

Enrichment and Determination of Trace Estradiol in Environmental Water Samples by Hollow-Fiber Liquid-Phase Microextraction Prior to HPLC

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Abstract

This paper presents a novel and simple cleanup procedure based on hollow fiber liquid-phase microextraction (HF-LPME) for the determination of trace estradiol in environmental. Estradiol was extracted from a 140-mL water sample (the donor phase) into the pores of the hollow fiber wall organic solvent, then into the organic solvent (the acceptor phase) in the lumen of the hollow fiber. Afterwards, the hollow fiber was eluted with methanol to capture estradiol from the acceptor phase. Different experimental parameters, including the organic phase type and its volume, compositions of the donor phases, ionic strength, stirring rate, temperature, and the extraction times were controlled and optimized based on the response of the HPLC instrument. Under the optimized experimental conditions, the proposed method was found to be linear in the concentration of 1–1000 ng/mL for estradiol, and the limit of detection was 0.1 ng/mL. Furthermore, the method provided a good enrichment factor of 300, and repeatability (relative standard deviation = 5.5). Finally, the proposed method was applied for the analysis of real environmental samples.

Introduction

For many years, it has been known that a wide range of organic micropollutants of anthropogenic origin are present in wastewater (1), and recently those with endocrine disrupting ability have become the focus of attention. It has been estimated that over 99% of the estrogenic activity in environmental water may be attributable to the presence of free steroid estrogens (2). As presently operated, the ability of wastewater treatment to remove steroid estrogens is limited (3,4). Once in receiving waters, the compounds have the potential to bio-concentrate (5) and accumulate in organisms (6). Estradiol, as one of the steroid estrogens, has a critical role in the development of breast cancer, which has been postulated for more than a century, ever since Herman demonstrated that oophorectomy induced tumor remission in human breast cancer (7). Substantial evidence supports a causal relationship between the risk of human breast

cancer and the levels of estrogen (8,9). On the other hand, estradiol can be transferred into a series of metabolites which are related to endometrial cancer or breast cancer (10). Hence, estradiol would pose an important threat to human health by polluting the living environment, especially water. In order to protect human health and environmental safety, it is very essential to establish simple and sensitive methods for monitoring low levels of estradiol in environmental water.

Radioimmunoassay (11,12) and high-performance liquid chromatography (HPLC) (13,14,15,16) methods are often employed for the determination and confirmation of the residual estradiol in various samples. However, in the last few years, the use of LC coupled with mass spectrometry detector (LC–MS) (17,18,19,20), and gas chromatography coupled with mass spectrometry detectors (GC–MS) (21,22,23) have improved the sensitivity and have been rapidly accepted techniques in estradiol analysis. Nevertheless, one of the main problems associated with this combination is the ion suppression due to matrix effects, so the selected extraction technique should minimize this effect (24).

Traditionally, routine methods involve several sample preparation steps such as extraction, cleanup, and concentration before instrumental analysis, using liquid–liquid extraction (LLE) or solid phase extraction (SPE) as extraction techniques (25,26,27). However, the main drawback of SPE or LLE is that they are time- and labor-intensive procedures and require large amount of high purity organic solvents, which are expensive, toxic and considered as environmental pollutants (28). In recent years, miniaturized techniques such as: solid phase microextraction (SPME) (29), supported liquid membrane (SLM) extraction (30,31), and liquid phase microextraction (LPME) (32) have been reported as alternatives to the conventional ones.

In 1992, S.B. Hawthorne introduced SPME (33) as a solvent-free extraction method, which has been used for the analysis of volatile and semi-volatile analytes (34). However, this method has some disadvantages; for example, SPME fibers are still comparatively expensive and have a limited lifetime. Additionally, the coatings currently available for SPME are either nonpolar or slightly polar; hence, SPME cannot be satisfactorily used for highly polar analytes. Furthermore, when SPME is coupled to HPLC, a special SPME–HPLC interface device has to be used for the solvent desorption (35,36).

