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Solid phase microextraction of esters: comparison of headspace and direct extraction

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Headspace solid phase microextraction and direct solid phase microextraction techniques for determination of ethyl acetate, n-butyl acetate, i-butyl acetate, n-pentyl acetate, i-pentyl acetate are suggested and compared. Solid phase microextraction was performed with a fiber coated with a 100 mm thick film of polydimethylsiloxane. Optimal extraction conditions were: the extraction was carried out at 30 °C or at room temperature for headspace and direct SPME, respectively; the extraction time in both cases was 10 min, the solutions were stirred at 200 rpm. Desorption of the analytes was carried out for 10 s at 200 °C. The precision, linearity, detection limits were determined. The proposed techniques could be applied for the analysis of wine.

Key words: headspace solid phase microextraction, direct solid phase microextraction, gas chromatography, esters

INTRODUCTION

Ultimately, one of the main arguments on which efforts of the analysts are focused is the miniaturisation of extraction techniques. When extracting liquid samples, traditional liquid–liquid extraction faces several limitations, such as use of an extractant non-miscible with the sample, difficulty in extracting polar and ionic compounds from water, large organic solvent volumes resulting in a diluted extract [1]. To prevent these drawbacks, two techniques are used: solid phase extraction and solid phase microextraction (SPME). In the first one, the sample is percolated through a solid phase that retains the solutes of interest. They are further eluted with a small solvent volume. The method is selective, provides an extract with a high concentration of the analytes; there is a wide choice of available solid phases (common inorganic adsorbents, siloxane-bonded silica materials, nonpolar and ion-exchange macroreticular resins). The solid phases are packed into cartridges or enmeshed in a web of microfibrils to create a membrane, which can be used with a simple vacuum-assisted filtration apparatus [2]. However, the necessity to use an eluting solvent can lead to a poor sensitivity in the case if only part of the extract is used for further analysis, time-consuming sample preparation in the case of the extract concentration after desorption, non-compatibility of the final extract solvent with the analytical system [3]. Those problems are overcome by the use of SPME. This technique uses a fused silica fiber coated with a thin layer of a selective coating. Analytes are adsorbed onto the fiber. The fiber is transferred to the injection port of the gas chromatograph, where thermal desorption and transfer of the analytes onto the GC column take place [4].

The commercially available coatings may be classified into homogeneous pure polymer coatings and porous particles imbedded in a polymeric phase [5]. At present, two homogeneous polymer coatings are available: polydimethylsiloxane (PDMS) and polyacrylate (PA). Those coatings behave similarly to organic solvents [6, 7]. In the case of porous particles imbedded in a polymer phase, the majority of interaction is determined by the adsorption process on the porous particles [5]. Those coatings have a lower mechanical stability than homogeneous polymer phases, but show a high selectivity. At present, the fibers available are: PDMS-divinylbenzene (DVB), PDMS- Carboxen (CAR) (carbon molecular sieves), Carbowax (CW) (polyethylene glycol)-DVB and DVB-CAR-PDMS.

The fibers differ in the coating polarity and volume. Polar fibers are effective for extracting polar analytes, and nonpolar fibers are effective for extracting nonpolar analytes from different martices. Coating volume determines method sensitivity, but thicker coatings result in longer extraction times.

Volatile organic compounds play an important role in the organoleptic characterization of food and drinks. Esters make the most important contribu-

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tion to what we usually perceive as fruit flavours. They are present in higher concentrations than any other class of volatile compounds, and they are present in a greater variety compared with any other class [8]. Extraction and concentration of esters is one of the important areas of analysis.

There are rather few articles on the analysis of esters using SPME. The fiber coatings used were PA [9–13], PDMS [10–12, 13], PDMS-DVB [11–13], CW-DVB [11, 12] and CAR-PDMS [12, 13, 15]. The opinions on the choice of the fiber coatings differ. In three [10,11,14] of the 7 [9–15] articles PDMS, in two [9, 13] PA and in two [12, 15] CAP-PDMS are suggested for esters SPME. Anyway, it seems that PDMS coating is among the best for esters extraction. However, in all the cases when PDMS was used, SPME of esters was accomplished using a headspace extraction mode. The aim of the present study was to optimise and to compare direct SPME and headspace SPME for the extraction of the selected esters using a PDMS coated fiber.

