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Application of Three-phase Hollow Fiber LPME using an Ionic Liquid as Supported Phase for Preconcentration of Malachite Green from Water Samples with HPLC Detection

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A novel three-phase hollow fiber liquid phase microextraction was developed for the determination of malachite green (MG) in environmental waters, which selected [BMIM][PF_6] mixed with 1% trioctylphosphine oxide (TOPO) as supported phase. Several parameters (accepter phase pH, sample pH, supported phase membrane, volume of accepter phase, salinity, extraction time) that could affect extraction performance were investigated. Under the optimal extraction conditions, the established approach showed excellent characters as: high enrichment factor (212), wide linear range (0.20-100 μg L^{-1}), low detection limit (0.01 μg L^{-1}), good reproducibility (RSD, 8.9%, n=5) and satisfactory recovery (84.0-106.2%). The method was applied to detect MG at Yangtze River and pond waters in Zhenjiang, Jiangsu province, and 4 sites among 15 sampling sites were found MG with the concentration of 1.73-11.06 μg L^{-1}, which confirmed that the proposed environmentally friendly method was simple and effective for monitoring MG in aquatic system.

Key Words : Malachite green, Three-phase hollow fiber liquid phase microextraction, Ionic liquid, Environmental water, HPLC/UV

Introduction

Malachite green (MG), a triphenylmethane dye,[1] originally used as a dyeing agent in the textile and paper industry. Since 1933, it was extensively found all over the world in the fish farming industry as a kind of fungicide, ectoparasiticide and disinfectant.[2] Because of its potential carcinogenic, genotoxic and mutagenic properties,[3-5] MG has been banned in aquaculture industry in European Commission, U.S. and China.[6] However, due to their low cost and high effectiveness, it is still illegally used at certain areas.[7-9] Therefore, it is necessary to establish an effective approach to monitor MG in waters.[10]

Because of environmental matrix effects and low concentration of MG occurring in water, the main step of the analytical process especially in the extraction procedures is the isolation and enrichment.[11] At present, solid phase extraction (SPE) is the most popular sample pretreatment method,[12] which uses a solid phase and a liquid phase to isolate one, or one type of analyte from a solution. SPE is usually used to cleanup a sample before using a chromatographic or other analytical method to quantitate the amount of analyte(s) in the sample.[13,14] However, the method often require complicated procedures and always need much toxic organic solvents. On the other hand, the disadvantages of the methods also showed being expensive, time-consuming, and labour-intensive, all of which limited its further application.[15]

As an alternative for analysis of MG in water, hollow fiber liquid-phase microextraction (HF-LPME) has the merits of simplicity, efficiency, negligible volume of solvents needed and excellent sample cleaning ability.[16-18] HF-LPME could be classified into three-phase or two-phase mode, the former was suitable for pre-concentration of acidic or basic compounds, especially for the sample with complex matrix. In order to make the method more effective and environmental friendly, room temperature ionic liquids (ILs) was introduced to the analyzing system, which possess many significant advantages, such as low vapor pressure, wide range of miscibility with other organic solvents, good thermal stability, moderate dissolvability of organic compounds and dual natural polarity.[19-22]

In this study, our aim was to develop a novel three-phase HF-LPME adopted [BMIM][PF_6] mixed with 1% trioctylphosphine oxide (TOPO) as supported phase combined with HPLC/UV to determine MG in environmental waters. At the process of pre-concentration, parameters that could impact the extraction efficiency were examined and evaluated in details, and the proposed method was successfully applied for monitoring trace MG in aqantic environments at Zhenjiang section of the Yangtze River and pond waters.