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By using the LLE at the miniaturized scale (31,37), the disadvantages of SPME can be omitted here. In the LPME, the analytes are extracted from a sample matrix into a micro drop organic solvent as an acceptor phase, which is suspended on the needle tip of a microsyringe immersed in the stirred sample solution (37). The acceptor phase may also be contained inside the lumen of a hollow fiber and immobilized inside the wall pores of the hollow fiber (32). Among these techniques, LPME using hollow fiber membranes (HF-LPME) (32) provides mechanical stability and protection to the organic phase because the use of a membrane or hollow fiber. It is simple, effective, and low-cost, due to the agitator that is reused, and the HF used at just 1 RMB for 100 cm. Meanwhile, it uses micro liters of organic solvents, provides excellent sample cleanup ability, and obtains very clean extract. One of the main advantages of HF-LPME over SPME is that the acceptor solution was effectively protected. Besides, HF-LPME can also show some selectivity because of the pores in its wall. In this sense, large molecules, which can be soluble in extracting solvent, may not be extracted.

HF-LPME has been successfully used for the extraction of pesticides (38), aromatic amines (39), tetracycline antibiotics (32), metal (40), non-steroidal anti-inflammatory (41), and benzophenone (42), et al. As far as is known, there has been no report on the combination of HF-LPME and HPLC for the determination of estradiol in water samples.

Environmental water was regionally polluted by dejecta of livestock which had seriously influenced the security of water resources. This phenomenon usually occurred in undeveloped regions, such as rural areas, where low technological and poor testing capabilities exist. Therefore, it is necessary to establish a simple, rapid, and inexpensive method without well-trained analysts for the elimination of regional and accidental pollution events. M.H. Liu et al. (43) proposed a method using three-phase HF-mediated LPME for the determination of synthetic estrogens. However, static three-phase HF-LPME may cause poor reproducibility because of its tedious operation and multiple factors. To overcome these shortages, a two-phase, dynamic, and sensitive HF-LPME procedure in combination with HPLC-UV was developed for the extraction and determination of estradiol in environmental water samples.

It efficaciously reduced the instability of the acceptor solution which is prone to release from the HF to the liquid extract. Meanwhile, it also earned many merits such as simplicity, ease of operation, low-cost, suitability for developing regions, etc. The parameters affecting the extraction efficiency were investigated and determinations were carried out under the most appropriate conditions.

Experimental

Reagents and materials

An analytical standard of estradiol was purchased from Sigma-Aldrich (Beijing, China). *N*-octyl alcohol was obtained from Heng Xing (Tianjin, China). HPLC-grade methanol was provided by Tedia (Shijiazhuang, China). Ultrapure water was prepared in the lab using Ultra-Clear (18.2 M Ω /cm, Heal Force) and all the other solvents were analytical reagent grade unless otherwise stated.

Standard solutions of 10 μ g/mL were prepared using stock standards at 1 mg/mL in HPLC-grade methanol. All the standard solutions were stored at 4°C in the refrigerator. All glassware used in the experiments was cleaned with ultrapure water. Ten M of hydrochloric acid was used for adjusting the pH value of the water samples.

For HF-LPME, the polyvinylidene difluoride (PVDF) (FoShan, China) to the HF membrane was used. The wall thickness of this fiber was 200 μ m, and the inner diameter was 1000 μ m.

Instruments and apparatus

An HPLC system, which consisted of a Spectra-physics 8810 precision isocratic pump (Spectra Physics), and a Biosystems 785A programmable absorbance detector (Applied Biosystems) was used for the analysis and separation. A reversed-phase Kromasil C₁₈ column (150 mm \times 4.6 mm i.d., particle size 5 μ m) was used for the separation at ambient temperature, and a Qianpu CT22 for the LC system was employed to acquire and process the chromatographic data. The mobile phase was a mixture of methanol–water (75:25, v/v), delivered at a flow rate of 1.0 mL/min; the injection volume was 20 μ L, and the detection wavelength was set at 280 nm.

Sampling

The tap water sample was freshly collected from the laboratory. An aquaculture water sample was obtained from a fishpond in Shijiazhuang (Hebei, China). Other water samples were surface water collected from the MinXin River in the Shijiazhuang suburbs. All samples were stored in 1 L glass bottles at 4°C.