EXPERIMENTAL

Reagents

Ethyl acetate (99.5%), n-butyl acetate (99%), i-butyl acetate (99%), n-pentyl acetate (99%) and i-pentyl acetate (99%) were purchased from Aldrich, ethanol (GC grade) and NaCl (analytical grade) were purchased from Reachim (Ukraine). All the reagents were used without further purification. A standard stock solution of ethyl acetate, n-butyl acetate, i-butyl acetate, n-pentyl acetate and i-pentyl acetate was prepared in ethanol by weighing and contained 1.76–1.79 mg ml^{-1} of each analyte. The stock solution was stored refrigerated at +4 °C. Working standard solutions were prepared daily by diluting the stock standard solution with distilled water to the required concentrations.

Instrumentation

SPME was carried out in a 13- ml vial closed with a silicone rubber septum containing cap. The vial was positioned in a water-jacketed vessel on a magnetic stirrer (RH3, MLV, Germany) and kept at a selected temperature with a circulating water-bath (UH, MLW, Germany).

SPME was performed with a 100 μm film thickness PDMS fiber housed in its manual holder (Supelco Bellefonte, PA, USA). New fibers were conditioned under a nitrogen stream at 250 °C for 30 min.

Gas chromatography was carried out in a Chrom 5 (Czech Republic) gas chromatograph equipped with a flame ionisation detector coupled with integrator. A 1.2 m length and 3 mm i.d. glass column packed with Separon CHN (150 μm) was employed. The following gas flow rates were used: nitrogen 45, hydrogen 30 and air 300 ml min^{-1}. The temperature of the injector was 200 °C, of the detector 190 °C and of the column 140 °C.

RESULTS AND DISCUSSION

Desorption conditions

The diffusion coefficient of the analytes in the coating increases and the gas/coating distribution constant rapidly decreases with temperature increase [5]. On the other hand, volatile analytes (as are the esters studied) are readily removed from the coating at relatively low temperatures. To determine the optimal desorption temperature for the esters examined, the injector temperature ranged from 200 to 280 °C (recommended operating temperature range for the fiber used). The fiber was immersed into 5 ml of standard esters solution for 15 min at room temperature and then thermally desorbed for 2 min. As is shown in Fig. 1, the ester desorption efficiency was the same for all the temperatures studied. For the further experiments, the desorption temperature of 200 °C was selected. Higher temperatures were avoided to minimise the thermal bleed of the fiber coating.

At 200 °C the effect of desorption time on desorption efficiency was studied. Desorption times from 1 s to 60 s were investigated. The most volatile ethyl acetate is completely desorbed in 3 s. For the analytes with higher boiling points the desorption period is a bit longer, however, 10 s are sufficient for their complete desorption (Fig. 2). There-

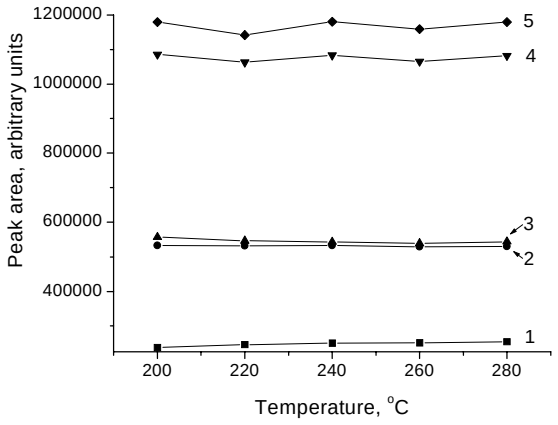

Fig. 1. Effect of desorption temperature on the peak area of *1* – ethyl acetate (81.1 μg ml^{-1}), *2* – i-butyl acetate (81.1 μg ml^{-1}), *3* – n-butyl acetate (85.5 μg ml^{-1}), *4* – i-pentyl acetate (85.1 μg ml^{-1}) and *5* – n-pentyl acetate (85.3 μg ml^{-1}). The fiber was exposed for 15 min to headspace at room temperature and desorbed for 2 min

