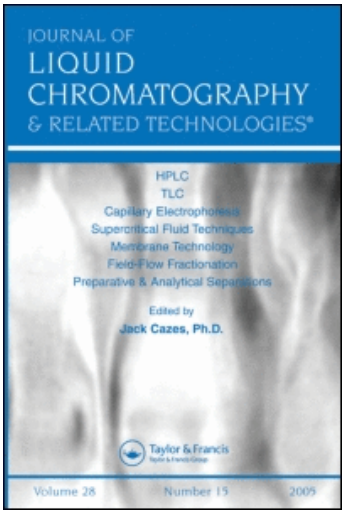

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Solid-Phase Extraction Coupled to High Performance Liquid Chromatography Using a Micro Device Packed with Triacontyl-Bonded Silica

Yoriko Morishima[ab]; Chuzo Fujimoto[b]; Kiyokatsu Jinno[a]
[a] School of Materials Science, Toyohashi University of Technology, Toyohashi, Japan [b] Department of Chemistry, Hamamatsu University School of Medicine, Hamamatsu, Japan

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Solid-Phase Extraction Coupled to High Performance Liquid Chromatography Using a Micro Device Packed with Triacontyl-Bonded Silica

Yoriko Morishima and Kiyokatsu Jinno
School of Materials Science, Toyohashi University of Technology, Toyohashi, Japan

Yoriko Morishima and Chuzo Fujimoto
Department of Chemistry, Hamamatsu University School of Medicine, Hamamatsu, Japan

Abstract: A comparison study of the adsorption capacity of octadecyl- and triacontyl-bonded phases was conducted. The latter exhibited greater capacity for phthalates dissolved in water than the former. A new device containing triacontyl packing was developed to couple solid-phase extraction (SPE) to narrow-bore column high performance liquid chromatography (HPLC) with ultraviolet absorption detection. The device consisted of a syringe filter unit packed with triacontyl-bonded silica, a gas-tight syringe (no needle), and an HPLC injection syringe needle. After a sample solution filled in a 5 mL syringe was delivered to the device by use of a syringe pump, the syringe was replaced with a 250 μL syringe filled with an eluting solvent. By inserting the needle of the device into the needle port of an injection valve followed by forcing the solvent through the packed bed, the analyte was desorbed from the bed and introduced into the sample loop of the valve. On rotating the valve, the extract was introduced into an HPLC column and separation was started. The performance of the device was evaluated by using diheptyl phthalate (DHP) and di-2-ethylhexyl phthalate (DEHP), spiked in water as samples. Preconcentration factors of 58 and 51 were attained with the device for DEP and DEHP, respectively. The method

Address correspondence to Chuzo Fujimoto, Department of Chemistry, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan. E-mail: fujimoto@hama-med.ac.jp

was linear in the range 1.0–50 ng/mL. Limits of detection (LOD)s were found to be 0.8 and 0.9 ng/mL for DHP and DEHP, respectively.

Keywords: Solid-phase extraction, phthalates, water analysis

INTRODUCTION

Solid-phase extraction (SPE) is a widely used technique to extract and concentrate analytes from aqueous samples.[1,2] The most popular SPE uses a syringe-barrel type cartridge containing an appropriate sorbent and, in recent years, membrane disks and microfibers have became commercially available. Although various high performance liquid chromatography (HPLC) packing materials have been utilized as a sorbent for SPE, alkyl-chain bonded phases such as octadecyl (C_{18})- or octyl (C_8)-bonded silica, usually of 40–60 μm in particle diameter, at present, are by far the most popular sorbents. However, such reversed-phase sorbents have been shown to provide low recoveries and poor reproducibilities, unless they are conditioned with a wetting solvent and kept wet before loading an aqueous sample. New functionalized polymeric sorbents that require no preconditioning have been introduced to solve these problems.[3]

Because of the unique characteristics, triacontyl (C_{30})-bonded phases have recently been attracting great interest in reversed-phase HPLC separations.[4,5] C_{30} phases show significantly greater shape selectivity compared with C_{18} phases due to their rigid, highly ordered triacontyl chains. There can be better alternatives to normal phases for the separation of isomers, in view of the fact that they are not as sensitive as normal phases to the mobile phase composition (particularly the water content) and are not as susceptible to column fouling. By virtue of these advantages, C_{30} phases have been employed for separating stereoisomers such as carotenoids[6,7] and tocopherol derivatives,[8–10] and for the separation of polycyclic aromatic hydrocarbons[11] and fullerenes.[12] Under highly aqueous mobile phase conditions, C_{30} phases exhibit excellent hydrolytic stability and are more retentive than most C_{18} phases. A more specific difference between C_{30} and other alkyl phases is that C_{30} phases provide excellent chromatographic reproducibilities even with mobile phases containing less than 5% organic modifier in water, which are often required to separate very polar, water-soluble compounds, but this is not the case for most C_{18} phases.