HF-LPME procedure

The optimization procedure was conducted using 20 ng/mL standard solutions. The extraction and pre-concentration procedure are described as follows: (i) the hollow fiber was cut into segments with a length of 11 cm. The fiber segment was cleaned with acetone by ultrasonication for 2 min to remove any possible impurities and directly dried in air; (ii) the fiber was submerged in the *n*-octyl and ultrasonicated for 10 min to fill the membrane pores of the HF wall with *n*-octyl. After that, using a Micro-Fine Syringe, 100 μ L *n*-octyl was injected into the lumen of the fiber, to make sure that the lumen was full of *n*-octyl. Then, the two ends of the fiber were enveloped with a capillary, which was sealed by heating and had a similar volume in comparison to the lumen of the fiber. The two ends were binded by a strand and introduced into a 140-mL glass bottle with a screw cap. It is worth noting that the fiber must be fully immersed in the aqueous water. The extraction and the enrichment was performed by agitation using a YL-1000 agitator (Shanghai Hengxing Co., Shanghai, China) at 500 rpm at 25°C for 1 h. At the end of the extraction, the HF extraction device was taken out from the bottle and both of the sealed ends were carefully cut, and the organic solvent was carefully withdrawn into the micro-syringe. Then, 100 μ L methanol was slowly flushed through the lumen to transfer the estradiol in the membrane phase into a clean and dry polytetrafluoroethylene insert tube. The extracted solution was evaporated to dryness at 90°C under nitrogen gas for about 20 min. After the sample dried, 100 μ L methanol was added to redissolve before HPLC analysis with an injection volume of 20 μ L.

HPLC analysis

A reversed-phase Diamonsil C₁₈ column (150 mm × 4.6 mm i.d., particle size 5 μm, Dikma Technologies, Lake Forest, CA) was used as the analytical column. HPLC separation was conducted by using a mixture of methanol–water (75:25, v/v) at a flow rate of 1 mL/min, and the detection was carried out at a wavelength of 280 nm. Aliquots of 20 μL of the sample extract were injected.

Results and Discussions

Various parameters were investigated to determine the optimal sample extraction procedure. All the optimization experiments were performed in water at an estradiol concentration of 20.0 ng/mL.

Selection of acceptor phase

In order to obtain an efficient extraction, the type of organic solvent used in LPME is an essential factor to be considered. Generally, there are several requirements for the choice of organic solvent. Firstly, it should be able to provide a high distribution coefficient for the target analytes. Secondly, it should have a low solubility in water and non-volatile to prevent solvent loss during extraction, especially when faster stirring rates and longer extraction time are applied. Finally, it should have a polarity matching with the polyvinylidene difluoride (PVDF) HF in order to enhance the transfer of analytes into the acceptor phase, since extraction occurs on the surface of the immobilized organic solvent. In this work, four kinds of organic solvents, including toluene, isooctane, *n*-butyl ether, and *n*-octanol were investigated for the extraction of estradiol; five repetitions of each solvent were done at the same time (relative standard deviation: 4.8–5.7%). The obtained results for estradiol are shown in Figure 1. When *n*-octanol was used as the organic solvent, better results were obtained. It can be observed that lower levels were obtained when toluene, isooctane, and *n*-Butyl ether were used, maybe due to the volatility of these solvents. Therefore, *n*-octanol was selected as the extraction solvent for HF-LPME in the following experiments.

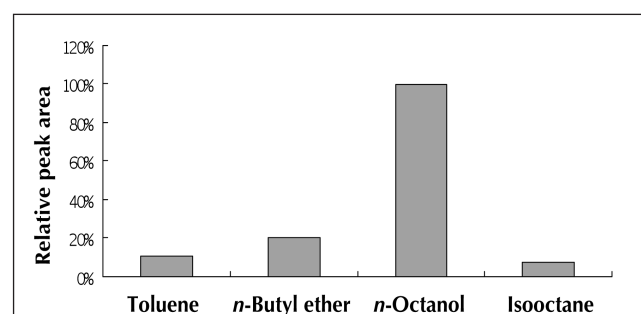


Figure 1. The effect of the organic solvent on the extraction efficiency of estradiol. Extraction conditions: aqueous sample, 140 mL of deionized water with the pH of 3.0; stripping solvent, methanol; stirring speed, 500 rpm; extraction time, 60 min; extraction temperature, 25°C; 10% (w/v) NaCl and 30 μL of *n*-octyl alcohol to the aqueous sample.

