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Headspace extraction of alcohols into a single drop

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The possibility of applying headspace microextraction into a single drop for the determination of alcohols in aqueous solutions is demonstrated. A drop of ethylene glycol containing butan-2-one as an internal standard is used for extraction. The analytes are extracted by suspending a 1 μl extracting drop directly from the tip of a microsyringe fixed above an extraction vial with a septum such that the needle passes through the septum and the needle tip appears above the surface of the solution. After the extraction is finished the drop is retracted back into the needle and injected directly into a GC column. Optimization of experimental conditions (sampling time, sampling temperature, stirring rate and ionic strength of the solution) with respect to the extraction efficiency were investigated and the linear range and the precision were examined. This headspace single drop microextraction method was applied to the analysis of beer.

Introduction

The most commonly used extraction techniques such as liquid–liquid and solid-phase extraction have several significant disadvantages. The major disadvantage of liquid–liquid extraction is the use of large volumes of expensive, toxic, high-purity organic solvents. Also, it is extremely time consuming. The requirements for solid-phase extraction solvents are less stringent than those for liquid–liquid extraction, but they are not eliminated.[1] Because of the disadvantages of conventional extraction techniques, solvent free sample preparation methods or those employing less organic solvents are becoming more and more important.

Solid-phase microextraction (SPME) was proposed in 1989 by Belardi and Pawliszyn.[2] For SPME, a small-dimension fused-silica fiber coated with a high-temperature phase is applied. The analytes are adsorbed on the fiber and the fiber is inserted directly into the injector of a gas chromatograph for thermal desorption. This technique eliminates most drawbacks of conventional extraction techniques. It requires no solvents, is experimentally simple and fast and the sampling can be carried out directly under field conditions or on-line. SPME can also be coupled with HPLC or capillary electrophoresis, but in this case a solvent desorption step is needed.[3]

Recently, Jeannot and Cantwell proposed solvent extraction from water into an 8 μl drop of organic solvent located at the end of a Teflon rod.[4] Then the liquid phase microextraction (LPME) method was simplified by suspending a drop directly from the tip of a microsyringe needle immersed in the aqueous phase.[5–9] He and Lee[10] investigated static and dynamic LPME from water.

Comparison of SPME and LPME showed that the two techniques are comparable in terms of precision, sensitivity and analysis time.[4] SPME has the advantage that there is no solvent peak in the gas chromatogram. On the other hand, SPME lifetime is limited as the solid-phase materials degrade with usage. Desorption of the analyte from the fiber in the GC injector is slower than conventional solvent evaporation and sometimes leads to analyte peak tailing. Moreover, the partial loss of the SPME fiber stationary phase can result into peaks that may co-elute with the analytes, thus affecting accuracy and precision. When SPME is coupled with HPLC, special equipment for a solvent desorption step is required and sometimes a lengthy process is needed to recover all sorbed analytes and to avoid carry-over. Liquid microextraction overcomes the problems of the fiber degradation and relatively slow desorption when coupled with GC. With HPLC it can be used without any additional desorption step. Solvent microextraction can be performed with the simplest of devices, a conventional microsyringe, whereas the apparatus employed in SPME is more elaborate and expensive. Moreover, although the variety of commercially available SPME fibers is constantly increasing, the choice of solvents for liquid microextraction is much broader and there are more possibilities for optimizing the extraction conditions.

The easy adaptability of SPME system to headspace analysis enables complex matrices to be extracted selectively. However, because the coatings available are non-polar or slightly polar, the current applications are mostly limited to non-polar compounds or compounds of medium polarity[11] and there is relatively little information about the SPME of polar compounds.[12] Even from this point of view, because of a wide choice of polar extraction solvents, headspace LPME seems to be an attractive technique. Moreover, the solvents need not even be water immiscible as in direct LPME from aqueous solutions. Headspace LPME can also be applied for volatile analytes determination in solid matrices.

Recently, the use of a liquid drop has been reported for sampling of gas streams. Dasgupta *et al*. discussed the collection and determination of trace gases using a static[13] or dynamically falling[14] drop. However, to our knowledge, there have been no reports concerning the adaptation to headspace analysis of solvent extraction into a single drop.

In this work, an attempt was made to apply headspace LPME into a single drop for the determination of polar organic compounds. Low molecular weight alcohols were used as target analytes.

Experimental

Reagents

Methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-1-ol, pentan-2-ol, butan-2-one and ethylene glycol were of analytical-reagent grade and were used without further purification. A stock standard solution of methanol, ethanol,

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propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-1-ol and pentan-2-ol was prepared by weighting of 0.75–0.85 mg of each analyte. The stock standard 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.

