CHEMIJA. 2007. Vol. 18. No. 3. P. 25–29

Single-drop microextraction for the determination of phthalate esters

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A possibility to apply direct microextraction into a single drop for the determination of some phthalates has been demonstrated. A drop of toluene containing nonadecane as an internal standard was used for the extraction. The analytes were extracted by suspending an extracting drop directly from the tip of a microsyringe fixed above the extraction vial so that the needle could pass the septum, and the needle tip appeared below the surface of the solution. After the extraction, the drop was retracted back into the needle and injected into the GC. Optimisation of the experimental conditions (sampling time, stirring rate and ionic strength of the solution) with respect to the extraction efficiency was accomplished. The method suggested was evaluated in terms of repeatability, detection limits and linear response range. The technique was tested for the analysis of cologne, shaving lotion and distilled water.

Key words: single drop microextraction, gas chromatography, phthalate esters

INTRODUCTION

Phthalates are esters of phthalic acid with a structure presented in Fig. 1. For many years phthalates have been used as plastifying agents, mainly to make polyvinyl chloride supple and flexible. They are present in the environment, food samples, medical devices, perfume and cosmetics [1]. Phthalates tend to bioaccumulate, they are poorly degradable and toxic. They can cause short-term effects such as allergies, astmas or long-term effects such as disruptions in nervous and endocrine systems, increased risk of cancer, decrease of fertility, disruptions in children development [2]. Because of their wide use and toxicity, monitoring of phthalates is of considerable importance. Gas chromatography and high performance liquid chromatography are commonly used to detect phthalates. However, low concentrations of phthalates require a pre-concentracion step, and determination of phthalates in complex matrices requires a selective extraction of the analytes.

Traditional extraction techniques, such as liquid–liquid extraction and solid-phase extraction have several significant disadvantages. The major disadvantage of the liquid–liquid extraction is the use of large volumes of expensive, toxic, high-purity organic solvents. Also, it is extremely time consuming. Requirements for the solid-phase extraction solvents are reduced if compared with the liquid–liquid extraction, but they are not eliminated [3].

Because of the disadvantages of conventional extraction techniques, microextraction techniques are gaining a growing interest. In 1996 Jeannot and Cantwell proposed a single-drop microextraction (SDME) based on the extraction of analytes from water into a drop of an organic solvent [4]. In the simplest

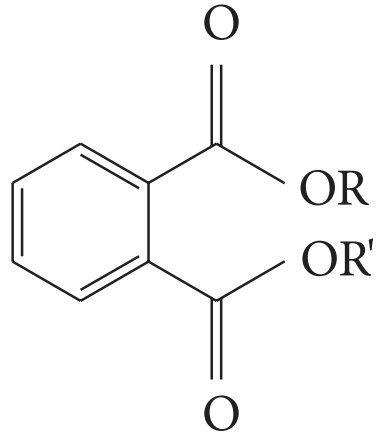

Fig. 1. General formula for phthalates

version of the SDME method, a drop is suspended directly from the tip of a microsyringe needle immersed into the aqueous phase [5–9] or held in the headspace [10–14]. The method is very uncostly and simple, carry-over free, uses especially small quantities of organic solvents (up to a few μL), the choice of organic solvents is broad and it gives many possibilities to optimize the extraction conditions. In the case of headspace SDME, the solvents should not even be water immiscible as in the case of direct LPME from water solutions. Headspace LPME should also be applied for the determination of volatile analytes in solid matrices. LPME has been applied for the determination of a wide variety of analytes, such as organophosphorus pesticides, aromatic hydrocarbons, phenols, aldehydes, alcohols, esters, etc. [6, 9, 10, 13, 15–18]. However, there is only one article dealing with the applicability of SDME to the extraction of phthalates [19]. The authors apply a mixture of three solvents as an extracting droplet. In this work we have simplified the LPME of phthalates by using one organic solvent.

