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APPLICATION OF SOLID-PHASE MICROEXTRACTION (SPME) TO CHROMATOGRAPHIC DETERMINATION OF FUSEL ALCOHOLS IN WINES

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ABSTRACT

A majority of the methods used for determination of fusel alcohols in wines by gas chromatography requires separation of the analysed substances from other components, mainly extract constituents, which can hamper the measurements. The aim of this study was to establish whether SPME method can be used for quick chromatographic determination of fusel alcohols in red wines. The studies were conducted on different red wines commercially available on domestic market. It was shown that the results for isobutanol were usually overestimated, while butanol recovery ranged from 97 to 116%, and approximated actual content of this component in the test sample. Sorption of amyl alcohols on fibres was in the range from 82 to 95%. Addition of strong electrolyte, such as sodium chloride, to a wine increased mictoextraction efficiency and precision of chromatographic determination of fusel alcohols. CAR/PDMS copolymer-coated fibre was characterised by better sorption ability for fusel alcohols in red wines in comparison with polyacrylate sorbent. SPME is much quicker and simpler than distillation and can be a competitive method of sample preparation for chromatographic determination of fusel alcohols in wines.

Key words: red wine, SPME, fusel alcohols.

INTRODUCTION

A majority of the methods used for determination of alcohols in wines by gas chromatography requires separation of the analytes from other components, mainly extract constituents, which can hamper the measurements. The separation can be achieved with physical methods, distillation or extraction [8, 10, 12]. Moreover, head space analysis [14] or direct injection of a test sample [12] can be carried out.

In distillation method, wine distillate is analysed, and total amount of fusel compounds can be determined colorimetrically (reaction with salicylaldehyde) or individual elements are identified and quantified by chromatographic analysis [11]. During distillation, from 95 to 99% of fusel compounds is transferred to a receiver, depending on distillation conditions [4].

Another method of sample preparation for fusel alcohol determination with chromatographic method consists in liquid-liquid extraction with the following solvents: dichloromethane, carbon disulphide, trichlorofluoromethane (Freon 11) and mixture of dichloromethane or ether with pentane. The obtained extracts are additionally concentrated on Vigurex column and dehydrated [2, 16]. The main disadvantages of this method are time-consuming procedure of sample preparation and low cost-effectiveness due to the use of expensive solvents. These burdens of this method can be eliminated by application of solid phase extraction (SPE). In this method, the test sample of wine is injected on a column filled with appropriate material (e.g. Propak, Amberlite), then the column is washed with properly selected solvent to remove substances interfering with the analysis. After drying of the resign with absorbed analytes in a jet of neutral gas, the tested components are eluted with a solvent, e.g. dichloromethane [13, 16].

Other studies have suggested possibility of application of solid phase microextraction (SPME). In this technique, test components are adsorbed not on a column (as in SPE) but on silica fibre coated with a film selectively adsorbing the analytes. After immersion of the fibres in water solution, extraction occurs until equilibrium is reached. Amount of the adsorbed substance depends on matrix chemical composition, analyte concentration and physico-chemical properties, extraction time, type and thickness of the layer coating the fibre, and temperature of the process. The procedure can be carried out automatically using autosampler.

SPME analysis can also be conducted by direct placing the fibre in head space (HS-SPME). HS-SPME has been used to analyse volatile aroma compounds [2, 9] and pesticides in wines [1].

Undoubted advantages of SPME technique consist in short time of sample preparation for analysis, possibility to automation of the procedure and good limit of detection approximating 5 - 50 ppt [14].

The aim of this study was to establish whether SPME can be used for quick chromatographic determination of fusel alcohols in red wines. The procedure involved direct immersion of the fibre in the test solution. Wine samples after distillation, in which the same alcohols were determined chromatographically served as the control.

MATERIALS AND METHODS

The study was conducted on different red wines commercially available on domestic market. Wine samples were prepared for chromatographic analysis by microextraction (method **I**) and distillation (method **II**).
Method I: solid phase microextraction (SPME).

SPME kits including silicon fibres coated with appropriate polymer were purchased from Supelco (Table 1).