Experimental

Reagents and Materials. Individual standard of MG and HPLC-grade acetonitrile were bought from Aladdin Co. Ltd (Shanghai, China). 1-Octyl-3-methylimidazolium hexafluorophosphate ([C8MIM][PF_6]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF_6]) with purity of 99% was obtained from Shanghai Barium Strontium Magnesium Industry Co. Ltd. Trioctylphosphine oxide (TOPO) and humic acid (contain 35.1% dissolved organic carbon) was purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was prepared by a Milli-Q SP reagent water system (Millipore, Milford, MA, USA). Standard stock solutions of MG (1000 μg mL^{-1}) were prepared with pure water and stored at 4 °C. Working standard solutions were prepared by diluting the standard solutions with ultrapure water just before use. All other chemicals were purchased from Nanjing Chemicals Co. Ltd (Nanjing, China) with analytical grade or better. Samples of the Yangtze River water and pond water were collected from Zhenjiang, Jiangsu province (China).

Polypropylene hollow fibers (280 mm id, 50 μm wall thickness, and 0.1 μm pore size) were obtained from Membrana GmbH (Wuppertal, Germany). Micro syringe (needle OD: 0.3 mm, long: 8 mm, cylinder volume: 0.5 mL) was obtained from BD Consumer Healthcare company (Franklin Lakes, NJ, USA), used to flush out the acceptor phase into a small glass vial (200 μL, Alltech, Deerfield, IL, USA) after extraction.

Supported Liquid Membrane Preparation and Extraction Procedure. Hollow fibers were cut into segments of certain length, washed with acetone in an ultrasonic bath and dried. The segment was immersed in IL ultra sound for 10s to impregnate the pores and rinsed with water on the outside for 5s in order to remove the excess of IL. The lumen of the prepared fiber piece was filled with aqueous pH 10 acceptor phase using a syringe. Both open ends of the fiber were heat-sealed by sintering. Then the hollow fiber segment was submerged into the 20 mL sample solution (donor phase) contained in a 50 mL glass beaker. The aqueous sample was stirred at 200 rpm at room temperature (25 °C) for tens of minutes. After extraction, the hollow fiber was removed from the sample. Its closed ends were cut and one end put in glass vial then the acceptor phase (volume calculated by the segment length and inner diameter) was pushed out with a syringe employed 50 μL methanol from the other end; and finally 20 μL of the analyte-enriched solvent was injected into the HPLC for analysis directly without further manipulation. The used hollow fiber was discarded and a fresh one was used for the next extraction. All experimental results were the average of three or more times parallel experimental determination results. The operation of the extraction was evaluated by the concentration enrichment factor (*EF*) which was calculated by this formula: $EF=C_{after}/C_{before}$, where C_{after}/C_{before} represents the ratio of concentration after and before extraction. C_{before} was calculated from the calibration curve by determination of different concentrations of standard solutions into the HPLC system. Under the optimal conditions, C_{after} was determined by the proposed procedure and calculated from the standard curve then multiplied by the dilution multiple.

HPLC Analysis. Samples were analyzed by HPLC on a SHIMADZU SPA-20 instrument consisting of a dual pump, a column oven, a column (150 mm × 4.6 mm, i.d. 5 μm, Eclipse XDB-C18) and UV-detector with wavelength at 600 nm. A mixture of 60% acetonitrile and 40% CH_3COONa-CH_3COOH buffer (0.02 mol L^{-1}, pH 4.26) was used as mobile phase at a flow rate of 1.0 mL min^{-1}. The column oven was set at 30 °C.

Results and Discussion

Optimization of Microextraction Conditions. Considering the extraction agent was the key parameter that could influence extraction efficiency, [BMIM][PF_6], [HMIM][PF_6] and [C8MIM][PF_6] were selected to evaluate the extraction efficiency of the system, which used as supported phase during the three-phase HF-LPME, and the result shows that enrichment factors were 205, 67 and 27, respectively. This result is consistent with Guo and Lee's experiment which selected [BMIM][PF_6] from six kinds of ILs as supported phase and developed three-phase liquid-liquid-liquid solvent bar microextraction for the analysis of phenolic acids substances in seawater samples.[23] Compared to other ionic liquids studied here, the more hydrophobic of [BMIM][PF_6], as well as its insolubility in the aqueous solution, should contribute to its better extraction efficiency,[24] or borne out by its better performance. So [BMIM][PF_6] was chosen as suitable extraction solvent in the following study. In order to obtain better extraction performance, other parameters that could affect extraction performance were investigated, including accepter phase pH, sample pH, supported phase membrane, volume of accepter phase, salinity, extraction time.

pH of Donor and Acceptor Phase: In the three-phase HF-LPME procedure, the pH of the donor phase and accepter phase plays an important role in the extraction performance by adjusting the existing state of analyte which must be in non-ionized form in the donor phase to cross the IL membrane, and ensures that the analyte molecules are irreversibly trapped and thereby concentrated.[25] MG is a weak acid (pK_a = 6.9), so low pH value could avoid MG ionized.