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EXPERIMENTAL

Reagents and solutions

Dimethyl phthalate (DMP) (99%), diethyl phthalate (DEP) (99%), dibutyl phthalate (DBP) (99%), benzyl-*n*-butyl phthalate (BBP) (98,2%), diethylhexyl phthalate (DEHP) (99,8%), di-*n*-octyl phthalate (DOP) (98%) and di-*n*-nonyl phthalate (DNP) (98%) were purchased from Alfa Aesar. Acetone (99,9%), *n*-octane (98%), tetradecane (99%), heptadecane (99%), nonadecane (99%), toluene (99%), *p*-xylene (98%) and amyl acetate (98%) were purchased from Sigma-Aldrich. NaCl (analytical grade) was purchased from Reachim (Ukraine). All the reagents were used without further purification.

A standard stock solution of the analytes (DMP, DEP, DBP, BBP, DEHP, DOP and DNP) of a concentration $1*10^{-2}$ mol L^{-1} was prepared in acetone. The stock solution was stored refrigerated at +4 °C. Working standard solutions were prepared just before use by diluting the stock standard solution with distilled water to the required concentrations.

Instrumentation

Single-drop microextraction was performed in a 13 ml volume vial closed with a silicone rubber septum placed in the cap. The vial was placed on a magnetic stirrer (MLW RH3, Germany). Single-drop microextraction was performed with a commercially available 10 μl microsyringe (Hamilton Microliter 700 series syringe). During the extraction, the syringe was fixed above the extraction vial so that the needle could pass the septum, and the needle tip appeared about 1 cm under the surface of the solution. Then a drop of the extracting solvent was suspended from the needle tip. After the extraction, the drop was retracted back into the needle and injected directly into the GC.

Gas chromatography was carried out in a Varian 3400 gas chromatograph equipped with a flame ionisation detector coupled with an integrator SP4290 (Spectra-Physics) and two connected fused silica capillary columns – HP-5 (5% Ph Me Silicone) (10 m × 0.53 mm, 2.65 μm in film thickness) and HP-17 (croslinked 50% Ph Me Silicone) (10 m × 0.53 mm, 2.0 μm in film thickness). The injector's temperature was 280 °C and the detector's temperature was also 280 °C. The oven temperature was programmed as follows: it was initially set at 100 °C for 4 min, then gradually ramped to 150 °C (10 °C min^{-1}), 230 °C (5 °C min^{-1}), to 280 °C (3 °C min^{-1}) and held for 10 min. The following gas flow rates were used: carrier (nitrogen) 10, make-up gas 20, hydrogen 30 and air 300 mL min^{-1}.

RESULTS AND DISCUSSION

The extracting solvent had to meet two requirements: to extract the analytes quite well and to be separated from the analyte peaks in the chromatogram. Four different solvents, *n*-octane, toluene, *p*-xylene and amyl acetate, were tested for the extraction of phthalates. Toluene and *p*-xylene showed the best extracting efficiency. However, toluene had a shorter retention time and, thus, its peak was better separated from the analyte peaks. So, toluene was chosen as an extracting solvent for the further work. In order to correct variable injection volumes, an internal standard was required. For this reason, tetradecane, heptadecane and nonadecane were tested. Nonadecane was the best one because it was eluted between the analytes, and its peak was well separated from the analyte peaks. An analytical signal was taken as the peak area ratio of the analyte to nonadecane. A chromatogram of a standard phthalate solution containing nonadecane as an internal standard is presented in Fig. 2.