Selection of the pH conditions

To obtain a high extraction efficiency for the weak acid compound estradiol, the sample solution should be acidified to effectively deionize the analyte, consequently reducing the water solubility and increasing the extractability. Hydrochloric acid was used to adjust the sample pH to the target value, measured by the pH meter. The best extraction efficiency for estradiol from water was observed at pH 2–5 (Figure 2). The reason is that the compound, which is a weak acid, was not molecular at an alkaline environment. Hence, pH 2–5 was selected for the subsequent analysis.

Stirring rate

In HF-LPME, the change of flow rate plays a role in affecting extraction dynamics, decreasing the thickness of the interfacial layer surrounding the solvent droplet, increasing the mass transfer of the analytes, and speeding up the extraction (44–46). In the current work, the two-end sealed, round HF was laid in the sample solution, and could rotate around a symmetrical axis when the magnetic stirrer was switched on. The free movement of the fiber contributed to the mass transfer process. Therefore, the stirring speed was also optimized for better extraction. The stirring speed was in the range of 100–1000 rpm. Agitation increased the extraction efficiency, but in very high speed (more than 500 rpm) a vortex was created in the sample solution, which reduced the effective extraction surface between the HF and the aqueous sample, and also lost solvent that could have affected the precision. Consequently, the stirring speed of 500 rpm was selected as a suitable agitation speeds in the experiments (Figure 3).

Extraction temperature

Temperature affected the kinetics of the extraction. At higher temperatures, the diffusion coefficient of the analyte increased and the viscosity of the organic membrane decreased, thus the time required to achieve equilibrium decreased. On the other hand, higher temperatures led to some practical difficulties, such as the instability of the acceptor phase and the reduction of the organic phase. Therefore, in order to achieve a stable and considerable acceptor phase, the temperature was adjusted at 25°C in further studies.

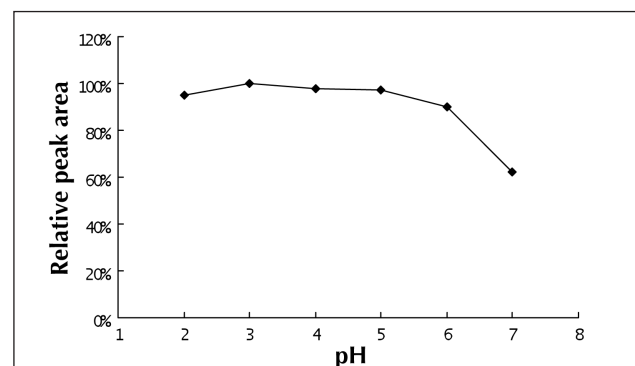


Figure 2. The effect of pH on the extraction efficiency of estradiol. Extraction conditions: aqueous sample, 140 mL of deionized water; organic solvent, *n*-octanol; stripping solvent, methanol; stirring speed, 500 rpm; extraction time, 60 min; extraction temperature, 25°C; 10% (w/v) NaCl and 30 μL of *n*-octyl alcohol to the donor phase.

Addition of *n*-octyl alcohol to the donor phase

The application of HF-LPME, which was utilized in the aqueous sample, usually is preferred to the LLE method, which is always used for the extraction of organic solvents. It was decided to add a co-solvent in the donor phase and decrease the release of *n*-octyl alcohol from the HF to the liquid extract. Similar experimental strategies have been applied in other studies (47). Accordingly, different content of *n*-octyl alcohol, varying in the range of 0 to 50 μL , were added into a 140 mL aqueous solution and the mixture was agitated for 60 min. Figure 4 depicts the results of this study. As shown, the presence of *n*-octyl alcohol enhanced the extraction of estradiol, reaching maximums at 30 μL , after which the response of the instrument was found to decrease. Although increasing the amount of co-solvent was expected to increase the total amount of *n*-octyl alcohol extracted, the presence of the high concentrations of the co-solvent were also expected to substantially decrease the diffusion of the analyte into the acceptor phase during the HF-LPME cleanup step. Therefore, there must be a tradeoff between the extraction efficiency of the target analyte and the transfer of the