The effect of donor phase pH 2-7 upon extraction efficiency was investigated. The results (Figure 1) showed that the peak area had no significant change at the range of pH 2-4, then jumped to maximum at pH 5 and decreased when pH value extended over 5. So pH 5 was selected in the following study.

As to acceptor phase aqueous, NaOH solutions with pH values ranged between 8 and 13 were added in the system to test the extraction performance. Figure 2 indicated that the maximum extraction efficiency was observed at pH 10. So aqueous NaOH solution with pH 10 was chosen as acceptor phase in the following study.

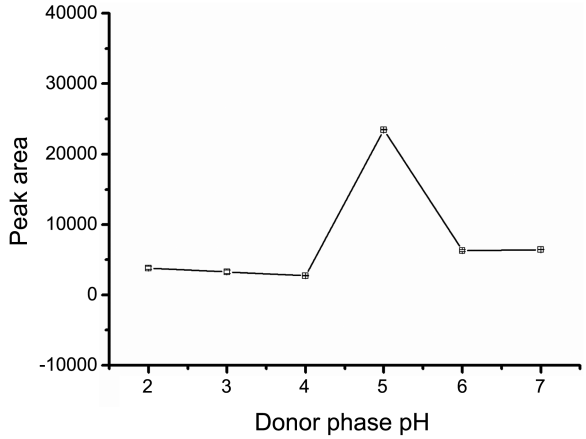


Figure 1. Effect of Donor phase pH on extraction efficiency. donor phase (DP): 20 mL, spiked concentration: 50 μg L^{-1}; acceptor phase (AP): 2.5 μL, aqueous NaOH solutions pH 10; supported phase: [BMIM][PF_6] added 1% TOPO (w/v); extraction time: 30 min; shaking speed: 200 rpm; temperature: 25 °C. Each point represents the average of three replicates.

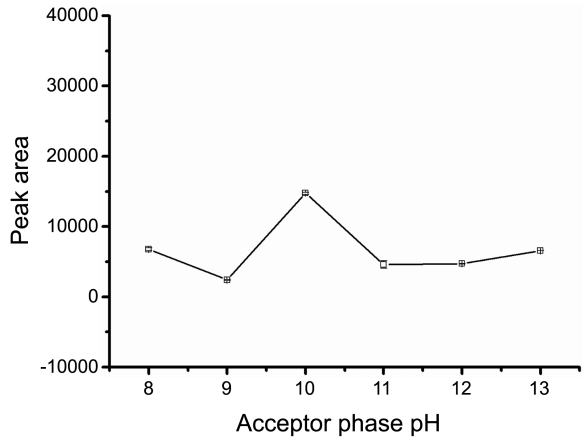


Figure 2. Effect of Acceptor phase pH on extraction efficiency. DP: 20 mL, spiked concentration: 50 μg L^{-1}; AP: 2.5 μL, aqueous NaOH solutions; supported phase: [BMIM][PF_6]; extraction time: 30 min; shaking speed: 200 rpm; temperature: 25 °C. Each point represents the average of three replicates.