The organic extractant was ethylene glycol containing a fixed concentration of butan-2-one.

Instrumentation

Single drop microextraction was performed in a 13 ml vial closed with a silicone rubber septum placed in the cap. The vial was placed in a water-jacketed vessel on a magnetic stirrer (RH3, MLW, Germany) and maintained at a desirable temperature with a circulating water-bath (UH, MLW).

Single drop microextraction was performed with a commercially available 10 μl microsyringe (Hamilton Microliter 700). During the extraction the syringe was fixed above the extraction vial with a septum such that the needle passed through the septum and the needle tip was located about 1 cm above the surface of the solution. Then a drop of the extraction solvent was suspended from the needle tip. After the extraction was finished the drop was retracted back into the needle and injected directly into the GC column.

Gas chromatography was carried out with a Chrom 5 (Czech Republic) gas chromatograph equipped with a flame ionization detector coupled with an integrator. A glass column of 2.5 m × 3 mm id packed with Separon SDA (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 and of the detector was 190 °C. The column temperature program was initial temperature 150 °C held for 26 min, then increased to 190 °C at 20 °C min^{-1} and held at 190 °C for 8 min.

Results and discussion

The extraction solvent had to conform to two requirements: to extract analytes well and to be separated from the analyte peaks in the chromatogram. Three solvents differing in polarity, decane, *o*-xylene and ethylene glycol, were tested. Preliminary trials showed that ethylene glycol gave the best extraction efficiency so it was chosen as the extraction solvent in further work. In order to correct for variable injection volumes butan-2-one was used as an internal standard. The analytical signal was taken as the peak area ratio of analyte to butan-2-one. A chromatogram of the standard solution alcohols after headspace extraction with a drop of ethylene glycol containing butan-2-one as internal standard is presented in Fig. 1.

Sampling temperature

We expected that an increase in sampling temperature would increase the absorption of the analytes on an ethylene glycol drop because of the increase in analyte concentration in the headspace. The effect of sampling temperature was studied by exposing an extracting drop for 15 min in the headspace at 20–80 °C. Measurements were performed on aqueous solutions containing 950–1000 μg ml^{-1} of each analyte. The extraction curves showed that the amount of the analytes absorbed increases with increase in temperature up to 60–70 °C (Fig. 2). This can be explained by the fact that at higher temperature the vapor pressure of the analytes and hence their concentrations in headspace increase. Above the temperature mentioned the amount of the analytes extracted decreases, probably because the partition coefficients to the extraction phase decrease. Hence the optimum sampling temperature for a fixed extraction time of 15 min was 60 °C.

Stirring rate

An equilibrium between the aqueous and vapor phases can be achieved more rapidly by stirring the aqueous sample. In the case of headspace sampling, it is essential to obtain a linear relationship between the concentrations of analytes in the water and in the vapor phase. In our experiments water samples were continuously agitated at 60 °C at different stirring rates with a magnetic stir bar on a stir plate. As can be seen in Fig. 3, the peak areas of all the analytes increase with increase in stirring rate up to 600 rpm. At 800 rpm the peak areas of some analytes remain stable and those of the rest of the analytes continue to increase. Higher stirring rates were not used because of spattering, which damaged the drop. Hence in further work a stirring rate of 600 rpm was chosen.

Sampling time

For optimum repeatability of the analysis, it is necessary to choose a time in which equilibrium between the extracting liquid and the headspace and water is reached. The equilibrium was examined by exposing the solvent drop to the headspace for an absorption time of up to 50 min. As can be seen in Fig. 4, the

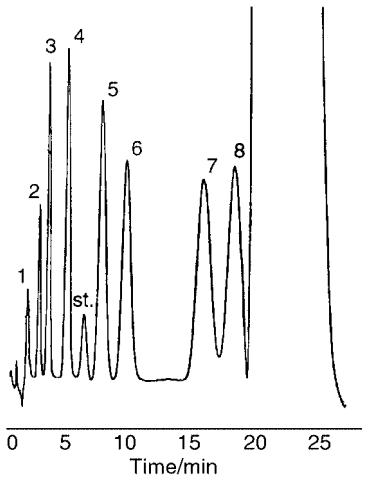

Fig. 1 Chromatogram of a standard solution of alcohols. (1) Methanol, (2) ethanol, (3) propan-2-ol, (4) propan-1-ol, (5) butan-2-ol, (6) butan-1-ol, (7) pentan-2-ol, (8) pentan-1-ol and (st.) butan-2-one.