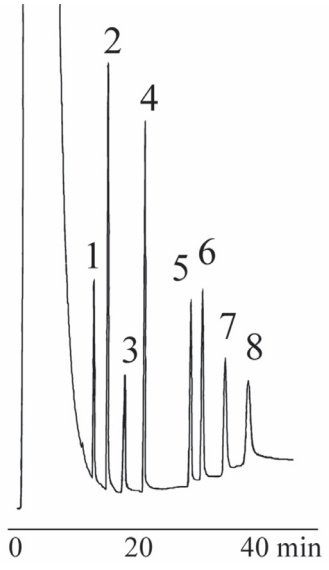

Fig. 2. Chromatogram of standard solution of phthalates. (1) DMP, (2) DEP, (3) nonadecane, (4) DBP, (5) BBP, (6) DEHP, (7) DOP and (8) DNP. Concentration of each compound in the sample: $1*10^{-3}$ mol L^{-1}. GC conditions: injector's temperature was 280 °C, detector's temperature was 280 °C. Oven temperature: 100 °C for 4 min, ramped to 150 °C (10 °C min^{-1}), 230 °C (5 °C min^{-1}), to 280 °C (3 °C min^{-1}) and held for 10 min. Gas flow rates: carrier (nitrogen) 10, make-up gas 20, hydrogen 30 and air 300 mL min^{-1}

Extraction conditions

Preliminary experiments showed that even at elevated temperatures the sensitivity of the headspace SDME was rather low and there was no pre-concentration of the analytes. Evidently, the volatility of phthalates is too low (boiling ranges are 282–370 °C) and thus their concentrations in the headspace are negligible. For this reason, phthalates were extracted using direct SDME.

Equilibrium between the aqueous and organic phases can be achieved more rapidly by agitating the aqueous sample. On the other hand, fast stirring rates can result in dislodgment of the organic drop from the needle tip. In our experiments, water samples were continuously agitated at room temperature at different stirring rates with a magnetic stirring bar using a 1 μL toluene drop. The extraction was carried out for 15 min. With an increase in the stirring rate, the peak areas of the analytes increased. However, at stirring rates exceeding 600 rpm the stability of the drop was poor. So, for the further experiments 600 rpm stirring rate was chosen.

Solvent microextraction is not an exhaustive extraction method and the analytes are partitioned between the bulk aqueous phase and the organic microdrop. The total amount of the analytes transferred in the drop reaches its maximum when the equilibrium between the two immiscible phases is established. As can be seen in Fig. 3, the analytes studied in our case did not reach the equilibrium even after 60 min. In order to have an acceptable analysis time, for the further work we chose non-equilibrium conditions and established a thirty-minute extraction time constantly maintaining the extraction time precisely the same.

Literary data on the influence of ionic strength on the extraction efficiency are contradictory. Some authors state that with an increase in the ionic strength, the extraction efficiency decreases [20, 21], others [22], on the contrary, state that it in-

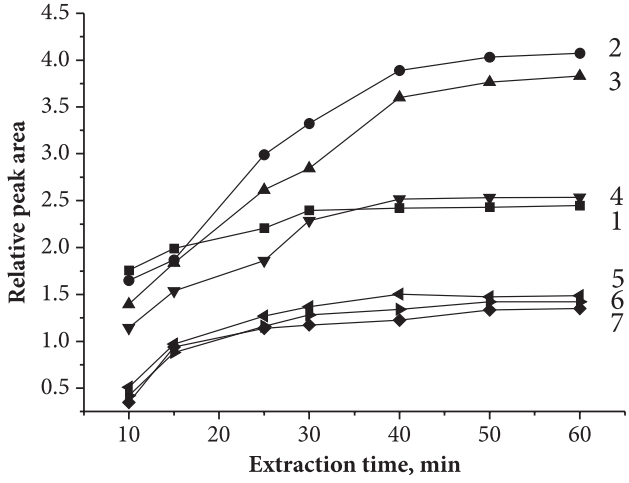

Fig. 3. Effect of the extraction time on the peak area of (1) DMP, (2) DEP, (3) DBP, (4) BBP, (5) DEHP, (6) DOP and (7) DNP. Concentration of each compound in the sample: $1*10^{-5}$ mol L^{-1}. Direct SDME at room temperature, solution stirring rate 600 rpm

creases. So, we had to examine this dependence for the case of the phthalates studied.

The ionic strength of the solution was modified by adding NaCl that is commonly used for this purpose. To 10 ml of water solution from 0.1 to 0.4 g mL^{-1} of NaCl was added. The plot of relative peak areas vs. the amount of NaCl added is shown in Fig. 4.

