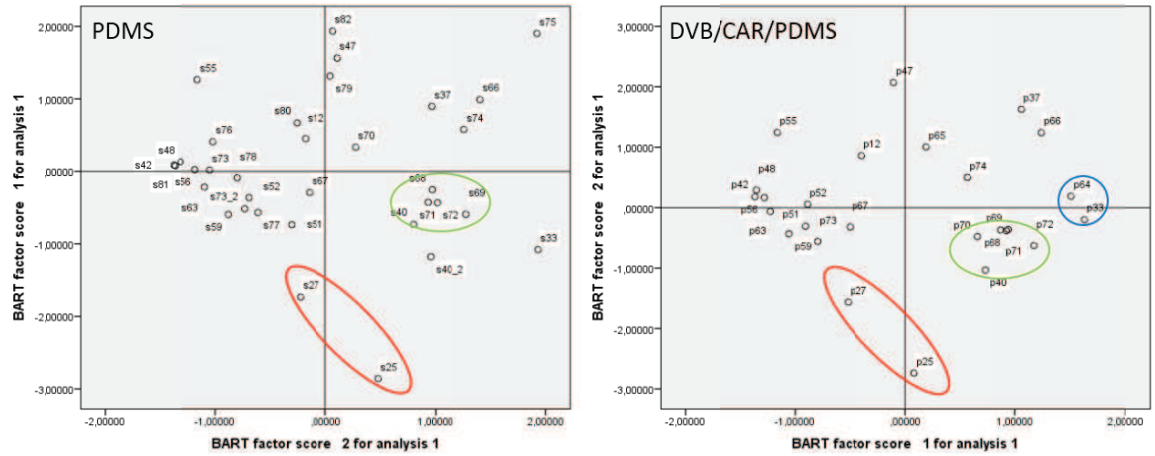

Figure 7 – Statistical distribution diagram taking into account peak area of all compounds (96.97% variance for PDMS (a) and 97.45% variance for DVB/CAR/PDMS (b)). *Note:* samples of surrogate cognacs and whiskies are encircled by ellipses with colored contours

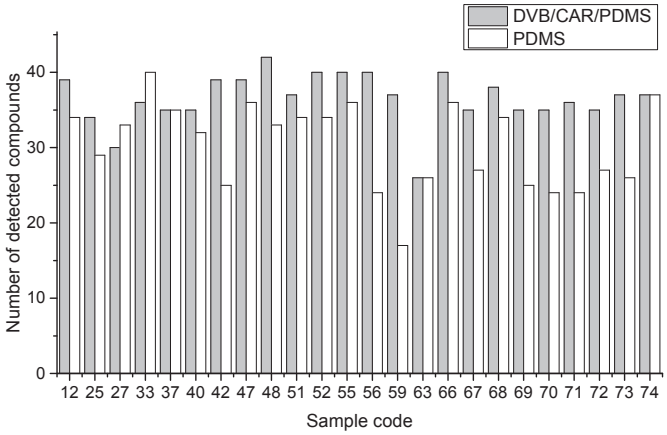

Figure 8 – Number of compounds detected in each sample using studied extraction coatings

ThePCA plots obtained basedon the data collected usingtwo different fibers appear relatively similar. However, DVB/CAR/PDMS provided slightly higher differentiation efficiency of adulterated and original whisky samples. In most cases, DVB/CAR/PDMS fiber allowed detectionof a higher number of target compounds compared to the PDMS fiber (Figure 8). Extraction selectivity of the DVB/CAR/PDMS coating to higher numbers of compounds can be explained by its multicomponent chemical composition and higher selectivity to polar compounds mostly present in alcoholic samples.

Conclusion

New SPME-GC-MS method for discovery of adulterated alcoholic beverages has been developed. The method parameters were experimentally optimized to provide high precision and reliability. According to the experiments, increase of extraction temperature leads to the increase in response of the most VOCs. However, to avoid transformation/ degradation of the samples, the extraction temperature must be limited to 30^0C (or to the minimum temperature of the autosampler). Increase of the extraction time to 60 min leads to the increase of total peak area. However, longer extraction times (>10 min for PDMS and >2 min for DVB/ CAR/PDMS) caused an undesired displacement of analytes on the SPME fiber. For mass spectrometric detector, the extraction time should be limited to 1 min while flame ionization detector may be used if longer extraction times appear necessary. Increase of NaCl mass up to 1.7 g added to the extracted sample leads to the increase of total peak area. However, the salt addition promotes competition between ethanol and the analytes for adsorption sites and causes problems with reproducibility. Therefore, it is concluded that an addition of salt prior to extraction is undesirable for the here presented method.

The developed method was successfully applied for discovery of adulterated samples of brandy and whisky. Principal component analysis plots obtained using the data collected by both 100 μm PDMS and DVB/ CAR/PDMS coatings were virtually similar. However, the DVB/CAR/PDMS fiber appears more efficient for detection of adulteration markers. Adulteration of potentially adulterated brandy and whisky samples was confirmed. One whiskey sample was regarded as potentially adulterated one, but the geographic origin may play a role in this context. Further two potentially identical samples appeared at significant different locations in the PCA map. The developed method is recommended for application in forensic laboratories.

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