Table 1. Characteristics of fibres used in SPME experiments

Method	Fibre description	Stationary phase	Film thickness [μ m]
I a	Black	Carboxen/polydimethylsiloxane (**CAR/PDMS**)	75
I b	White	Polyacrylate (**PA**)	85

A wine sample (3 cm^3) was transferred to a vial (4 cm^3), containing 0.6 g of NaCl, then 0.3 cm^3 of internal standard solution (1-pentanol), which does not occur in the tested wines, was added, and after mixing, microextraction fibre was placed in the liquid for 10 min (25°C, constant mixing on magnetic stirrer).

Chromatographic analysis was carried out immediately after extraction was finished. For this purpose, the fibre was placed for 3 min in the injector of gas chromatograph heated to 250°C (desorption of the adsorbed congeners).

Chromatographic analysis was performed using HP 5880 series II gas chromatograph, equipped with HP-INNOvax capillary column (30 m, 0.53 mm, 1.9 μm), and flame-ionisation detector (FID). Working conditions were: helium was the carrier gas (20 cm^3/min), injector and detector temperature was 250°C, the column was maintained at 60°C for 1.5 min, and then temperature was increased at 10°C/min up to 100°C and at 30°C/min up to 200°C.

Standard solutions of target alcohols in 10% ethyl alcohol were used for calibration. They were processed in the same way as test samples of wines, and conditions of chromatographic separation were maintained unchanged.
To determine influence of sugars on the results, a number of analyses were conducted on standard solution of fusel alcohols supplemented with saccharose (Fig. 3).

Method II: distillation of wine samples

To 50 cm^3 sample of the tested wine, 10 cm^3 of H_2O and 1 cm^3 of internal standard solution (pentanol) were added, and the mixture was subjected to distillation. The 40 cm^3 of distillate was collected to a measuring flask placed in ice bath. The obtained solution was filled with water to starting volume (50 cm^3) and subjected to chromatographic analysis. The same procedure was applied to standard solutions of fusel alcohols supplemented with pentanol (calibration).

RESULTS AND DISCUSSION

Fusel compounds in wines were analysed after sample preparation using two procedures, distillation or microextraction on fibres coated with two different polymers: carboxen-poly(dimethylsiloxane) (**Ia**) or poly(acrylate) (**Ib**). These fibres were chosen due to high efficiency of extraction of volatile low molecular weight compounds [5]. The results are presented in Tables 4 - 6 and in the Figures 1-3.

Fig. 1. Influence of NaCl addition on detector signal intensity
Left *y*-axis relates to amyl alcohols, while left *y*-axis shows remaining fusel compounds

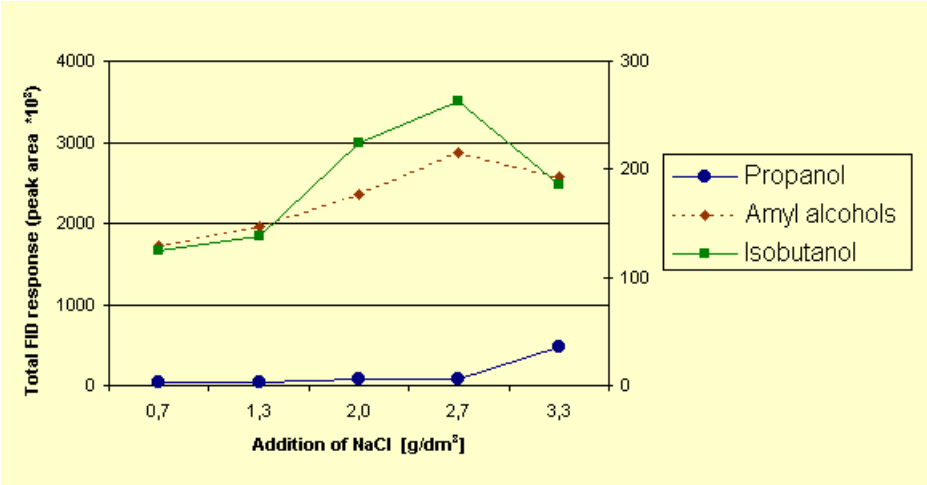

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Fig. 2. Comparison of the results obtained after preliminary SPME using CAR/PDME copolymer-coated fibre and distillation
Dry wine, Cabernet Sauvignon, 12.5% alc., France, 1998