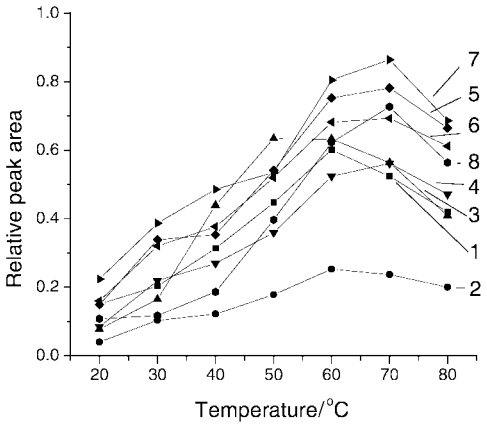

Fig. 2 Effect of sampling temperature on the relative peak areas of (1) methanol, (2) ethanol, (3) propan-2-ol, (4) propan-1-ol, (5) butan-2-ol, (6) butan-1-ol, (7) pentan-2-ol and (8) pentan-1-ol. The ethylene glycol drop was exposed to the headspace of 5 ml of aqueous solution for 15 min.

relative peak areas increase with increase in exposure time without reaching equilibrium. One of the possible reasons may be the slow equilibrium rate between the headspace and the drop. On the other hand, we noticed that with increase in the exposure time the drop volume increases significantly and after a 50 min exposure time it grew from an initial 1.9 μl (1 μl in the syringe glass barrel and 0.9 μl in the needle) to 5.4 μl. This is because ethylene glycol is hydrophilic and together with the analytes also absorbs water. Moreover, at longer sampling times the ethylene glycol drop, because of the gravity force, in many cases lost touch with the needle. Because of the increase in the drop size during the sampling, it was also not possible to increase the initial drop volume. Using 2 μl of ethylene glycol in the syringe barrel, after a 15 min exposure time the volume of the drop became too large to remain fixed to the needle tip and in two cases out of three the drop became detached. However, it is not necessary to reach equilibrium provided that the extraction conditions are reproduced. Therefore, in further work a compromise expose time of 15 min that allowed incidents of drop detachment to be avoided was chosen, even though the analytes had not reached equilibrium.

Ionic strength of solution

The addition of salt often improves the extraction of analytes in SPME.[15] On the other hand, it has been shown[9,16] that in the case of LPME from water the extraction efficiency decreases with increase in salt concentration. It was of interest to examine the influence of salt concentration on the efficiency of headspace LPME.

The ionic strength of solution was modified by addition of NaCl, which is commonly used for this purpose. To 5 ml of water solution, 0.15–0.5 g ml^{-1} of NaCl were added. The plot of relative peak area *versus* amount of NaCl added is shown in Fig. 5. It is evident that the addition of NaCl promotes the transport of the analytes to the headspace and hence to the extracting drop. This can be explained by the fact that water molecules form hydration spheres around the salt ions. These hydration spheres reduce the concentration of water available to dissolve analyte molecules; hence it is expected that this will drive additional analytes into the extraction phase.[15] However, at NaCl concentrations above 0.4 g ml^{-1} the extraction efficiency did not change any further. This can be explained by the fact that the solubility of NaCl at 60 °C is <0.4 g ml^{-1}. Therefore, in further work saturated salt conditions with an NaCl concentration of 0.4 g ml^{-1} were chosen.

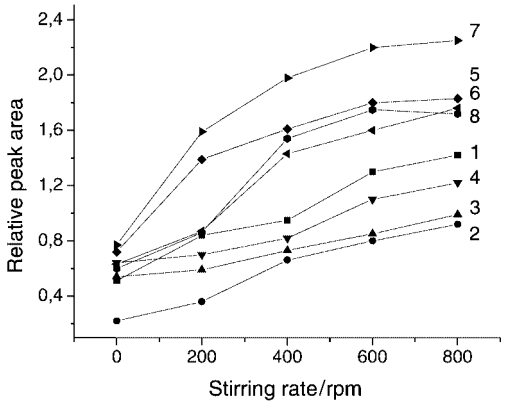

Fig. 3 Effect of stirring rate on the relative peak areas of (1) methanol, (2) ethanol, (3) propan-2-ol, (4) propan-1-ol, (5) butan-2-ol, (6) butan-1-ol, (7) pentan-2-ol and (8) pentan-1-ol. The ethylene glycol drop was exposed to the headspace of 5 ml of aqueous solution for 15 min at 60 °C.