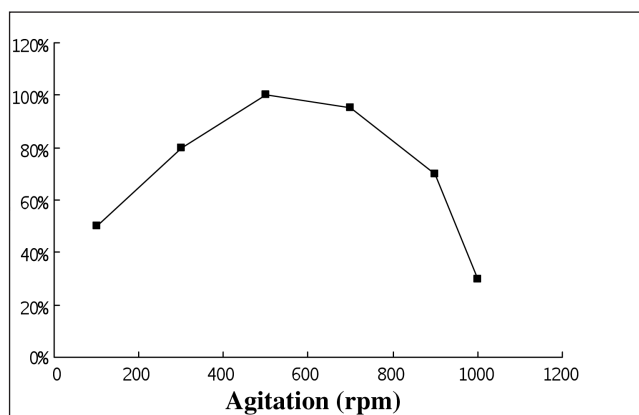


Figure 3. The effect of agitation on the extraction efficiency of estradiol. Extraction conditions: aqueous sample, 140 mL of deionized water with the pH range of 3.0; organic solvent, *n*-octanol; stripping solvent, methanol; extraction time, 60 min; extraction temperature, 25°C; 10% (w/v) NaCl and 30 μL of *n*-octyl alcohol to the donor phase.

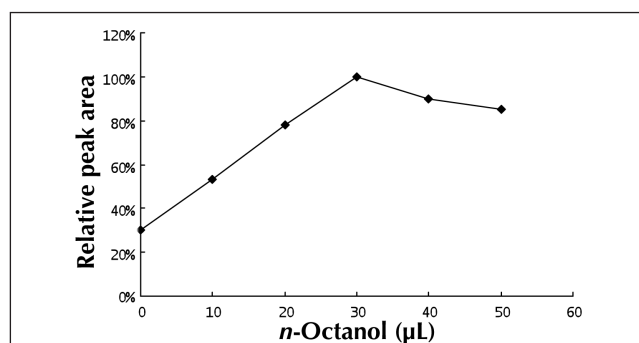


Figure 4. The effect of *n*-octyl alcohol in the donor phase on the extraction efficiency of estradiol. Extraction conditions: aqueous sample, 140 mL of deionized water with the pH range of 3.0; organic solvent, *n*-octanol; stripping solvent, methanol; stirring speed, 500 rpm; extraction time, 60 min; extraction temperature, 25°C; 10% (w/v) NaCl.

analyte across the membrane; hence, the optimum co-solvent concentration should correspond to the maximum overall sensitivity of the HF-LPME procedure. Hence, it was decided to use 30 μL *n*-octyl alcohol added into a 140 mL aqueous solution for the extraction. The present finding is similar to the work of Jingfu Liu et al. (47), who developed an HF-LPME based method used for the analysis of partitioning coefficients and acid dissociation constants.

Salt effect

In general, the addition of salt to the sample and accordingly increasing the ionic strength of the aqueous solution may have several effects on HF-LPME, mainly due to the salting-out effect (44). In a separate set of experiments, the effect of the ionic strength on the HF-LPME cleanup step was investigated by adjusting the salt content of the agitated extract to values ranging from 0 to 20% (w/v) NaCl. All other experimental parameters were similar to the ones previously described. Figure 5 shows the results of this study. As shown, the response of the instrument increased whilst increasing the salt content of the aqueous samples up to 10% (w/v) NaCl, and then decreased as the ionic strength of the solution increased. This phenomenon may be attributed to the decrease of the sample viscosity when a great quantity of salt was added. Therefore, a 10% (w/v) NaCl content was selected for all subsequent experiments.