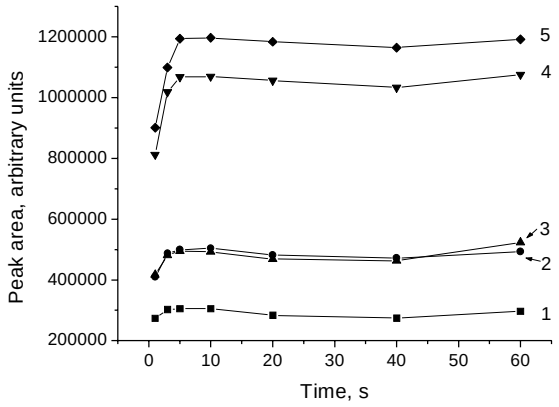

Fig. 2. Effect of desorption time on the peak area of *1* – ethyl acetate (81.1 μg ml^{-1}), *2* – i-butyl acetate (81.1 μg ml^{-1}), *3* – n-butyl acetate (85.5 μg ml^{-1}), *4* – i-pentyl acetate (85.1 μg ml^{-1}) and *5* – n-pentyl acetate (85.3 μg ml^{-1}). The fiber was exposed for 15 min to headspace at room temperature and desorbed at 200 °C

fore in the further work desorption time of 10 s was used.

Headspace SPME conditions

In order to optimise the headspace SPME, 5 ml of standard esters solution was placed into extracting vial. The SPME fiber was fixed in the headspace above the solution. The sirring rate, extraction temperature, extraction time and ionic strength of the solution were examined.

When volatile compounds are analysed by the headspace technique, most of the analytes are abundant in the headspace, which results in a relatively fast extraction even without agitation [5]. In most cases agitation is required to facilitate equilibration between the bulk of the aqueous sample and the fiber.

In our experiments, water samples were continuously agitated using a magnetic stirrer at a different stirring rate for 15 min. As is shown in Fig. 3a, a 200 rpm stirring rate is sufficient to reach constant peak areas. This stirring rate was used in all the further experiments. Higher stirring rates were not used due to sputtering, which may cause a negative effect on the reproducibility of the sorption conditions.

Optimisation of the sorption temperature was studied by exposing the SPME fiber in the headspace for 15 min. Dependence of the peak area on the temperature of the sample vial was studied. The sample temperature ranged within 20–45 °C. The plot showed that the peak area for all the compounds studied increased up to 30 °C (Fig. 4). Therefore this value was selected as the optimum temperature.

For optimum repeatability of the analysis it is necessary to adjust a proper sampling time. The

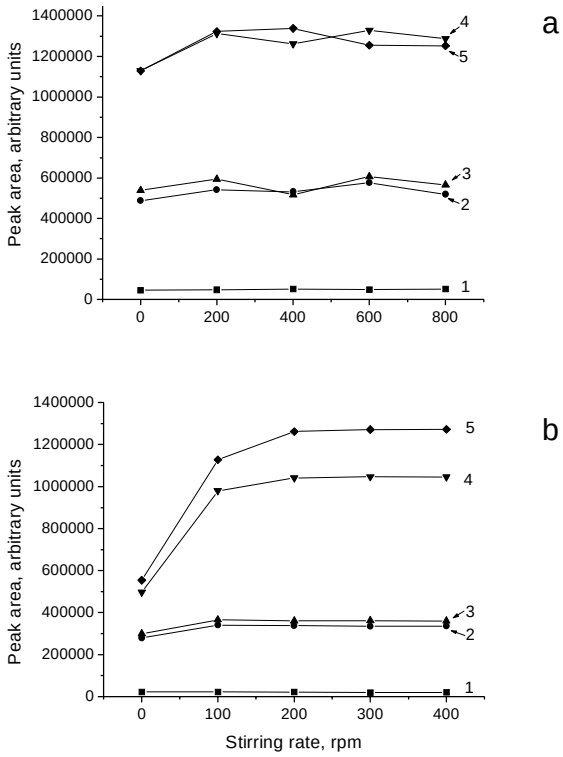

Fig. 3. Effect of stirring rate on the peak area of *1* – ethyl acetate (81.1 μg ml^{-1}), *2* – i-butyl acetate (81.1 μg ml^{-1}), *3* – n-butyl acetate (85.5 μg ml^{-1}), *4* – i-pentyl acetate (85.1 μg ml^{-1}) and *5* – n-pentyl acetate (85.3 μg ml^{-1}). *a* – Headspace SPME, *b* – direct SPME. Desorption at 200 °C for 10 s

