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Application of dispersive liquid–liquid microextraction and high-performance liquid chromatography for the determination of cetrimonium bromide in water samples

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Abstract

A simple, rapid and sensitive method for the determination of cetrimonium bromide at trace levels in water samples was developed by combining a dispersive liquid –liquid microextraction (DLLME) technique with high performance liquid chromatography (HPLC-UV). A mixture of extraction solvent and dispersive solvent were rapidly injected into 10.0 mL aqueous sample, and a cloudy solution was formed. After extraction of the analyte into the fine droplets of extractant, phase separation was performed by centrifugation and the enriched analyte in the sedimented phase was determined by HPLC-UV. The factors relevant to the microextraction efficiency, such as the kind and volume of extraction and dispersive solvent, and the extraction time were optimized. Under the optimum conditions (extraction solvent: chlorobenzene, volume, 70 µL; dispersive solvent: ethanol, volume, 0.75 mL), the enrichment factor of 200 was obtained. The linear range was 0.005–100 µg L^{-1}. The relative standard deviation of 5.9% (n= 5), and detection limit (signal-to-noise ratio of 3) of 0.10 pg mL^{-1} were obtained.

Keywords: Dispersive liquid-liquid microextraction, Cetrimonium bromide, High performance liquid chromatography.

1. Introduction

Cetrimoniumbromide(hexadecyltrimethylammonium bromide, CTAB) is one of the components of the topical antiseptic cetrimide. The cetrimonium (or hexadecyltrimethylammonium) cation is an effective antiseptic agent against bacteria and fungi. This cationic surfactant uses include providing a buffer solution for the extraction of DNA. It has been widely used in synthesis of gold nanoparticles (e.g., spheres, rods, bipyramids). It is also widely used in hair conditioning products. Based on the available data, cetrimonium bromide is considered safe for use in rinse-off cosmetic products but is safe only at concentrations of up to 0.25% in leave-on products. Potentiometric methods, using a different ion-selective electrodes, were investigated for determination of cetrimonium bromide [1]. The aim of our investigation was to develop a sensitive technique for determination of low levels of CTAB in different natural waters. High-performance liquid chromatography (HPLC) as a very efficient separation technique with a low cost UV spectrophotometer detector could be a good selection. However, in many cases, owing to matrix interference and insufficient instrumental detection limit for trace determination in real environmental samples, direct chromatographic separation and determination of those species is difficult. Therefore, in order to obtain accurate, reliable and sensitive results, a separation/preconcentration method is required prior to chromatographic separation of the target analytes.

Conventional sample preparation techniques such as liquid –liquid extraction (LLE) have the disadvantages of being time-consuming and expensive, and of requiring large volumes of toxic organic solvents. Therefore, in recent years several liquid phase microextraction (LPME) techniques with negligible volumes of extractant and the minimum number of steps have been developed such as, single drop microextraction (SDME) [2-4], solvent bar microextraction (SBME) [5], hollow fiber liquid-phase microextraction (HF-LPME) [6] and dispersive liquid–liquid microextraction (DLLME) [7-12]. Among all these kinds of LPME, DLLME was first introduced by Assadi and co-workers in 2006 [11], has

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attracted much attention in the recent years due to simplicity of the operation, rapidity, low sample volume, low cost and high recovery and enrichment factor. DLLME is based on ternary component solvent system such as Homogeneous liquid–liquid extraction (HLLE) and Cloud-point extraction (CPE). In this method, the appropriate mixture of extraction solvent and disperser solvent is rapidly injected into aqueous sample containing the analyte. At the moment of injection, fine droplets of the extraction solvent are dispersed in aqueous phase and a cloudy solution is formed. The analyte is quickly extracted into fine droplets of extraction solvent due to an infinitely large surface area between extraction solvent and aqueous phase. Then the cloudy solution is exposed to centrifugation to separate two phases. Finally the enriched analyte in the sediment phase is determined by proper instrumental method. Since its introduction in 2006, DLLME has been portrayed to be an efficient, fast, and sensitive microextraction technique for both inorganic [13, 14]and organic [15] compounds. However, to the best our knowledge, there is no report concerning cetrimonium bromide analysis using the DLLME method.