When separations are performed using C_{18} phases with such highly aqueous mobile phases, solute retention decreases over time and, at worst, all retention and resolution are lost. This is, generally, believed to be the result of the phenomenon called “phase collapse”, which occurs under highly aqueous conditions.[4,13,14] The alkyl chains of C_{18} phases are folded over on themselves in a water-rich environment, thereby the alkyl phase

available to interact with solutes decreases, resulting in the decrease in retention. The problem of phase collapse can be overcome by using wide-pore silicas at the expense of retention. On the contrary, in the case of C_{30} phases, the alkyl chains are thought to remain fully extended under 100% aqueous conditions, allowing effective retention of even highly polar compounds.[4,13,14]

In view of the fact that under highly aqueous conditions, C_{30} phases are stable and do not undergo phase collapse, it seems probable that they are ideal sorbents to be used for the SPE of analytes in aqueous media. The use of C_{30} phases as SPE sorbents should make it unnecessary to add organic modifiers to water samples. For these reasons, we examined the utility of a C_{30} phase as sorbent in SPE. To our knowledge, C_{30} phases have not been previously used for water sample extraction. A new device was developed for carrying out sample extraction followed by sample injection into an HPLC column. With this device, just as analytes adsorbed on the C_{30} phase are desorbed, they are introduced into the sample loop of an HPLC injection valve. The performance of the device was studied using phthalates as analytes. Analysis of phthalates in water has been a challenging task because these compounds are present everywhere at trace levels and they may be etiological agents in several human diseases.[15,16] Especially, di-2-ethylhexyl phthalate (DEHP) examined here is most commonly used as a plasticizer in diverse applications and is suspected of being a reproductive toxicant.[17]

EXPERIMENTAL

Chemicals and Materials

DEHP (purity >98%) and diheptyl phthalate (DHP; purity >95%) were purchased from Tokyo Chemical Industries (Tokyo, Japan). HPLC-grade acetonitrile was obtained from Kanto Kagaku (Tokyo, Japan). The C_{30} and C_{18} phases used were Develosil C30-UG and Develosil ODS-UG, respectively, from Nomura Chemical (Seto, Japan). The physical properties of these sorbents are as follows: particle diameter, 15–30 μm with an average diameter of 20 μm; mean pore size, 14 nm; pore volume, 1.15 mL/g; surface area, 300 m^2/g; carbon content, 18%. Water used for HPLC and sample preparation was obtained on a Nihon Millipore (Yonezawa, Japan) Milli-Q SP water purification system. Stock solutions were prepared by dissolving the phthalates in water at a concentration of 1.0 mg/mL for each compound. Standard solutions for the experiments were freshly made by dilution of the stock solutions with acetonitrile and, subsequently, with water.

SPE Device

The SPE device developed in this study was fabricated by utilizing a disposable filter unit, Sample Prep-LCR4-LH (Nihon Millipore), which had a female Luer-Lok inlet and a male slip outlet. The housing was made of polypropylene and the membrane filter was a hydrophilic PTFE membrane filter with 4 mm diameter and 0.5 μm pore size (Figure 1). The membrane filter lodged in the bottom of the filter unit served to retain sorbents. An amount of 15 mg of the C_{30} phase were packed in the filter unit. A piece of hydrophilic PTFE membrane filter with 0.45 μm pore size (Nihon Millipore) and two pieces of cellulose paper filters (Advantec Toyo, Tokyo, Japan) were cut out to match the inside diameter of the filter unit and were placed on the sorbent. The filters are replaceable with a new one when they become blocked by particulate matter in water samples. When a 250 μL gas-tight syringe was attached to the filter unit via Luer-Lok connection, no free space was generated between the packed bed and the barrel tip.

The male slip outlet of the filter unit was shortened to reduce extra column dispersion. A stainless steel (SS) tube of 0.15 mm i.d., 0.81 mm o.d. and 14 mm length was pressed into the male slip outlet to provide a tight fitting connection. This SS tube was then connected butt-to-butt to a SS tube of 0.13 mm i.d., 0.71 mm o.d., and 55 mm length (manufactured in-house) with a short section of PTFE tubing. Note that the o.d. of the latter SS tube matches that of a 22-gage needle, which the needle port of the injection valve used can accommodate. Before use, the entire assembly was thoroughly rinsed with acetonitrile and water and it was confirmed that no analyte peaks appeared in the chromatogram of a blank acetonitrile eluate.