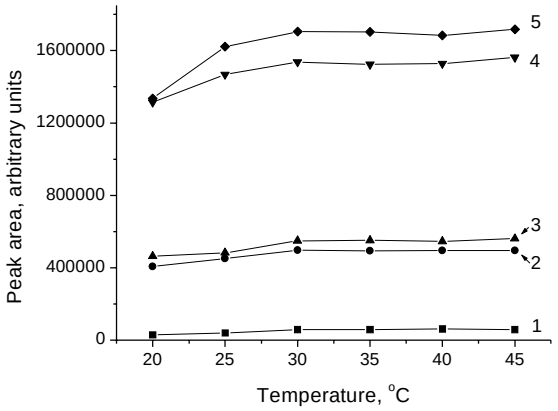

Fig. 4. Effect of extraction temperature on the peak area of *1* – ethyl acetate (81.1 μg ml^{-1}), *2* – i-butyl acetate (81.1 μg ml^{-1}), *3* – n-butyl acetate (85.5 μg ml^{-1}), *4* – i-pentyl acetate (85.1 μg ml^{-1}) and *5* – n-pentyl acetate (85.3 μg ml^{-1}). Sample stirring rate was 200 rpm. The fiber was exposed to headspace for 15 min and desorbed at 200 °C for 10 s

equilibration period was examined by exposing the fiber to the headspace for different periods of time at 30 °C. Ten minutes were a sufficient extraction time for all the analytes investigated (Fig. 5a).

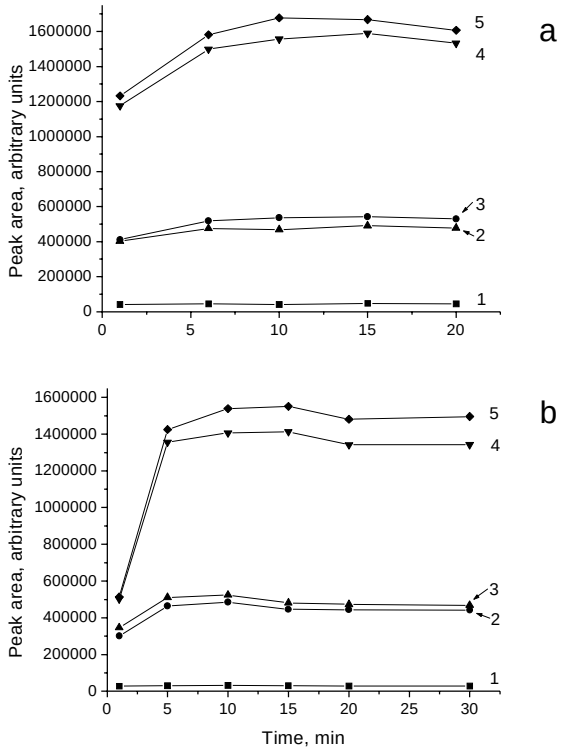

Fig. 5. Effect of extraction time on the peak area of *1* – ethyl acetate (81.1 μg ml^{-1}), *2* – i-butyl acetate (81.1 μg ml^{-1}), *3* – n-butyl acetate (85.5 μg ml^{-1}), *4* – i-pentyl acetate (85.1 μg ml^{-1}) and *5* – n-pentyl acetate (85.3 μg ml^{-1}). *a* – headspace SPME at 30 °C, *b* – direct SPME at room temperature. Sample stirring rate was 200 rpm. Desorption at 200 °C for 10 s

Next, a study of the influence of ionic strength on the extraction was performed, because the increase of the ionic strength improves the extraction efficiency in SPME [7]. To increase the ionic strength, we added NaCl which is commonly used for this purpose. To the standard esters solution, different portions up to saturation of NaCl were added. From the curves presented in Fig. 5a it is evident that the addition of NaCl enhances the extraction efficiency. In further experiments, 0.4 g ml^{-1} of NaCl was added.

Direct SPME conditions

In the direct extraction mode, the fiber is immersed directly into the sample solution and the analytes are transferred directly from the sample matrix to the extracting phase. As in the case of headspace SPME stirring rate of the solution, the extraction time and ionic strength of the solution were examined. Extraction at elevated temperatures was not studied, because there was no need to transfer the analytes into the headspace. Ten millilitres of standard solution was used for the experiments.