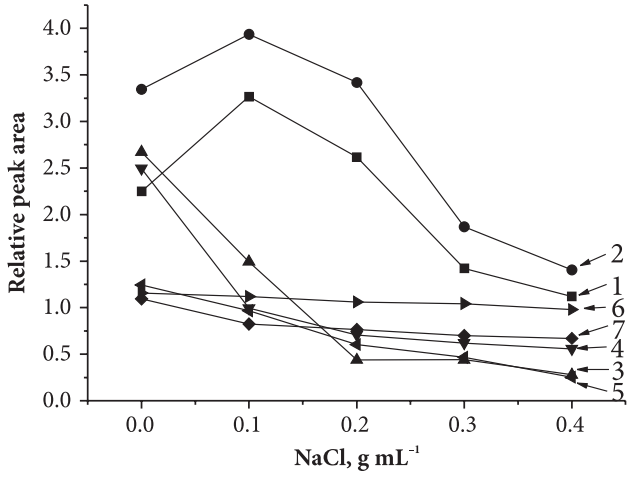

Fig. 4. Effect of NaCl on the peak area of (1) DMP, (2) DEP, (3) DBP, (4) BBP, (5) DEHP, (6) DOP and (7) DNP. Concentration of each compound in the sample: $1*10^{-5}$ mol L^{-1}. Direct SDME for 30 min at room temperature, solution stirring rate 600 rpm

For most of the analytes at the presence of NaCl, the peak areas decreased. Only for DMP and DEF small quantities of NaCl promoted the transport of the analytes to the extracting drop. However, with the further increase of NaCl, the concentration and the quantity of DMP and DEP in the droplet diminished. An explanation for this may be that the dissolved NaCl may have changed the physical properties of Nernst diffusion film and reduced the rate of diffusion of the analytes into the drop [21, 23]. Thus, the extraction efficiency depends on two concurrent effects – diffusion decrease and salting-out effect. Likely, for larger molecules the diffusion into the drop is impeded more than for smaller ones.

Quality parameters

Linear response ranges for the direct single-drop microextraction were examined. Extraction conditions were the following: direct SDME was carried out from 10 mL of aqueous solution into 1 μL toluene drop for 30 minutes at room temperature at 600 rpm stirring rate. The linear ranges for all the phthalates investigated were up to $1*10^{-5}$ mol L^{-1}. The correlation coefficients for all the analytes were 0.997–0.999 (n = 6). The limits of detection, defined as three times of base-line noise are presented in Table 1. The repeatabilities were determined by the analysis of five replicates. Relative standard deviations (RSDs) are listed in Table 2.

Table 1. **Detection limits of phthalate esters, mol L^{-1}**

Analyte	Detection limit, mol L^{-1}
DMP	$5.0 \cdot 10^{-7}$
DEP	$1.0 \cdot 10^{-7}$
DBP	$7.5 \cdot 10^{-8}$
BBP	$2.0 \cdot 10^{-7}$
DEHP	$7.5 \cdot 10^{-7}$
DOP	$1.0 \cdot 10^{-6}$
DNP	$2.5 \cdot 10^{-6}$

Table 2. **Repeatabilities of SDME of phthalate esters ($5 \cdot 10^{-6}$ mol L^{-1}) , n = 5**

Analyte	RSD, %
DMP	8.4
DEP	6.5
DBP	6.8
BBP	8.2
DEHP	9.1
DOP	9.9
DNP	11.4

Application

The developed method was used for the analysis of a cologne "Trojnoj" (Kiev, Ukraine) and a shaving lotion "Lemon" (Kiev, Ukraine). The extraction conditions were as described above. A direct microextraction from undiluted products turned out to be impossible because the drop detached from the needle tip immediately. It can be explained by the presence of a significant amount of ethanol (56.62 %) in the matrix, which leads to a change in the surface tension of the extracting drop and hence to a change in the adhesion forces resulted from the surface tension. However, low detection limits allow to analyze diluted samples. Moreover, in order to work in the linear concentration range, a 1000-fold dilution of the samples is required. In Fig. 5,