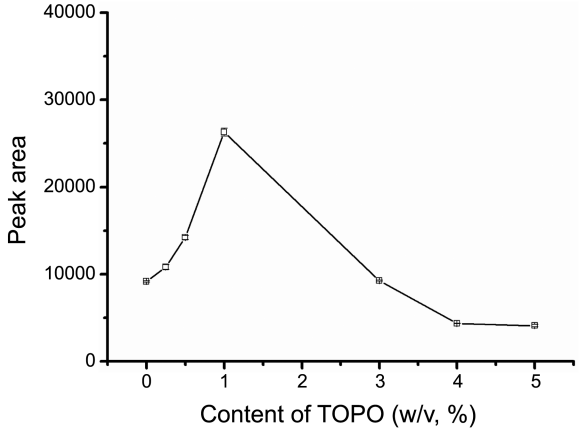


Figure 3. Effect of the concentration of TOPO in supported phase membrane on extraction efficiency. DP: 20 mL, spiked concentration: 50 μg L^{-1}; AP: 2.5 μL, aqueous NaOH solutions pH 10; supported phase: [BMIM][PF_6] added TOPO; extraction time: 30 min; shaking speed: 200 rpm; temperature: 25 °C. Each point represents the average of three replicates.

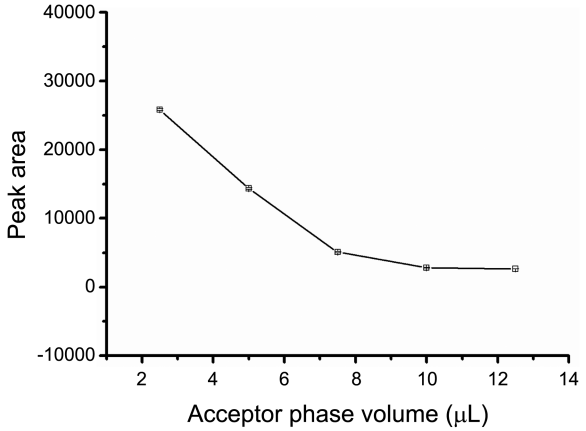


Figure 4. Effect of Acceptor phase volume on extraction efficiency. DP: 20 mL, pH 5, spiked concentration: 50 μg L^{-1}; AP: 2.5-12.5 μL, aqueous NaOH solutions, pH 10; supported phase: [BMIM][PF_6] added 1% TOPO (w/v); extraction time: 30 min; shaking speed: 200 rpm; temperature: 25 °C. Each point represents the average of three replicates.

Supported Phase Membrane: TOPO was known as a polar organic compound with low pK_a and K_{OW} through the hydrogen bonding between TOPO and target compounds improved enrichment factor.[26] In this study, TOPO was added as a carrier compound in the liquid membrane which is a innovation point compared with the similar study,[23] and the different concentrations (0-5%, w/v) was investigated in detail to obtain the best extraction efficience. It was seen from Figure 3 that the peak area increased at the range of 0-1%, reached maximum when the concentration was 1%, and decreased with TOPO in excess of 1%, so 1% TOPO in the member liquid was adopted for the subsequent experiments.

Effect of Acceptor Phase Volume: In a three-phase HF-LPME system, a smaller volume of acceptor phase involves a higher enrichment factor in the acceptor phase. The acceptor phase should be of a sufficiently large volume to promote analyte transport into the acceptor phase.[27] In considerarion of the limitations of the volume of hollow fiber lumen and the convenience of experimental operating, the influence of volume of acceptor phase was investigated in 2.5-12.5 μL. The result, shown in Figure 4, indicated that 2.5 μL of acceptor phase yields the maximum extraction efficiency and this volume was used in the following study.

Effect of the Saline Concentration in the Extraction Efficiency: In usual, salts would lead to a decrease of solubility of the analyte in solution, and improves the extraction efficiency depending on salting-out effect.[28,29] On the other hand, the salt addition can suppress the extraction by electrostatic interaction,[30] the occurrence of ion exchange procedure could enhance the solubility of [BMIM][PF_6] in aqueous phase and decrease the extraction efficiency.[31] The results in Figure 5 showed that the extraction efficiency decreased with improvement of salts, which indicated that the ion exchange effect played a major role in this system.