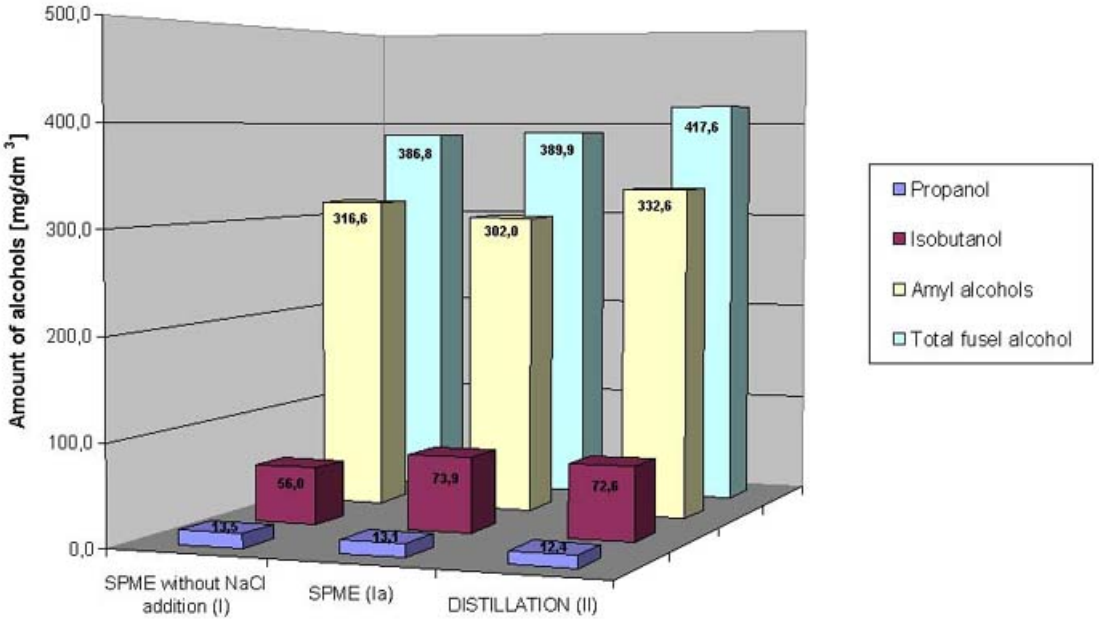
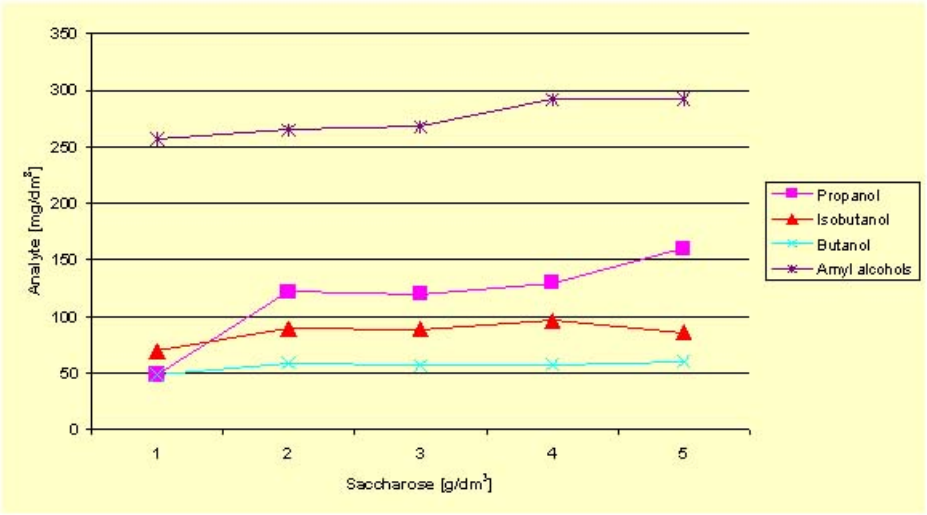


Fig. 3. Influence of saccharose content on the results of determination of fusel alcohols in model solutions



Influence of NaCl addition on the results of determination of fusel compounds

Strong electrolyte, such as sodium chloride affects adsorption of a compound in two-phase system, decreasing solubility of hydrophobic substances in water phase and causing so called "salting out" effect. This property has been frequently used in different analytical methods to improve detection limit [15]. Sensitivity of SPME of fusel alcohols rose with elevation of the amount of dissolved salt, reaching maximum when 3 cm^3 of wine were supplemented with 0.6 - 0.8 g of NaCl (Fig. 1). At the same time, intensity of detector response signal increased from 1.4 to 11 times. Further increase in sodium chloride concentration weakened signal from the majority of the tested alcohols, except for propanol, since for this alcohol, the highest sensitivity was achieved at as high NaCl concentration as about 30 g/dm^3.