Extraction time

In general, mass transfer is a time-dependent process, and the maximum absorbance signal is attained when the system is at an equilibrium. Since in some range the equilibrium is steady and satisfied for reproducibility and precise analysis, the complete equilibrium, which needs a long time, was not necessary. The effect of the sampling time upon the HF-LPME cleanup step was investigated and the results are depicted in Figure 6, where the response of the analytical instrument is given as a function of time (ranging from 0 to 120 min). As can be seen, the extraction efficiencies of estradiol enhanced with increasing the extraction time, and reached equilibrium at 60 min. Thus, it was decided to use a 60 min sampling time.

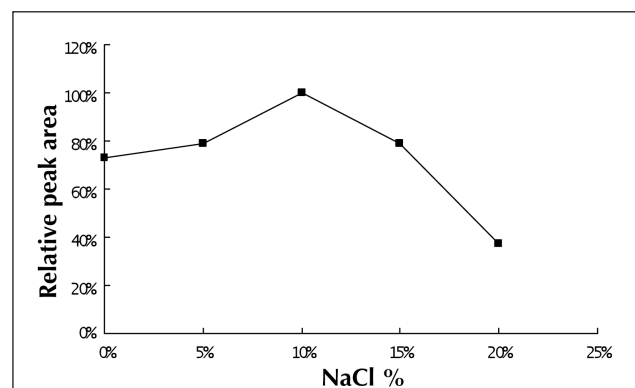


Figure 5. The effect of salt upon the extraction efficiency of estradiol. Extraction conditions: aqueous sample, 140 mL of deionized water with the pH range of 3.0; organic solvent, *n*-octanol; stripping solvent, methanol; stirring speed, 500 rpm; extraction time, 60 min; extraction temperature, 25°C; 30 μL of *n*-octyl alcohol to the donor phase.

Performance of the method

The quantitative parameters of the proposed HF-LPME method were calculated under the optimized conditions described in the previous sections [aqueous sample: 140 mL of water with the pH 3.0; organic solvent: *n*-octanol; stripping solvent: methanol; stirring speed: 500 rpm; extraction time: 60 min; extraction temperature: 25°C; 10% (w/v) NaCl and 30 μ L of *n*-octyl alcohol to the donor phase]. The calculated figures of merit are summarized in Table I. Each standard sample was extracted by the proposed method under the optimized conditions. For each level, three replicate extractions were performed at the same time. The calibration curve was obtained by plotting the

LOD (limit of detection) (ng/mL)	0.1	
Regression equation	$A = 1579C + 7171$	
Slope \pm SD	0.0238 ± 0.0009	
Intercept \pm SD	0.0124 ± 0.0011	
DLR (ng/m ³)	1–1000	
R ²	0.9995	
EF (enrichment factor)	300	
RSD% ($n = 6, 20$ ng/mL)	5.5	
Recovery ($n = 5, R\%$)	5 ng/mL	120.5 (7.3%)
(RSD: relative standard deviation)	100 ng/mL	100.9 (4.7%)
	500 ng/mL	97.5 (5.1%)

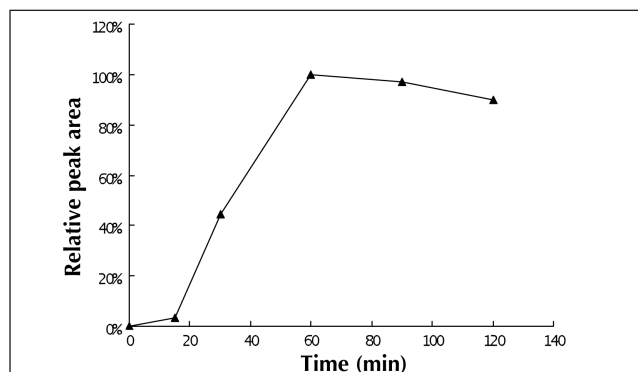


Figure 6. The effect of sampling time upon the extraction efficiency of estradiol. Extraction conditions: aqueous sample, 140 mL of deionized water with the pH range of 3.0; organic solvent, *n*-octanol; stripping solvent, methanol; stirring speed, 500 rpm; extraction temperature, 25°C; 10% (w/v) NaCl and 30 μ L of *n*-octyl alcohol to the donor phase.