In the present work, DLLME followed by HPLC with UV detection was applied for extraction and determination of cetrimonium bromide in water samples. The effects of various experimental parameters, such as the kind and volume of extraction solvent and dispersive solvent, as well as extraction time were studied and optimized.

2. Materials and methods

2.1. Instrumentation

A Knauer HPLC system (Berlin, Germany), equipped with a K-1001 HPLC pump, D-14163 degasser, and a K-2600 UV detector was used. Chromgate software (version 3.1) for HPLC system was employed to acquire and process chromatographic data. The analytical column was ODS III (250 mm × ID 4.6 mm, 5 µm) from MZ-Analysentechnik (Mainz, Germany). The pH of the solutions was measured by a PHS-3BW model pH-meter (Bell, Italy). An EBA20 model centrifuge (Hettich, Germany) was used to accelerate phase separation.

A mobile phase comprised of water/acetonitrile (70:30, v/v) at a flow rate of 1 mL/min was found to be optimum. Water phase was prepared by mixing of 835 mL of distilled water and 65 mL of 1.0 mol L^{-1} sodium hydroxide solution. The pH was then adjusted to pH=3 with 85% ortophosphoric acid and then the sufficient amount of distilled water was added up to 1000 mL. Prior to use, the mobile phases were filtered through a 0.45 µm membrane filter and degassed under vacuum. The sample injection volume was 20 µL and the analytes were monitored at 208 nm (at room temperature).

2.2. Reagents and solutions

Cetrimonium bromide (Figure. 1), ethanol, chlorobenzene, acetone, acetonitrile, methanol, chloroform, carbon tetrachloride, carbon disulfide and ultra-pure water were all from Merck (Darmstadt, Germany). A mixture of stock solution containing cetrimonium bromide at 1000 µg mL^{-1} was prepared in HPLC grade methanol. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with ultra-pure water in a 10 mL volumetric flask. The aqueous solutions were prepared daily by diluting the standard mixture with ultra-pure water. All the standard solutions were stored at 4 ◦C in the dark. Other reagents and solvents were also obtained from Merck.

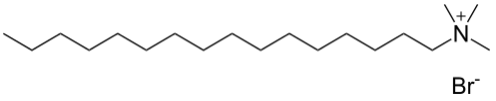

Figure 1. Structural formula of Cetrimonium bromide.

2.3. Extraction procedure

A 10 mL aqueous sample solution containing 0.01 mg L^{-1} cetrimonium bromide was placed in a 12 mL screw cap glass test tube with conical bottom. 750 µL ethanol (disperser solvent), and 70 µL chlorobenzene (extraction solvent), were injected rapidly into the sample solution and the mixture was gently shaken. A cloudy mixture was formed in the test tube. The mixture was then centrifuged for 5 min at 4000 rpm. The fine droplets of chlorobenzene including the analyte were sedimented at the bottom of the test tube. The sedimented phase was transferred to another test tube with a conical bottom using a 100 µL HPLC syringe. The organic phase was evaporated in room temperature. Then 50 µL acetonitrile was added to the residue and a 20- µL aliquot was injected into the HPLC system for analysis.

3. Results and discussion

The effect of different parameters such as type and volume of extraction solvent, disperser solvent, and extraction time were investigated. In this experiment, 10 mL of the model sample was used to study the extraction efficiency, based on peak area, under different experimental conditions.

3.1. Optimization of DLLME procedure

3.1.1. Selection of extraction solvent

To select an appropriate extraction solvent for DLLME, certain requirements must be met. First, the solvent must have good affinity for the target compounds. Second, it should have a low solubility in water. Third, it should have a higher density than water. Finally, the organic solvent should have no interferences with the analyte peaks when directly injected for chromatographic analysis. On the basis of these considerations, four different extraction solvents including CCl_4 (density 1.59 g mL^{-1}), CS_2 (density 1.26 g mL^{-1}), $CHCl_3$ (density 1.48 g mL^{-1}), and C_6H_5Cl (density 1.11 g mL^{-1}) were tested in this work.