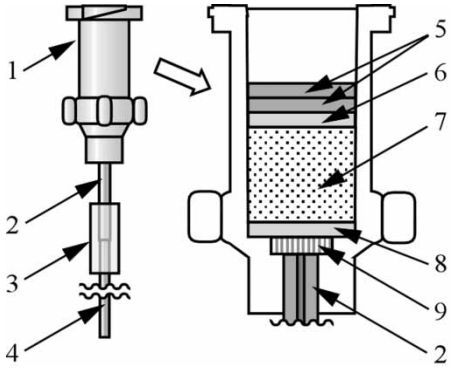

Figure 1. Construction of the SPE device: 1, filter unit; 2, SS tube; 3, PTFE tube; 4, 22-gage syringe needle; 5, cellulose paper filter; 6, hydrophilic PTFE membrane filter; 7, C_{30}-silica packings; 8, hydrophilic PTFE membrane filter (originally attached to the filter unit); 9, filter support.

HPLC Instrumentation

The HPLC system consisted of a Jasco (Tokyo, Japan) 880-PU pump, a Jasco 870 UV detector equipped with a 1.0 μL flow cell, a Rheodyne (Cotati, CA, USA) 7125 six-port injection valve, and a System Instruments (Tokyo, Japan) Labchart 180 integrator. Detection was performed at 230 nm. The injection valve was fitted with a sample collection loop made from a 0.15 mm i.d. PEEK tubing (distributed by GL Sciences, Tokyo, Japan), the volume of which had been calibrated. All separations were carried out on a Shiseido (Tokyo, Japan) Capcell Pak C_{18} UG 120 column (2.0 mm i.d. × 15 cm, 5 μm particle size). A mixture of acetonitrile and water (90/10, v/v) was used as the mobile phase at a flow rate of 200 μL/min.

Procedure

A 5 mL gas-tight syringe (Ito, Shizuoka, Japan) filled with a sample solution was attached to the SPE device via the Luer-Lok connection. The device was mounted on an Azumadenki Kogyo (Tokyo, Japan) MF-2 syringe pump and the sample solution was pumped to the device for a certain period of time. Subsequently, the device was dismounted from the syringe pump and the 5 mL syringe was replaced with a 250 μL gas-tight syringe (Hamilton, Reno, NV, USA) filled with a desorption solvent. The needle of the device was inserted into the needle port of the injection valve in the load position; the device, as such, acts as a sample injection syringe. As the syringe plunger was pressed manually, and the desorption solvent was thereby forced through the SPE device, analytes were desorbed and transferred into the loop of the valve. By switching the injection valve to the injection position, the contents of the loop were carried to the column and LC separation was started. After every extraction, the SPE device was washed with 2.5 mL of acetonitrile and then equilibrated with 1 mL of water.

RESULTS AND DISCUSSION

Comparison of Sorbents

The adsorption capacity was compared for the C_{30} and C_{18} phases. A sample solution containing DHP and DEHP at 50 ng/mL of each analyte was delivered to a SPE device containing 15 mg of sorbent at 110 μL/min for 45 min. Subsequently, the analytes were desorbed with 250 μL of acetonitrile and the eluate was collected in a vial. An aliquot of 10 μL of the collected eluate was injected into the HPLC system. As expected, the C_{30} phase exhibited higher adsorption capacity than the C_{18} phase (Figure 2). The

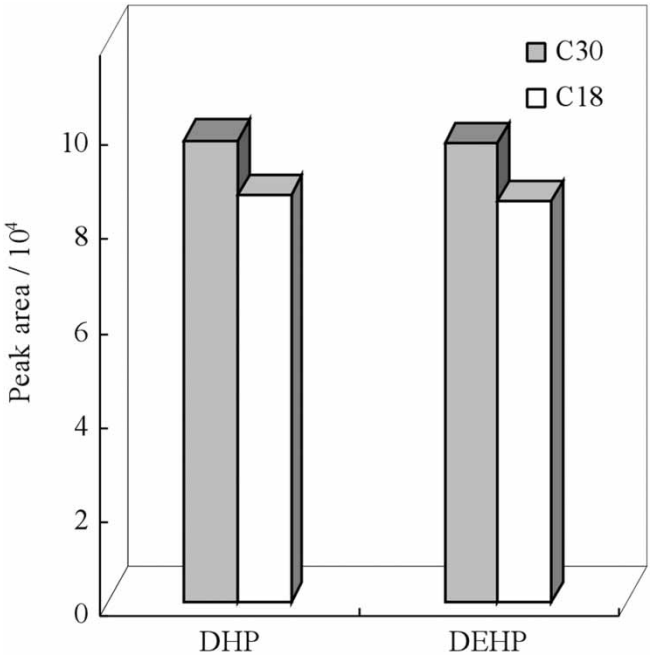

Figure 2. Comparison of adsorption capacity between C_{30} and C_{18} phases. A sample solution containing DHP and DEHP at 50 ng/mL of each analyte was passed through the SPE device at 110 μL/min for 45 min.