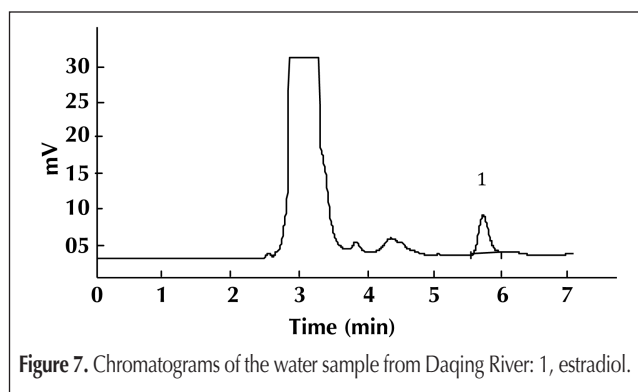


Figure 7. Chromatograms of the water sample from Daqing River: 1, estradiol.

peak areas of estradiol against the concentration of the estradiol in the aqueous sample. The linear range was 1–1000 ng/mL, with a correlation coefficient of 0.9995 by using a weighted linear regression method. The calibration equation is shown in Table I, where C is the concentration of estradiol in ng/mL.

The repeatability of the method, expressed as relative standard deviation, was calculated from six replicates of a river water sample containing the analyte at a concentration of 20 ng/mL, and it was achieved at 5.5%. The limit of detection was calculated according to the $S/N = 3$ ratio, using, in this case, analyte-free samples spiked with the analytes. The obtained value was 0.1 ng/mL.

The recovery studies were performed by analyzing the proposed method for diverse, analyte-free samples, and environmental samples spiked with the analytes at different concentration levels (5, 100, and 500 ng/mL). The obtained peaks areas for each analyte were interpolated in the calibration graphs constructed using standards. In this case, the recovery value was calculated by the known equation: $R\% = [(analyte\ found / analyte\ added) \times 100]$. The average values are summarized in Table I.

Application to environmental samples

Six environmental water samples, including tap water, the Minxin River, the Jing River, the Taiping River, a polluted pool (in the suburban area of Shijiazhuang, China), and aquaculture water (collected in Tian Le Cauf, Shijiazhuang, China) were studied using the developed method. The concentration of estradiol in the water sample from a Minqing pool, which had been polluted by excrement and urine in a suburban area, was 45.8 ng/mL, and is shown in Figure 7; however, no target compound could be detected from the other environmental water

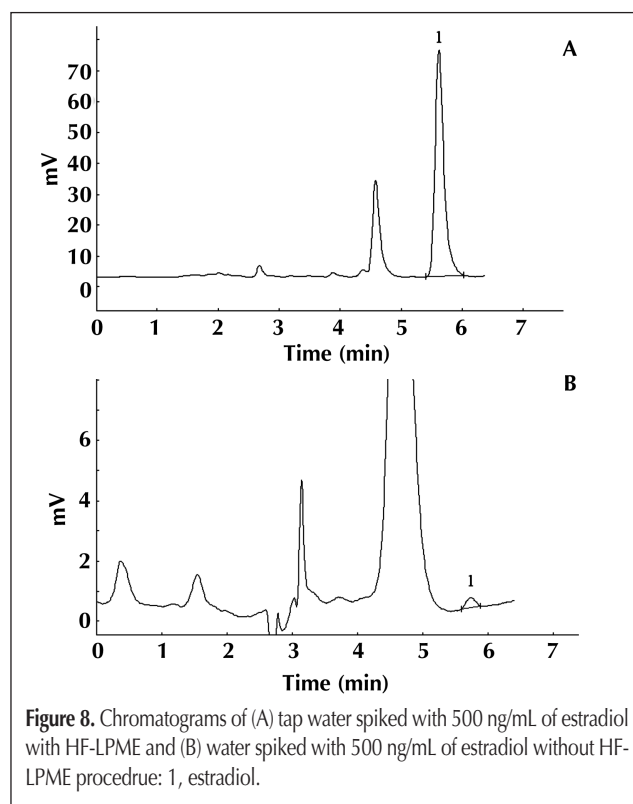


Figure 8. Chromatograms of (A) tap water spiked with 500 ng/mL of estradiol with HF-LPME and (B) water spiked with 500 ng/mL of estradiol without HF-LPME procedure: 1, estradiol.