To pick up a constant volume of the sedimented phase (70 µL), different volumes of the extraction solvents were added into the sample. Thus a series of sample solutions was studied by using 0.5 mL of ethanol containing 100, 125, 100 and 160 µL volumes of CCl_4, $CHCl_3$, C_6H_5Cl and CS_2, respectively. The results (Fig. 1) revealed that chlorobenzene has the highest extraction recovery in comparison with the other tested solvents. Additionally, with this solvent, using the lowest volume among all four solvents was possible. Therefore, C_6H_5Cl was selected as the extraction solvent.

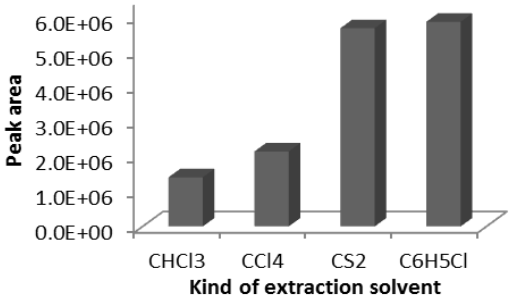

Figure 2. Effect of different extraction solvents on the efficiency of DLLME. Extraction conditions- concentration of analyte: 0.01 mg L^{-1}; sample volume: 10.0 mL; volume of settled phase: 70.0 µL; disperser solvent (ethanol) volume: 0.5 mL, extraction time: 1 min.

3.1.2. Selection of dispersive solvent

The miscibility of the dispersive solvent in organic phase (extraction solvent) and aqueous phase (sample solution), is the main factor affecting the selection of dispersive solvent in DLLME process. In addition, dispersive solvents should disperse extraction solvent as very fine droplets in aqueous phase to obtain immediately a transfer of analytes from aqueous phase to the extraction phase. Therefore, methanol, ethanol, acetonitrile and acetone were tested to investigate the influence of these solvents on the DLLME performance. The experiments were performed by using 0.50 mL of each dispersive solvent containing 70 µL C_6H_5Cl (as extraction solvent). The results, illustrated in Figure. 3, indicated that ethanol exhibited the highest extraction efficiency. Thus, ethanol was chosen as the dispersive solvent for subsequent experiments.

3.1.3. Effect of extraction solvent volume

To study the effect of extraction solvent volume, solutions containing increasing volumes (20–100 µL) of chlorobenzene dissolved in a fixed volume of ethanol (0.50 mL) were subjected to the same DLLME procedure. It was observed that by increasing the volume of extraction solvent from 20 to 70 µL, the volume of sedimented phase increased from 6 to 56 µL. Figure. 4 indicates that by increasing the chlorobenzene volume up to 70 µL, and therefore, sedimented phase volume, the peak area of the analyte increased.

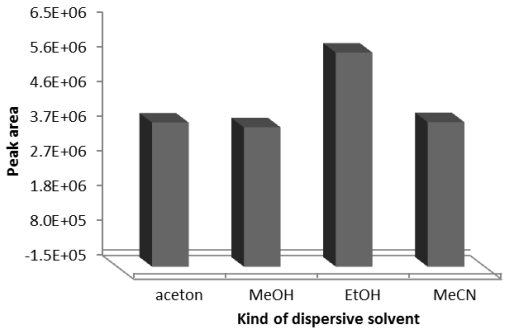

Figure 3. Effects of different dispersive solvents on the efficiency of DLLME. Extraction conditions- concentration of analyte: 0.01 mg L^{-1}; sample volume: 10.0 mL; extraction solvent (C_6H_5Cl) volume: 70.0 µL; disperser solvent volume: 0.5 mL, extraction time: 1 min.

A decrease in efficiency was observed above 70 µL. This is probably due to a large droplet formation at low ratio of the dispersive solvent to the extraction solvent volume. Large solvent droplets were rapidly settled at the bottom of the tube and low extraction efficiencies accrued. Therefore, the extraction efficiency was decreased [16]. On the basis of these results, 70 µL of chlorobenzene was selected as optimal solvent extraction volume.

3.1.4. Effect of dispersive solvent volume

The volume of the dispersive solvent is one of the important factors which should be considered in DLLME process. To evaluate the optimum volume of the dispersive solvent, various experiments were performed using different volumes of ethanol (0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mL) with optimum amount of chlorobenzene (70 µL). It was observed that the extraction efficiency was increased by increasing the volumes of ethanol up to 0.75 mL and then decreased (Figure. 5). At low volume, ethanol cannot disperse extraction solvent properly, and cloudy solution does not form completely, and the extraction recoveries are low. On the other hand, the solubility of analyte in water sample increases at high volume of ethanol, therefore, extraction recovery decreases too. Thus, 0.75 mL of ethanol was chosen as the optimum volume for next experiments.