In the case of direct SPME, an efficient stirring of the solution is extremely important in order to reduce the effect caused by the "depletion zone". This zone is formed close to the fiber as a result of fluid shielding and small diffusion coefficients of analytes in liquid matrices [5]. For stirring rate studies, the water samples were continuously agitated for 15 min at 100–400 rpm (Fig. 3b). Above 200 rpm the peak areas were constant, therefore this stirring rate was used in the further experiments.

The sampling time was examined exposing the fiber to a solution stirred at 200 rpm for up to 30 min. As is shown in Fig. 5b, a 10 min extraction time is sufficient to reach an equilibrium between the solution and the fiber.

As in the case of headspace SPME, the ionic strength of the solution was modified by addition of NaCl. To 10 ml of the standard esters solution up to 4 g (0.4 g ml^{-1}) of NaCl was added. The addition of NaCl enhances extraction efficiency (Fig. 6b), so as in the case of headspace SPME for further work, 0.4 g ml^{-1} of NaCl was used.

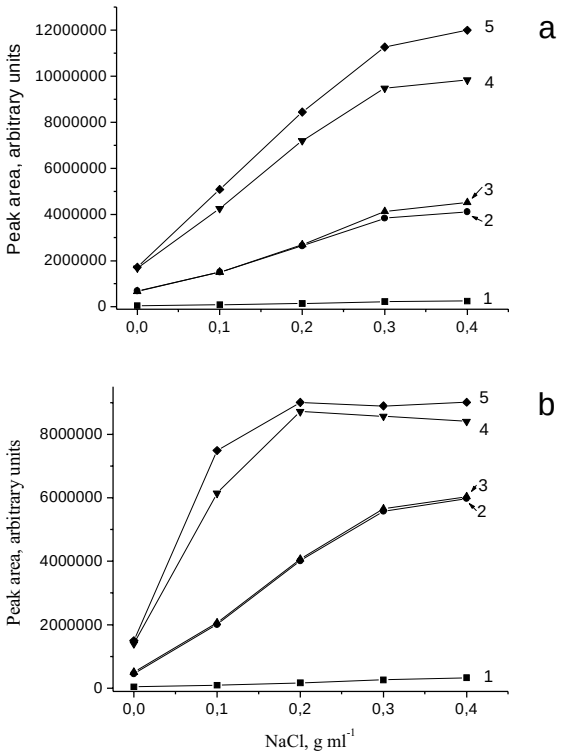

Fig. 6. Effect of NaCl content on the peak area of *1* – ethyl acetate (81.1 μg ml^{-1}), *2* – i-butyl acetate (81.1 μg ml^{-1}), *3* – n-butyl acetate (85.5 μg ml^{-1}), *4* – i-pentyl acetate (85.1 μg ml^{-1}) and *5* – n-pentyl acetate (85.3 μg ml^{-1}). *a* – headspace SPME at 30 °C, *b* – direct SPME at room temperature. Sample stirring rate was 200 rpm. Desorption at 200 °C for 10 s

Comparison of headspace and direct SPME

The quality parameters of the SPME methods such as linearity, repeatability and limits of detection (LOD) were calculated under the optimised condi-

tions described above. For the headspace SPME, the linear ranges for the determination of the esters studied were within 0.16 mg ml^{-1} (except ethyl acetate 0.36 mg ml^{-1}). Using the direct SPME, the linear ranges for the most of the analytes studied were smaller and were within 0.08 mg ml^{-1}, and only for n-pentyl acetate and i-pentyl acetate they were within 0.16 mg ml^{-1}. For all the analytes good linearities were observed, with correlation coefficients > 0.996 (n = 7). Limits of detection were defined as the concentration of the analyte that produces a peak three times higher than the baseline noise. As is shown in Table 1, LOD for the first three analytes in the both methods studied are comparable. For n-pentyl acetate and i-pentyl acetate lower LOD were obtained using headspace SPME.

In order to calculate the concentration factor, the limits of detection without SPME were also determined by direct injection of 2 µl of standard solutions of esters. Comparison of the detection limits obtained by direct GC and GC hyphenated with SPME showed that for ethyl acetate, which is rather soluble in water (solubility in water is 8.6 g/100 ml at 25 °C), LOD were of the same order. On the other hand, for i-pentyl acetate and n-pentyl acetate (solubility in water is about 0.1 g/100 ml at 25 °C) in the case of headspace SPME more than a 20-fold preconcentration was achieved. So the combination of SPME with GC allows much smaller LOD and is a promising technique for the detection of analytes with a low water solubility.