lower adsorption capacity of the C_{18} phase would be ascribed to the phase collapse phenomenon.[4,13,14] The C_{30} phase was consequently employed in subsequent experiments.

Optimization of Desorption Conditions

Three different desorption solvents were tested: chloroform, acetonitrile, and an (90/10, v/v) acetonitrile/water mixture. A sample solution containing DHP and DEHP at 50 ng/mL of each analyte was passed at 110 μL/min for 10 min through the SPE device, which was then eluted with 60 μL of the desorption solvent into a 50 μL loop of the injection valve. The acetonitrile/water mixture resulted in poor analyte elution. Both chloroform and acetonitrile were effective for eluting the analytes. However, the use of chloroform brought about a strong solvent peak close to the DHP peak in the chromatogram, because not only does chloroform exhibit strong absorption at the wavelength used, but also its elution strength is so strong in reversed-phase chromatography that the retention of DHP is weakened by injection of a

large amount of chloroform. This was not the case of acetonitrile. Consequently, pure acetonitrile was selected as the desorption solvent.

To find the required volume of acetonitrile to elute the phthalates from the C_{30} phase, the collection loop volumes of 20, 50, 70, and 100 μL were investigated, and the phthalates were eluted with an eluent volume 10 μL greater than the loop volume used. The largest peak areas were obtained with a 70 μL loop for both analytes. Next, with the 70 μL loop, the volume of the eluent was varied from 60 to 90 μL. A maximum peak area was obtained with an eluent volume of 80 μL for individual analytes, as shown in Figure 3. Therefore, 80 μL of acetonitrile was hereafter used to desorb and transfer the analytes to the 70 μL loop.

Effect of the Flow Rate of Sample Solution

In general, the flow rate of the sample solution can affect the recovery of the analyte as well as the total analysis time. Holding the sample volume constant

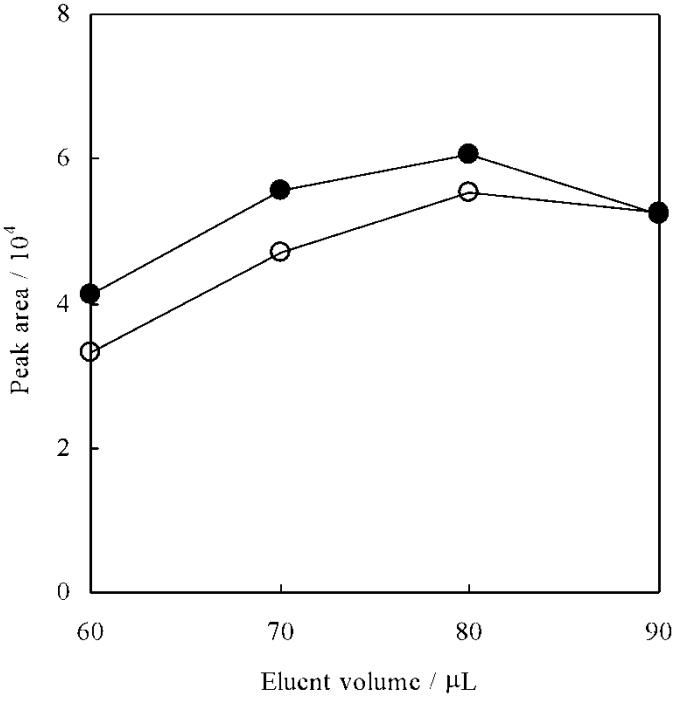

Figure 3. Effect of the eluent volume on the peak area in the chromatogram: (•), DHP; (○), DEHP. A 70 μL loop was used. A sample solution containing DHP and DEHP at 50 ng/mL of each analyte was passed through the SPE device at 110 μL/min for 10 min.

(1.67 mL), the flow rate of the standard solution was varied from 10 to 167 μL/min. It was found that the flow rate had no significant effect on the recovery of the analyte up to 167 μL/min, which was the maximum flow rate obtainable with the syringe pump equipped with the 5 mL syringe. Because of the relatively low flow resistance, further increase in the flow rate appeared to be feasible with this device.