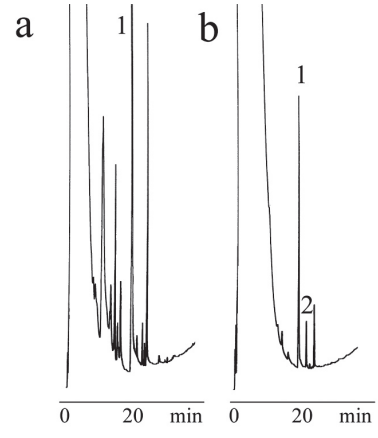

Fig. 5. Chromatograms of cologne "Trojnoj" obtained by direct syringe injection (1 μL) (a) and by SDME after 1000-fold dilution (b). (1) DEP, (2) nonadecane. GC conditions: injector's temperature was 280 °C, detector's temperature was 280 °C. Oven temperature: 100 °C for 10 min, ramped to 150 °C (10 °C min^{-1}), 230 °C (7 °C min^{-1}), to 280 °C (3 °C min^{-1}) and held for 10 min. Gas flow rates: carrier (nitrogen) 10, make-up gas 20, hydrogen 30 and air 300 mL min^{-1}

chromatograms of the cologne "Trojnoj" obtained after a direct syringe injection (a) and after SDME of the 1000-fold diluted sample are presented. In both samples DEP was detected. In the case of the direct injection, besides DEP, many extraneous peaks are observed in the chromatogram. On the other hand, the chromatogram obtained after the SDME was much cleaner and the DEP peak was completely separated from the interferences. The concentration of the DEP was determined by a standard addition method and was 3.38 and 2.93 g L^{-1} in "Trojnoj" and "Lemon", respectively.

Recovery testing was carried out by spiking 20 μL $1*10^{-2}$ mol L^{-1} of the standard DEP mixture to 10 mL of the diluted sample. The obtained results were compared with the known amounts of the standard DEF added to the matrix. The recoveries were 96–104%.

The proposed SDME method is even more useful when particularly small concentrations of phthalates have to be determined. In Fig. 6, a chromatogram of distilled water is presented and DEHP peak is evidenced in the chromatogram. The distilled water was held in a plastic bottle and DEHP that was used as a plasticizer leached from the bottle into the water. The concentration of DEHP ($1*10^{-6}$ mol L^{-1}) was determined by a standard addition method.

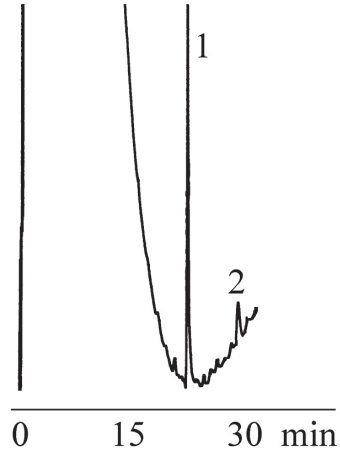

Fig. 6. Chromatogram of distilled water obtained after SDME. (1) nonadecane, (2) DEHP. GC conditions: injector's temperature was 280 °C, detector's temperature was 280 °C. Oven temperature: 100 °C for 4 min, ramped to 150 °C (10 °C min^{-1}), 230 °C (5 °C min^{-1}), to 280 °C (3 °C min^{-1}) and held for 10 min. Gas flow rates: carrier (nitrogen) 10, make-up gas 20, hydrogen 30 and air 300 mL min^{-1}

CONCLUSIONS

The paper describes the use of single-drop microextraction for phthalate sampling and pre-concentration. The proposed method reduces the amount of the solvent necessary for the extraction to 1 μL, shows a good reproducibility and low detection limits, allows selective extraction of analytes. The technique is compatible with GC. Due to its simplicity, velocity and low cost, the method is a promising technique for phthalate analysis.