3.1.5. Effect of extraction time

In DLLME, extraction time is defined as interval time between injecting the mixture of disperser solvent (ethanol) containing of extraction solvent (chlorobenzene) and before starting to centrifuge. The effect of extraction time was examined in the range of 0–50 min. The results indicated (Figure. 6) that the extraction was completed after 1 min from injection. It is revealed that after formation of the cloudy solution, the surface area between extraction solvent and water sample is infinitely large and shows that the transition of analytes from water sample to extraction solvent is fast and equilibrium state is achieved quickly. Therefore, this method is very fast and this is the most

important advantage of DLLME technique. Preliminary consideration of centrifugation time in the range 2 to 10 minutes showed that 5 minutes centrifugation leads to better aggregation of sedimented phase at bottom of the conical test tube and provided the highest extraction efficiency. So, in this method the most time-consuming step is the centrifuging of sample solution in the extraction procedure, which is 5 min.

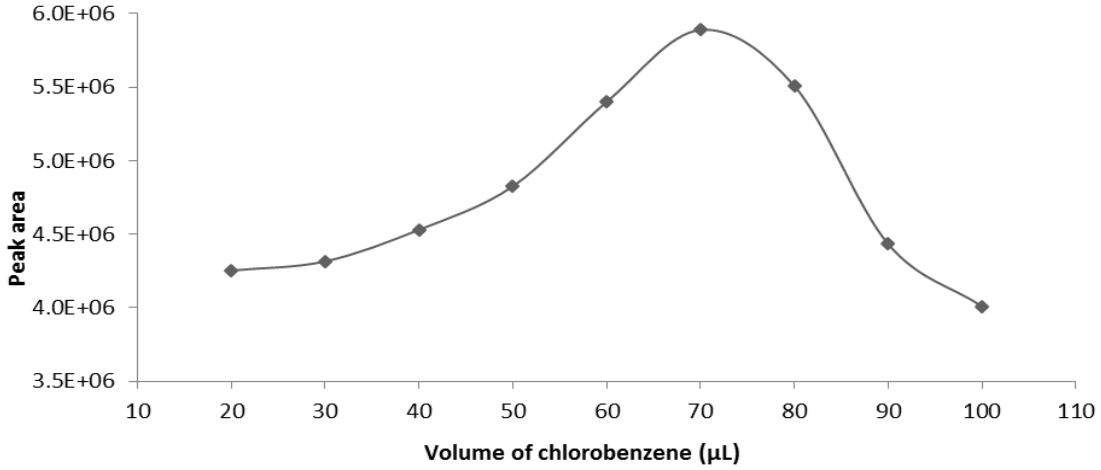

Figure. 4. Effect of extraction solvent volume on the efficiency of DLLME. Extraction conditions – concentration of analytes: 0.01 mg L^{-1}; sample volume: 10.0 mL; extraction solvent: C_6H_5Cl; disperser solvent (ethanol) volume: 0.5 mL, extraction time: 1 min.