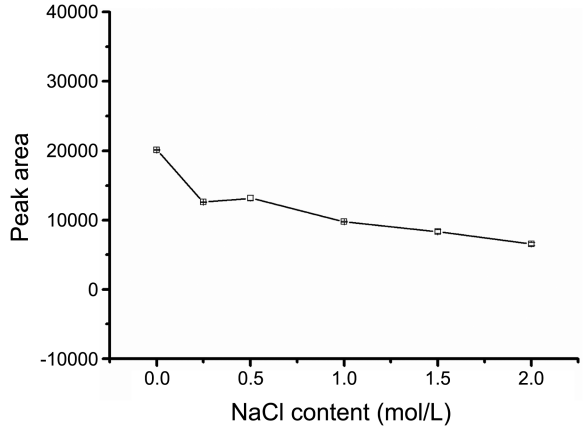


Figure 5. Effect of salinity on extraction efficiency. DP: 20 mL added NaCl 0-2.0 mol L^{-1}, pH 5, spiked concentration: 50 μg L^{-1}; AP: 2.5 μL, aqueous NaOH solutions pH 10; supported phase: [BMIM][PF_6] added 1% TOPO (w/v); extraction time: 30 min; shaking speed: 200 rpm; temperature: 25 °C. Each point represents the average of three replicates.

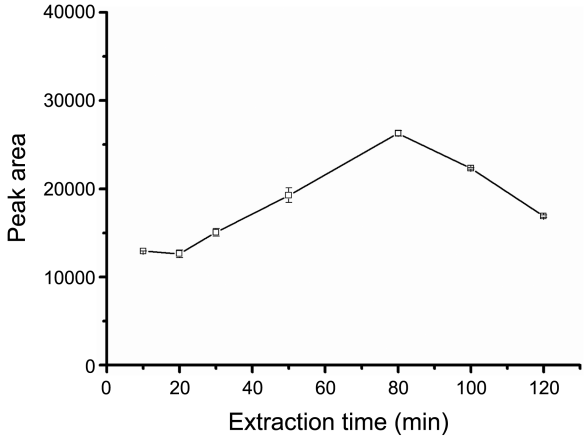


Figure 6. Effect of extraction time on extraction efficiency. DP: 20 mL, pH 5, spiked concentration: 50 μg L^{-1}; AP: 2.5 μL, aqueous NaOH solutions pH 10; supported phase: [BMIM][PF_6] added 1% TOPO (w/v); extraction time: 10-120 min; shaking speed: 200 rpm; temperature: 25 °C. Each point represents the average of three replicates.

So water samples served as donor phase no adding any salt in the following study.

Effect of the Extraction Time: HF-LPME is a kind of time-dependent method,[32] the extraction efficiency depends on the amounts of analytes transferred from donor phase to supported membrane and then acceptor solution. In most cases, the extraction efficiency would increase with longer extraction time before the extracting process reaches equilibrium.[33] In this study a series of extraction times over the range of 10-120 min was evaluated to obtain the equilibrium time. As shown in Figure 6, when extraction time exceed 80 min, no better performance was found, so 80 min was selected as the optimal extraction time.

Effect of Humic Acid: Natural dissolved organic matter (DOM) are commonly existed in the aquatic environment, which are heterogenous, hardly separable, mixtures of polyelectrolytes with varying molecular sizes, and it would potentially influence extraction efficiency.[34] This study examined the effect of humic acid (used as model DOM, 0-25 mg L^{-1}) to the extraction efficiency of MG during the three-phase HF-LPME procedure.[35] Figure 7 indicated that the extraction efficiency had no obvious change during 0-15 mg L^{-1}, however, when humic acid exceed 15 mg L^{-1}, the peak area declined. Actually, DOM in most of environmental waters are less than 15 mg L^{-1}, which displayed the method have good tolerance to this substance.