For comparison, wine samples were analysed also with SMPE method without addition of sodium chloride to the sample (modification of method **Ia**). The results for butanol differed from those obtained with method **Ia** (Fig. 2). Variance uniformity testing confirmed improvement of precision of fusel alcohol determination after sample supplementation with NaCl.

Influence of saccharose addition on extraction of fusel compounds from model solutions

To establish the effect of saccharose concentration on the test results, a number of standard solutions of fusel compounds supplemented with different amounts of sugar were prepared. At higher saccharose concentrations in model solutions, slight shift of equilibrium between the solution and a fibre towards stationary phase was observed, causing overestimation of component concentration (Fig. 3). The results of determination of higher alcohols in the samples supplemented with saccharose were higher by 25%, except for propanol, whose extraction increased more than 2.5 times after saccharose addition at 10 g/dm^3. However, no significant effect of total extract of the analysed wines on determination of fusel alcohols was noted (Tables 4-6). Nevertheless, the obtained results indicate necessity to take extract content into consideration, while preparing standard solutions for determination of fusel compounds using SPME method, particularly if sweat wines are to be analysed.

Recovery of fusel alcohols during microextraction (SPME)

Recovery of the tested compounds in SPME method was examined on wine samples supplemented with known amounts of fusel alcohols. Qualitative and quantitative extraction significantly depended on fibre type and differed for different alcohols (Tables 4-6). It was shown that the data obtained for isobutanol were usually overestimated, while butanol recovery ranged from 97% to 116% and approximated actual content of this component in the test sample. Sorption of amyl alcohols on fibres ranged from 82% to 95% and did not significantly differ from the amount of a component added to the test sample (Table 2). To improve accuracy of the assay, appropriate correction factors can be introduced, resulting from recovery percentages for individual components: isobutyl alcohol (-10%) and amyl alcohol (+ 10%).

Table 2. Recovery of fusel alcohols during extraction using SPME fibres (n = 3)

Analyte	Amout of alcohol added [mg/dm^3]	Average recovery		SD
		[mg/dm^3]	[%]	
Isobutanol	32.00	36.24	113.24	3.47
Butanol	20.09	21.48	106.91	12.81
Amyl alcohols	100.22	90.03	89.84	7.66

Fusel alcohols are presented in wine at relatively high concentrations, and 10-minute extraction time, used in these studies was sufficient for adsorption of appropriate amount of the substances on the fibre to perform chromatographic analysis. Quantitative analysis was possible due to linear relationship between analyte concentration in absorption layer and initial content of the compound in the sample. Under such conditions, fibre is not saturated during dynamic equilibrium, and to increase SPME method sensitivity, time of fibre presence in the test solution can be prolonged [5].

The results obtained with two different fibres (method **Ia** and **Ib**) showed that the methods significantly differed in their precision and accuracy (Table 5). Standard deviations calculated on the basis of the results obtained with different alcohols ranged from 0.01 to 35.8. Other determinations of fusel alcohols in wines with HS-SPME technique using PDMS fibre indicated much larger standard deviations [9]. The application of direct immersion of a fibre in sample solution or a change in absorbing substance elevated precision of determination of isobutanol and amyl alcohols in our studies.

Total content of fusel alcohols estimated with method **Ia** and **Ib** differed by 8 - 37% (Table 5). If poly(acrylate) fibre was used (method **Ib**), usually determination of propanol was not possible (relatively low sensitivity), while butanol contents were even several times higher in comparison with the results obtained using carboxen-poly(dimethylsiloxane) fibre (method **Ia**)

Distillation method

The technique consisting in simple distillation of higher alcohols, among other things, to separate them from non-volatile wine congeners was hitherto used for sample preparation for areometris, pycnometric and refractometric measurements and for chromatographic analysis [4, 11]. For comparative analysis of accuracy assured by this method, model studies of 10% v/v ethanol solutions were carried out. Influence of simple distillation and steam distillation on recovery of alcohols from the tested model samples was studied, and no significant differences were found. Average recovery of individual alcohols ranged from 86% to 90% (Table 3). Therefore, only simple distillation was applied in further studies for sample preparation. This method is also used for determination of proof and extract of actual wines [7].