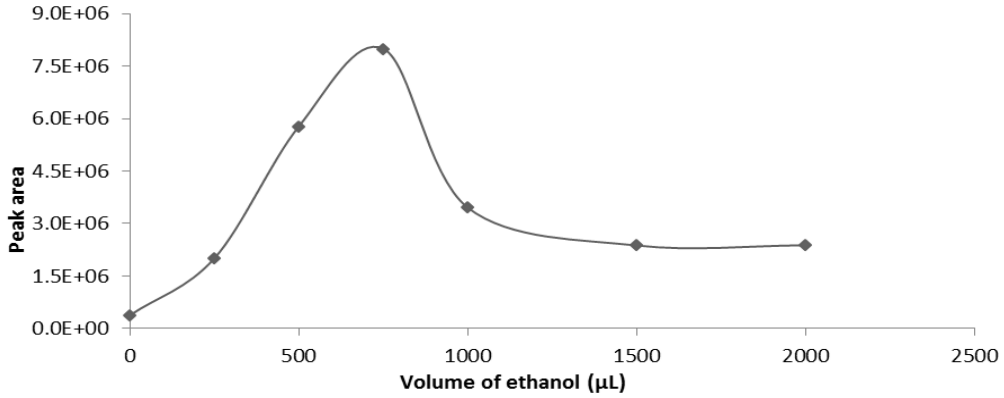

Figure. 5. Effect of dispersive solvent volume on the efficiency of DLLME. Extraction conditions – concentration of analytes: 0.01 mg L^{-1}; sample volume: 10.0 mL; extraction solvent (C_6H_5Cl) volume: 70 µL; disperser solvent: ethanol, extraction time: 1 min.

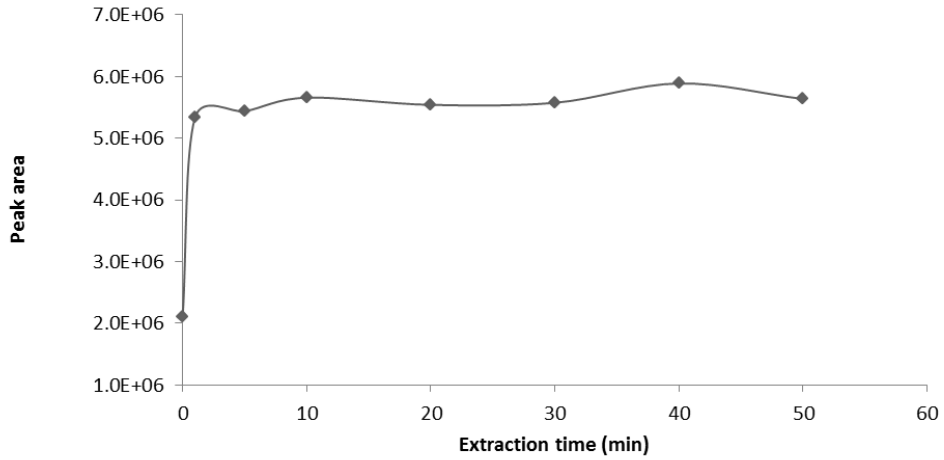

Figure. 6. Effect of extraction time on the efficiency of DLLME. Extraction conditions – concentration of analytes: 0.01 mg L^{-1}; sample volume: 10.0 mL; extraction solvent (C_6H_5Cl) volume: 70 µL; disperser solvent (ethanol) volume: 0.5 mL.

3.1.6. Analytical figures of merit

The dynamic linear range (DLR), the correlation coefficient (r^2), the limit of detection (LOD), the relative standard deviation (RSD), the preconcentration factor (PF), were determined under the optimal condition and the results were summarized in Table 1. The calibration graph was linear in the range of 0.005–100 μg L^{-1} of cetrimonium bromide with a good correlation coefficient (0.9994). The limit of detection (LOD) was calculated based on $LOD = 3S_b/m$ (where S_b is the standard deviation of the blank signal and m is the slope of calibration graph), was 0.1 pg mL^{-1}. The relative standard deviation (RSD) for five replicate measurements of cetrimonium bromide solution was 5.9%. The preconcentration factor, defined as the ratio between the volume of the initial sample and the final volume obtained after the extraction step, was 200 for 10 mL of the sample solution.

Table 1. Analytical characteristics of the proposed method for the determination of cetrimonium bromide.

Parameter	Analytical feature
Dynamic linear range (μg L^{-1})	0.005–100
Correlation coefficient (r^2)	0.9994
Limit of detection (LOD) (pg mL^{-1},3σ, n=10)	0.1
Relative standard deviation (RSD %) (n=5)	5.9
Preconcentration factor	200
Sample volume (mL)	10

4. Conclusion

This study describes a DLLME method combined with HPLC-UV for determination of trace amounts of cetrimonium bromide in water samples. The proposed method is simple, efficient, rapid, and inexpensive and the consumption of organic solvents is lower than the other conventional sample preparation methods. It also has a low detection limit, good calibration range and high preconcentration factor with a reduced amount of sample in comparison with the other reported methods.

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