Received 23 July 2007
Accepted 10 September 2007

References

1. B. Tienpont, F. David, E. Dewull and P. Sandra, *Chromatographia,* **61**, 365 (2005).
2. C. Sablayrolles, M. Montrejaud-Vignoles, D. Benanou, L. Patria and M. Treilhou, *J. Chromatogr. A,* **1072**, 233 (2005).
3. P. G. Desideri, L. Lepri, D. Heimler, S. Giannessi and L. Checchini, *J. Chromatogr.,* **284**, 167 (1984).
4. M. A. Jeannot and F. F. Cantwell, *Anal. Chem.,* **68**, 2236 (1996).
5. L. S. de Jager and A .R. J. Andrews, *Analyst,* **125**, 1943 (2000).
6. M. Saraji and M. Bakhshi, *J. Chromatogr. A,* **1098**, 30 (2005).
7. M. A. Jeannot and F. F. Cantwell, *Anal. Chem.,* **69**, 235 (1997).
8. Y. Wang, Y. Ch. Kwok, Y. He and H. K. Lee, *Anal. Chem.,* **70**, 4610 (1998).
9. E. Zhao, L. Han, S. Jiang, Q. Wang and Z. Zhou, *J. Chromatogr. A,* **1114**, 269 (2006).
10. J. M. Kokosa and A. Przyjazny, *J. Chromatogr. A,* **983**, 205 (2003).
11. Vidal, A. Canals, N. Kalogerakis and E. Psillakis, *J. Chromatogr. A,* **1089**, 25 (2005).
12. E. Hansson and M. Hakkarainen, *J. Chromatogr. A*, **1102**, 91 (2006).
13. Ch. Deng, N. Yao, N. Li and X. Zhang, *J. Sep. Sci.,* 280, 2301 (2005).
14. A. Tankevičiūtė, R. Kazlauskas and V. Vičkačkaitė, *Analyst,* 126, 1674 (2001).
15. E. Psillakis and N. Kalogerakis, *Trends Anal. Chem.,* **21**, 53 (2002).
16. L. Xu, C. Basheer and H. K. Lee, *J. Chromatogr. A*, **1152**, 184 (2007).
17. A. Tankevičiūtė, A. Rožkov, R. Kazlauskas and V. Vičkačkaitė, *Chem. Anal*., **47**, 935 (2002).
18. A. Tankevičiūtė, R. Bobnis, R. Kazlauskas and V. Vičkačkaitė. *Chem. Anal.*, **50**, 539 (2005).
19. R. Batlle and C. Nerin, *J. Chromatogr. A,* **1045**, 29 (2004).
20. Y. Wang, Y. Ch. Kwok, Y. He and H. K. Lee, *Anal. Chem.,* **70**, 4610 (1998).
21. E. Psillakis and N. Kalogerakis, *J. Chromatogr. A,* **907**, 211 (2001).
22. H. Lord and J. Pawliszyn, *J. Chromatogr.A,* **902**, 17 (2000).
23. M. C. Lopez-Blanco, S. Blanco-Cid, B. Cancho-Grande and J. Simal-Gandara, *J. Chromatogr. A,* **984**, 245 (2003).

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MIKROEKSTRAKCIJA TIRPIKLIO LAŠU FTALIO RŪGŠTIES ESTERIAMS NUSTATYTI

S a n t r a u k a

Parodyta ftalio rūgšties esterių nustatymo galimybė panaudojant mikroekstrakciją tirpiklio lašu. Ekstrakcijai naudojamas tolueno lašas su vidiniu standartu nonadekanu. Ekstrakcija atliekama mikrošvirkštu, pritvirtintu virš ekstrakcinio indo. Mikrošvirkšto adata praduriama ekstrakcinį indą dengianti tarpinė, ir tirpiklio lašas išstumiamas į tirpalą. Po ekstrakcijos lašas įtraukiamas į švirkštą ir įleidžiamas į dujų chromatografą. Optimizuotos ekstrakcijos sąlygos (ekstrakcijos trukmė, tirpalo maišymo greitis bei joninė jėga) ir nustatytas rezultatų pasikartojamumas, analičių aptikimo ribos, tiesinis koncentracijų intervalas. Metodas pritaikytas ftalatams odekolone, skutimosi losjone ir distiliuotame vandenyje nustatyti.