Method Validation. To estimate the propsosed method performance, calibration curves were plotted using 9 spiking levels of water ranged from 0.20 to 100 μg L^{-1} for MG. Each parameter was determined according to 5 replicates, as shown in Table 1, and good results were achieved as following:

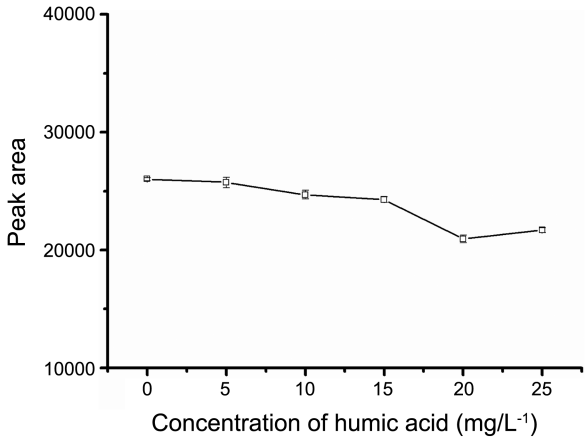


Figure 7. Effect of humic acid on extraction efficiency. DP: 20 mL, pH 5, spiked concentration: 50 μg L^{-1}; AP: 2.5 μL, aqueous NaOH solutions pH 10; supported phase: [BMIM][PF_6] added 1% TOPO (w/v); extraction time: 80 min; shaking speed: 200 rpm; temperature: 25 °C. Each point represents the average of three replicates.

enrichment factor (212), wide linear range (0.20-100 μg L^{-1}), low detection limit (0.01 μg L^{-1}) and reproducibility (relative standard deviation, MG, 8.9%). These results showed that the proposed approach could be used as an effective pretreatment technique to enrich and separate MG from waters. Furthermore, in comparison with solid phase extraction the most popular sample pretreatment method that has been used in the monitoring of MG (shown in Table 3), the established three-phases HF-LPME (HF-LLLME) indicated high enrichment factor, low detection limit and small

Table 1. The linear range, precision, detection limit and enrichment factors of the method

Analyte	Linear range (μg L^{-1})	Calibration curve	Precision (RSD, %, n=5)	Correlation coefficient, r^2	Enrichment factor	LOQ (μg L^{-1})	LOD (μg L^{-1})
MG	0.20-100	y = 547.39x + 62.79	8.9%	0.9908	212	0.15	0.01

Table 2. Analysis on the concentrations and spike recoveries of MG in samples by the proposed method (n = 3)

Water samples No.	Spiked (μg L^{-1})	Found /(μg L^{-1})	Recovery (± SD)/%
1[a]	0.00	3.72	–
	1.00	4.83	106.0 ± 6.4
	5.00	9.03	106.2 ± 4.7
	10.00	13.17	94.5 ± 6.5
2	0.00	ND	–
3	0.00	11.06	–
	1.00	12.00	94.0 ± 8.4
	5.00	14.92	87.2 ± 7.4
	10.00	19.48	84.2 ± 4.3
4	0.00	ND	–
5	0.00	ND	–
6	0.00	ND	–
7	0.00	1.98	–
	1.00	2.82	84.0 ± 9.6
	5.00	6.22	84.8 ± 3.3
	10.00	12.08	101.0 ± 9.8
8	0.00	ND	–
9	0.00	ND	–
10	0.00	1.73	–
	1.00	2.85	102.0 ± 2.8
	5.00	6.97	104.8 ± 5.0
	10.00	11.82	100.9 ± 8.1
11	0.00	ND	–
12	0.00	ND	–
13	0.00	ND	–
14	0.00	ND	–
15	0.00	ND	–

[a]1-6, water from pond; 7-15, water from river. ND: not detecte

Table 3. Characteristic performance data obtained by using the established method and solid phase extraction for determination of MG in water samples

Method	LOD (μg L^{-1})	RSD (%)	EF	Sample volume (mL)	Reference
MISPE[a]	0.1	4.15	10	1000	[36]
SPE	0.28	1.6	50	100	[37]
MSPE	0.5-1	4.2	–	1000	[38]
IL-HF-LLLME	0.01	8.9	212	20	The established method

[a]Molecular imprinting solid phase extraction (MISPE)

amount of water sample.