Table II. Comparison of Figures of Merit of the Proposed Method with the Other Method for the Analysis of Estradiol*

Method	Sample preparation	Derivation or not	LOD (ng/mL)	LDR (ng/mL)	R	Time (min)	Volume of organic solvent (mL)	Volume of sample (mL)	EF	Ref
LC-MS-MS	LLE-SPE	Y	0.0004	0.001-0.4	0.968	60	10.58	1.0	-	(17)
LC-MS-MS	LLE	Y	0.08	0.08-10.24	0.99	-	16	2.5	-	(18)
LC-MS-MS	SPE	N	0.0012-0.0051	-	-	-	-	-	-	(19)
HPLC	SPE	Y	2.7	2.7-10000	0.9996	-	0.5	0.5	-	(14)
GC-MS	LLE-SPE	Y	0.3	-	0.996	-	-	6	-	(22)
CLEIA	SPE	Y	0.001	0.0025-2	0.9965	> 240	23	10	-	(49)
MEKC	LLE	N	1000	3000-105000	0.9959	-	-	-	-	(45)
HPLC	SDME	N	0.4	1-200	0.9991	50	0.006	6	343	(15)
LC-MS-MS	SPME	N	0.007	0.01-0.2	0.9997	-	-	-	-	(20)
HPLC	SPME	N	0.04	0.2-50	0.9970	-	-	-	-	(16)
GC-MS	SPME	Y	0.0007	0.005-0.5	0.9980	-	-	-	-	(23)
proposed	HF-LPME	N	0.1	1-1000	0.9995	60	0.05	140	300	-

* LLE: liquid-liquid extraction; pSFC: packed column supercritical fluid chromatography; SPE: solid-phase extraction; CLEIA: chemiluminescent enzyme immunoassay; MEKC: micellar electrokinetic capillary chromatography; SDME: single-drop microextraction, SPME: solid-phase microextraction; Y: derivation; N: not derivatized; LOD: limit of detection; RSD: relative standard deviation; EF: enrichment factor.

samples. Therefore, six tap water samples were spiked with 700 µg/mL of estradiol to 100 µL, respectively. The chromatograms are shown in Figure 8 including (A) with the HF-LPME procedure, which has a high enrichment and a high cleanup efficiency, enriching 140 mL of tap water with 500 ng/mL of estradiol, and (B) without HF-LPME. For the comparison, the enrichment factor was known to be 300.

Table II compares the figures of merit generated by the proposed method and alternative methods for the extraction of estradiol from different samples. The HF-LPME method proposed shows a more widespread application in comparison with other methods except SPE-HPLC. The extraction time was significantly shorter than LLE-SPE-GC-MS (48) and SPE-CLEIA (49), although it was relatively longer than SDME-HPLC. The experiment time was shortened by carrying out simultaneous extractions. In the present work, many samples were extracted together. Although the developed method has less sensitivity (higher LOD) than other methods, reliable measurements of estradiol can be performed with the important difference that other methods are more tedious to derivatisation and more expensive to purchase and use. The proposed method is relatively sensitive and is satisfactory for the determination of estradiol in environmental samples. The most important factor is that if the high enrichment HF-LPME produced by the proposed method with LC-MS-MS is necessary, it will be much more sensitive than any other methods.

Conclusions

A HF-LPME method was developed for the determination of estradiol in environmental samples. Parameters such as organic phase type and its volume, compositions of the donor phases, ionic strength, stirring rate, temperature, and extraction times were studied and investigated. Compared with the reported sample preparation techniques, the proposed method seems to

be more preferred for its simplicity, analytical precision, cost effectiveness, minimization of organic waste, absence of memory effect (owing to the use of fresh HF), and there is no need for tedious steps such as with traditional LLE, which has the steps of phase separation and re-dissolution. Finally, it is concluded that this method is an effective technique for the enrichment of estradiol from environmental samples, and only simple sample preparation and UV-HPLC can satisfy the determination of estradiol for the elimination of regional and accidental pollution events in undeveloped regions.

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