Table 3. Mean recovery of fusel alcohols during distillation [%] (n = 4)

Distillation type	Propanol	Isobutanol	Amyl alcohols
Simple distillation (50 cm^3 wine + 10 cm^3 water)	90	90	90
Simple distillation (50 cm^3 wine + 25 cm^3 water)	87	86	88
Steam distillation	88	90	90
Average recovery	88	88	89

Although distillation method does not require additional specialised equipment, chemical reactions can occur in the sample during heating, leading to a change in analyte composition. Maintaining always identical conditions of distillation of volatile substances during each distillation and proper cooling the distillate, to prevent losses due to a release of the tested compounds to the atmosphere are decisive factors determining maximal recovery of alcohols and method precision.

Differences in total content of fusel alcohols between distillation and SPME method (**Ia** and **Ib**) range from 0.03% to 33%, depending of wine type. The largest divergences were seen in the contents of congeners, present at relatively low concentrations (propanol, butanol)

Table 4. Results of determination of fusel alcohol contents in wines after SPME using CAR/PDMS fibre (Ia) and simple distillation (II) [mg/dm^3]

Wine	Method	Propanol		Isobutanol		Butanol		Amyl alcohols		Total fusel alcohols
			SD		SD		SD		SD	
Semi-dry *Bear Blood* 11% alc. Bulgaria 1999	**I b**	14.5	7.1	64.3	2.1	1.00	0.02	250.3	4.7	330.1
	II	15.1	0.7	49.6	0.1	0.59	0.00	223.2	6.5	288.5
Dry *Bordoux Bon Baron* 11.5% alc. France 1998	**I b**	N	N	86.7	1.0	0.44	0.04	281.3	1.1	368.4
	II	16.4	0.3	81.5	1.9	0.58	0.65	307.2	6.4	405.7
Dry *Sophia Gamza* 11.5% alc. Bulgaria 1998	**I b**	11.9	16.3	73.0	0.9	0.97	0.09	242.3	4.7	328.2
	II	18.2	0.0	51.9	0.2	0.42	0.09	203.8	0.2	274.3
Dry *Cambras* 12% alc. France 1999	**I b**	N	N	70.4	0.3	1.20	0.82	233.3	2.8	304.9
	II	17.2	0.2	60.9	0.8	0.30	0.13	248.2	4.7	326.6
Dry *Cabernet Sauvignon* 12.5% alc. France 1999	**I b**	N	N	123.6	4.0	0.77	0.05	418.9	13.0	543.3
	II	23.6	0.3	87.1	1.7	0.29	0.03	377.6	6.8	488.6
Sweet *Cherry Wine* 14.6% alc. Poland 2000	**I b**	N	N	75.8	1.5	3.1	0.4	181.3	4.0	260.2
	II	13.9	0.2	46.6	1.1	1.9	0.1	165.5	3.2	227.9
Sweet *Trzech Muszkieterów* 15.6% alc. Poland 2000	**I b**	N	N	199.5	15.5	7.5	0.3	229.8	1.6	436.8
	II	26.6	0.2	100.9	1.8	3.6	0.1	208.6	4.1	339.7

N- not determined

Table 5. Results of determination of fusel alcohol contents in wines after SPME without NaCl (I), with NaCl (Ia) and after distillation (II) [mg/dm^3]

Wine	Method	Propanol		Isobutanol		Butanol		Amyl alcohols		Total fusel alcohols
			SD		SD		SD		SD	
Dry *Cabernet Sauvignon* 12.5% alc. France 1998	**I**	13.5	0.7	56.0	12.7	0.74	0.07	316.6	35.1	386.8
	I a	13.1	1.8	73.9	15.6	0.93	0.02	302.0	21.0	389.9
	II	12.4	0.5	72.6	4.8	-	-	332.6	10.1	417.6
Dry *Cambras* 12% alc. France 1999	**I**	15.4	1.4	67.5	10.5	0.70	0.02	281.9	37.5	365.5
	I a	15.5	0.7	76.5	15.4	0.90	0.03	305.9	35.8	398.8
	II	16.4	0.4	86.7	4.1	-	-	345.7	10.5	448.8
Dry *Egri Bikaver* 11.5% alc. Hungary 1996	**I**	26.3	10.5	93.4	4.8	1.04	0.09	251.8	6.3	372.5
	I a	60.5	1.6	110.6	0.1	0.95	0.06	208.2	23.0	380.2
	II	34.6	2.0	98.6	4.9	-	-	276.4	11.7	409.9