Application. To test the reliability of the proposed methodology, this method was applied to detect MG in Yangtze River and pond waters in Zhenjiang, Jiangsu province (China). To evaluate the veracity of the analysis, water samples were spiked with three concentrations of MG (1, 5 and 10 μg L^{-1}). It could be known from Table 2 that MG were found in 4 sampling sites ranged at 1.73-11.06 μg L^{-1}, and our results also displayed that the concentrations of MG in Pond waters are higher than in Yangtze River. Representative chromato-

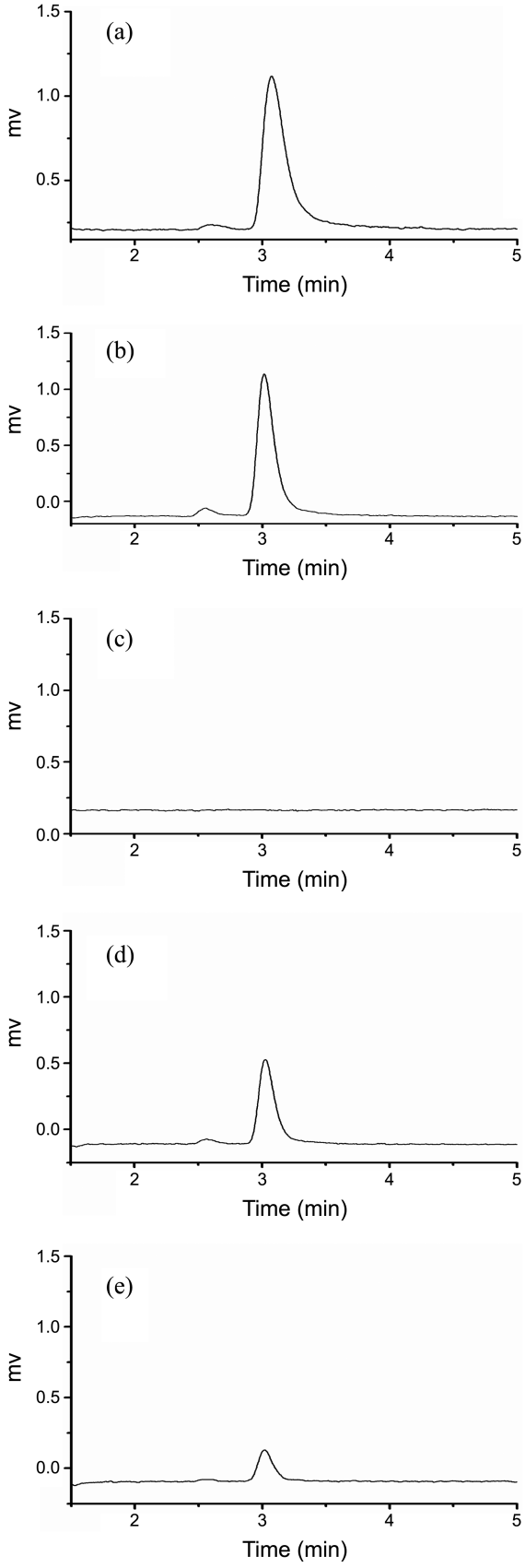


Figure 8. Representative chromatograms. (a) calibration standard solution of MG at 0.5 mg L^{-1}; (b) water sample fortified with MG at 1 μg L^{-1}; (c) blank control; (d) pond water sample; (e) river water sample.

grams of calibration standard solution of MG at 0.5 mg L^{-1}, water sample fortified with MG at 1 μg L^{-1} and water samples are shown in Figure 8.

Conclusion

In this paper, a novel three-phase HF-LPME was established for determination of trace MG in surface waters, and [BMIM][PF_6] mixted with 1% TOPO (w/v) was used as supported phase membrane, after extraction parameters were optimized, the method was demonstrated: (i) simple pre-separation procedures with low cost; (ii) low detection limits (0.01 μg L^{-1}) and good linear range (0.20 to 100 μg L^{-1}); (iii) good reproducibility (relative standard deviation, 8.9%; n=5) and good recoveries (84.0-106.2%); (iv) high enrichment factor (212); which indicated that the HF-LPME method has great potentials for the determination of MG in various environmental waters.

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