Table 1. **Limits of detection for headspace SPME and direct SPME of esters**

Compound	LOD, µg l^{-1}	
	Headspace SPME	Direct SPME
Ethyl acetate	660	494
n-Butyl acetate	135	203
i-Butyl acetate	625	179
n-Pentyl acetate	77.9	289
i-Pentyl acetate	84.3	506

The repeatability of the methods was calculated for two different concentrations analysing five replicate samples. Relative standard deviations (RSDs) are listed in Table 2. Headspace SPME demonstrated a better repeatability. In most cases RSDs were higher at a lower analyte concentration.

The optimised techniques were applied for analysis of white apple wine (Anykščių vynas, Lithuania). A chromatogram of the wine obtained after headspace SPME is presented in Fig. 7. The analyte concentrations were determined by the standard addition method using both direct SPME and headspace SPME and were 52.21 ± 8.52 mg l^{-1} for ethyl acetate, 7.12 ± 0.87 mg l^{-1} for i-butyl acetate and 1.16 ± 0.18 mg l^{-1} for i-pentyl acetate. To make sure that the peaks really represented the esters mentioned, chromatographic separation was carried out using a packed column with another stationary phase (Separon SDA). Addition of standard solutions confirmed the presence of the analytes.

Table 2. **Repeatabilities for headspace SPME and direct SPME of esters (n = 5, P = 0.95)**

Compound	Concentration, µg ml^{-1}	RSD, % (n = 5)	
		Headspace SPME	Direct SPME
Ethyl acetate	81.1	3.3	7.1
	2.46	5.6	14
n-Butyl acetate	85.5	5.6	6.0
	2.59	5.5	7.3
i-Butyl acetate	81.1	4.8	5.5
	2.46	3.4	4.5
n-Pentyl acetate	85.3	3.6	8.7
	2.58	8.9	18
i-Pentyl acetate	85.1	5.3	8.5
	2.58	5.0	14

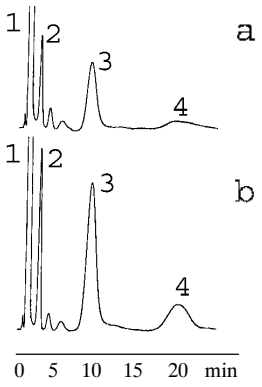

Fig. 7. The chromatogram of: *a* – white apple wine (Lithuania) and *b* – of the same wine spiked with esters. *1* – ethanol, *2* – ethyl acetate, *3* – i-butyl acetate, *4* – i-pentyl acetate. The fiber was exposed to headspace at at 30 °C for 10 min. Sample stirring rate was 200 rpm. Desorption at 200 °C for 10 s

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ESTERIŲ KIETAFAZĖ MIKROEKSTRAKCIJA: EKSTRAKCIJOS IŠ VIRŠERDVĖS IR TIESIOGINĖS EKSTRAKCIJOS PALYGINIMAS

S a n t r a u k a

Pasiūlyti ir palyginti kietafazės mikroekstrakcijos iš viršerdvės ir tiesioginės kietafazės mikroekstrakcijos metodai etilo acetatui, n-butilo acetatui, i-butilo acetatui, n-pentilo acetatui ir i-pentilo acetatui nustatyti. Kietafazė mikroekstrakcija atlikta strypeliu, padengtu 100 μm storio polidimetilsiloksano sluoksniu. Optimalios kietafazės mikroekstrakcijos iš viršerdvės ir tiesioginės kietafazės mikroekstrakcijos sąlygos yra šios: ekstrakcijos trukmė 10 min, tirpalo maišymo greitis 200 apsisukimų per minutę. Kietafazė mikroekstrakcija iš viršerdvės buvo atliekama 30°C, o tiesioginė kietafazė mikroekstrakcija – kambario temperatūroje. Analitės desorbuojamos 10 s 200°C temperatūroje. Nustatytos analičių aptikimo ribos, įvertintas rezultatų pasikartojamumas. Pasiūlytos metodikos gali būti pritaikytos vyno analizei.