Precision, linearity and detection limits

The linear response range was examined on 5 ml of aqueous solutions of alcohols. A 2 g amount of NaCl was added to the extraction vial before the analysis and sampling with 1 μl of ethylene glycol at 60 °C and a 600 rpm stirring rate for 15 min was carried out. The linear ranges for all the alcohols investigated were within 1.5 mg ml^{-1}. Correlation coefficients of the linear calibration graphs were 0.997–0.999 (n = 6), except for 0.987 for methanol. The detection limits for methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-1-ol and pentan-2-ol were 52, 8.4, 3.8, 4.9, 9.5, 6.3, 11.8 and 6.4 μg ml^{-1}, respectively. Although these detection limits are rather high, they probably could be significantly decreased by using more elaborate GC equipment.

The repeatabilities were calculated for five replicate measurements on standard solutions with two different concentrations of analytes (Table 1). For higher concentrations the RSDs (except for methanol) did not exceed 6%. For lower analyte concentrations the RSDs were up to 10%, except for 18.8% for methanol.

Application

The developed method was applied to the analysis of beer. Ethanol, propan-1-ol, butan-2-ol and pentan-2-ol were detected.

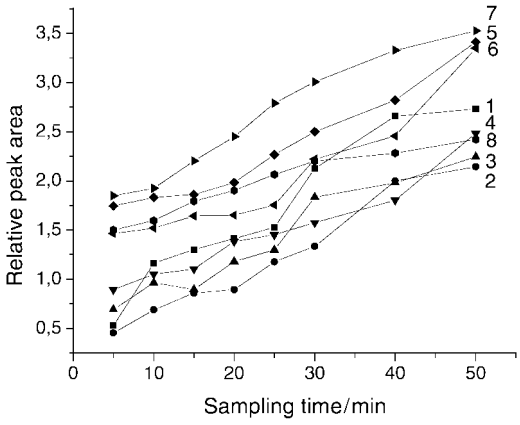

Fig. 4 Effect of sampling time on the relative peak areas of (1) methanol, (2) ethanol, (3) propan-2-ol, (4) propan-1-ol, (5) butan-2-ol, (6) butan-1-ol, (7) pentan-2-ol and (8) pentan-1-ol. The ethylene glycol drop was exposed to the headspace of 5 ml of aqueous solution at 60 °C at a stirring rate of 600 rpm.

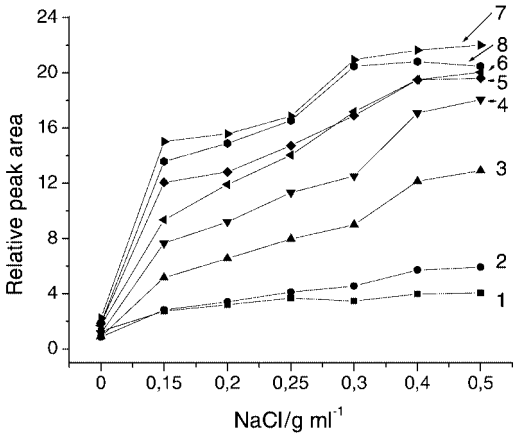

Fig. 5 Effect of addition of NaCl on the relative peak areas of (1) methanol, (2) ethanol, (3) propan-2-ol, (4) propan-1-ol, (5) butan-2-ol, (6) butan-1-ol, (7) pentan-2-ol and (8) pentan-1-ol. The ethylene glycol drop was exposed to the headspace of 5 ml of aqueous solution for 15 min at 60 °C at a stirring rate of 600 rpm.

The analyte concentrations were determined by the standard additions method and were 78 mg ml^{-1} for ethanol and 18, 48 and 192 μg ml^{-1} for propan-1-ol, butan-2-ol and pentan-2-ol, respectively. Ethanol was determined after a 100-fold dilution of beer; for the other analytes no dilution was required. To make sure that the peaks really represented the alcohols mentioned, the chromatographic resolution of the sample was carried out on a packed column with a liquid stationary phase, Separon CHN. Even in this case addition of standard solution showed the presence of the analytes.

Table 1 Repeatability of determination of alcohols by headspace LPME in aqueous solutions (n = 5)

Compound	Concentration/μg ml^{-1}	Repeatability (%)
Methanol	1020	10.6
	204	18.8
Ethanol	978	5.8
	196	10.0
Propan-2-ol	947	5.1
	189	8.6
Propan-1-ol	949	4.5
	190	7.8
Butan-2-ol	955	5.9
	191	9.6
Butan-1-ol	950	5.9
	190	8.0
Pentan-2-ol	962	6.0
	192	9.1
Pentan-1-ol	980	4.6
	196	9.4

This novel headspace LPME method selectively extracted the compounds of interest and so could be applied to the analysis of real samples even when followed by GC analysis with packed columns. We also believe that the approach used here can easily be expanded to the determination of other compounds and in combination with other determination techniques such as HPLC.

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