Table 6. Results of determination of fusel alcohol contents in wines after SPME using poly(acrylate) (Ia), and distillation (II) [mg/dm^3]

Wine	Method	Propanol		Isobutanol		Butanol		Amyl alcohols		Total fusel alcohols
			SD		SD		SD		SD	
Dry *Bordeaux Francois Delaville* 11% alc., France 1999	**I a**	16.6	2.2	110.0	2.4	0.77	0.12	408.9	32.2	536.3
	II	16.5	0.5	120.9	3.8	-	-	455.2	11.8	592.6
Dry *Merlot Delle Venezie* 11% alc., Italy 1999	**I a**	39.7	4.9	61.7	3.0	0.81	0.08	275.1	8.3	377.3
	II	43.1	0.7	58.6	4.5	-	-	279.4	9.3	381.1
Semi-dry *Bear Blood* 11% alc., Bulgaria 1999	**I a**	28.7	1.8	61.2	16.5	0.96	0.05	339.6	29.1	430.5
	II	16.7	1.0	66.4	3.3	-	-	264.7	10.8	347.8
Dry *Balaton* 11.5% alc., Hungary 1999	**I a**	27.5	3.9	102.1	3,5	0.78	0.17	280.8	6.9	411.2
	II	26.4	0.2	91.3	0.8	-	-	275.0	1.2	392.7
Dry *Merlot Sophia* 11.5% alc., Bulgaria 1998	**I a**	40.4	9.0	45.4	21.7	0.89	0.04	255.0	30.2	341.7
	II	15.4	0.4	66.6	1.7	-	-	259.8	2.5	341.8
Semi-sweet *Achaia Clauss* 11.5% alc., Greece 1999	**I a**	33.8	0.9	51.3	4.5	1.38	0.11	190.6	15.4	277.1
	II	34.2	1.3	53.7	0.9	-	-	186.3	3.9	274.2
Sweet *Makedonnikos Topikos* 12% alc., Greece 1998	**I a**	14.2	10.6	55.0	1.1	1.82	0.01	249.1	6.1	320.1
	II	23.3	0.6	65.4	1.0	-	-	284.0	4.2	372.7

Typical chromatograms of the test samples are presented in Figures 4 and 5.

Fig. 4. Chromatogram of an extract of Merlot Delle Venezie wine obtained by SPME on CAR/PDMS copolymer (Ia): 1 - propanol, 2 - isobutanol, 3 - butanol, 4 - amyl alcohols, 5 - n-pentanol (internal standard)

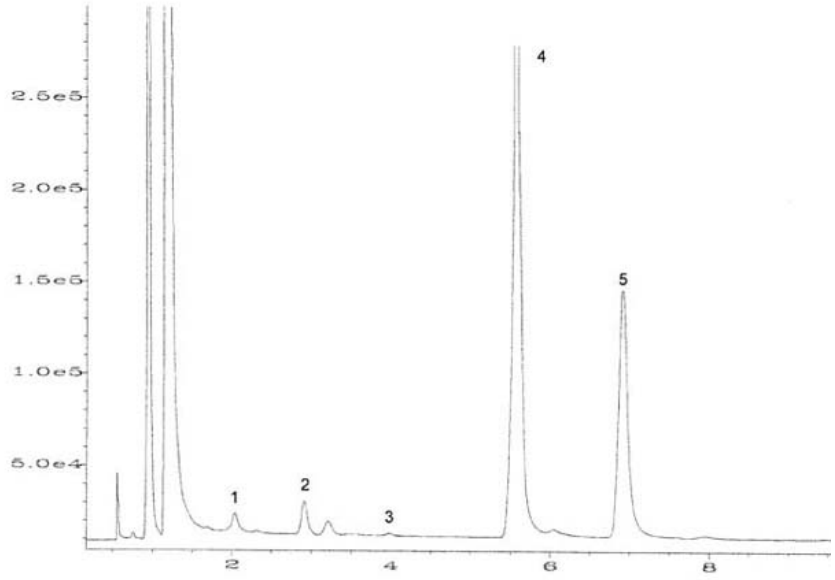
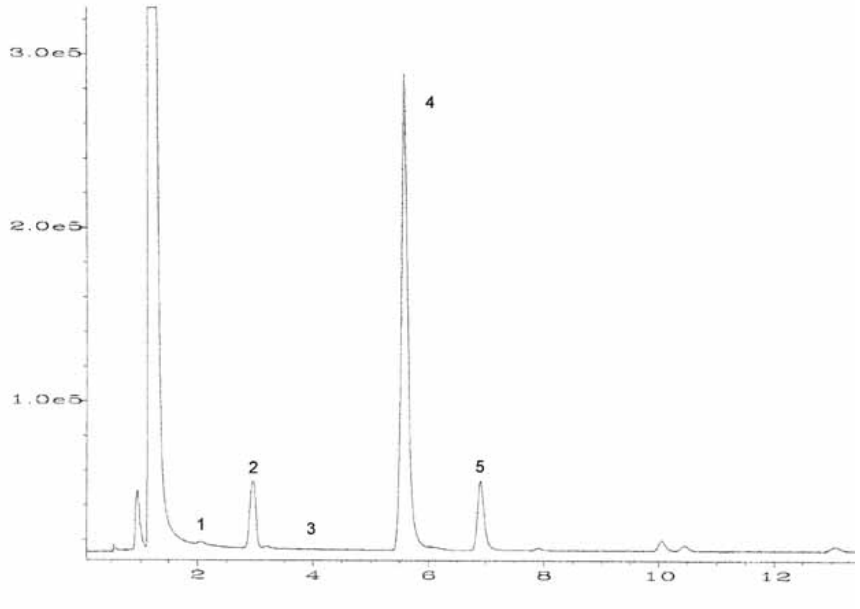