Effect of the Volume of Sample Solution

Standard solutions of DEP and DEHP (spiking level, 50 ng/mL each) were passed through the packed bed at a flow rate of 167 μL/min over periods of 5–30 min. It was found that the peak area of the analytes increased linearly with the extraction time, that is, the sample volume ($r = 0.999$). This indicates that no losses of these compounds by breakthrough occur for sample volumes up to 5.0 mL.

Extraction Performances

The preconcentration (enrichment) factor and recovery were evaluated for 30 min extractions of 5.0 mL water samples, spiked at 50 ng/mL of individual phthalates. The results are shown in Table 1, where the preconcentration factor is given by the ratio of the peak area obtained with the SPE device to that obtained by direct injection with a 70 μL loop; the recovery (%) was calculated by dividing the peak area obtained from the extracted samples with the product of the peak area obtained by the direct injection and the ratio of the loop volume to the sample volume delivered to the device. The obtained preconcentration factors are as high as 51 or more, meaning that the sensitivity of HPLC analysis is proportionately increased. The recovery values are much higher than those obtained using conventional SPE methods with reversed-phase materials.[18] The chromatograms obtained by using the SPE device, and by direct injection, are shown in Figure 4, from which it is quite obvious that the sensitivity is greatly improved by using the SPE device.

Table 1. Preconcentration factors and recoveries obtained with the C30-SPE devices ($n = 4$)

Compound	Preconcentration factor	Recovery (%)
DHP	58	81
DEHP	51	72

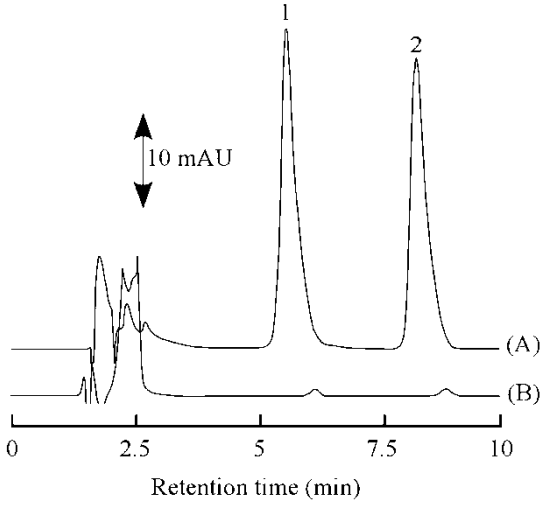

Figure 4. Comparison of chromatograms observed by preconcentration with the SPE device (A) and by direct injection (B) of DHP (peak1) and DEHP (peak 2). Extraction conditions: sample flow rate, 167 μL/min; extraction time, 30 min; loop volume, 70 μL; desorption solvent volume, 80 μL; analyte concentration, 50 ng/mL each. Chromatographic conditions are given in the text.

Calibration plots of peak area versus concentration were constructed for each analyte in the concentration range from 1.0 to 50 ng/L for 30 min extraction. The linearity was good with a correlation coefficient r of 0.999 ($n = 4$) for both phthalates.

The limit of detection (LOD) of the method was calculated as the concentration required to give a signal-to-noise ratio of 3. For a 30 min extraction, the LOD was found to be 0.8 and 0.9 ng/mL for DHP and DEHP, respectively. These values are higher than those obtained using conventional off-line SPE methods coupled to gas chromatography with an electron capture detector.[18] Further decreases in LOD would be possible by prolonging the extraction time or by the use of a more sensitive detection system.

The reproducibility of the method, expressed as relative standard deviation (RSD), was evaluated by using aqueous samples spiked with 5.0 ng/mL of each compound. The RSD values were 6.6% and 8.3% for DHP and DEHP ($n = 4$), respectively; similar values have been reported elsewhere.[18]

CONCLUSION

The C_{30} phase has been shown to be an efficient SPE sorbent for phthalates dissolved in water. The developed method is simple and convenient for the

preconcentration of analytes in aqueous samples before the HPLC analysis. Considering the fact that the values of preconcentration factor and LOD were obtained from sample volumes of less than the breakthrough volume, further improvement of the performance is anticipated. In addition, the method has the advantage of eliminating the risk of secondary contamination that can occur during some steps subsequent to extraction in traditional off-line SPE coupled to HPLC. Although it was found that the device can be used repeatedly (more than 100 times) by washing the packed bed with acetonitrile, one-time use of the device may be preferred, provided the packing material and the filter unit are more inexpensive than the washing solvents.

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