Fig. 5. Chromatogram of an extract of Merlot wine obtained by SPME on poly(acrylate) polymer (Ib): 1 - propanol, 2 - isobutanol, 3 - butanol, 4 - amyl alcohols, 5 - n-pentanol (internal standard)



Comparison of chromatograms and obtained results suggests that CAR/PDMS fibres are more valuable material for microextraction of fusel alcohols from wines than acrylate polymer (Figures 4 and 5).

SPME method enables to parallelly determine also other congeners composing wine bouquet, and can allow to demonstrate possible contamination with pesticides or other crop protection chemicals [6, 7].

SUMMARY

SMPE is much quicker and simpler method than distillation, which decreases probability of gross errors, and allows to avoid heating of the sample, thus eliminating a danger of changing wine chemical composition during analysis. A single fibre can be used for more than hundred analyses. However, it should be mentioned that SPME fibres are very vulnerable to mechanical damage and relatively expensive. One fibre costs about 70 euro. One unguarded moment or hurry can lead to a damage of fine fibre core. The application of autosampler with a fibre allows to automate analysis and should prolong fibre life, eliminating risk of mechanical damage. Total cost of purchase of SPME kit, calculated per one analysis repeated 3 times plus preparation of calibration curve should not exceed 3 euro. However, it should be noticed that diversity of wine chemical composition to higher degree influences results of analyses with SPME method. Practically each tested wine was distinguished from others by alcohol content and extracted congeners. These parameters directly affect affinity of analytes for stationary phase, and consequently, test result.

Variation uniformity testing showed that precision of distillation and SPME (**Ia**) method were similar in most cases, while concentrations of fusel alcohols were significantly different. Difference between average concentrations of amyl alcohols established with these two procedures ranged from 2% to 22%, while for isobutanol it was 1-50%. Total content of fusel alcohols determined in the samples prepared by distillation was usually higher in comparison with **Ia** method and lower in relation to **Ib** method. These discrepancies are caused by variable chemical composition of the tested wines and different physico-chemical processes occurring during sample preparation.

French wines, particularly Bordoux and Cabernet Sauvignon showed the highest contents of fusel alcohols in all tested samples, while Greek (Achaia Claus, Makedonnikos) and Polish (Cherry Wine) wines were characterised by relatively low content of amyl alcohols.

CONCLUSIONS

1. Addition of sodium chloride to wine samples increases microextraction efficiency and precision of chromatographic determination of fusel compounds.
2. CAR/PDMS copolymer-coated fibres are characterised by better sorption ability for fusel alcohols in red wines in comparison with poly(acrylate).
3. Accuracy of SPME method for different fusel alcohols can be increased by taking into account contents of ethyl alcohol and main wine congeners during preparation of standard solutions.
4. SPME can be a competitive method of sample preparation for chromatographic determination of fusel alcohols in wines in relation to distillation.

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