



Full Length Research Article

DETERMINATION OF DIETHYLSTILBESTROL IN WATER SAMPLES BY SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION-UV-VIS

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ABSTRACT

A salting-out assisted liquid-liquid extraction (SALLE) technique was successfully developed for extraction diethylstilbestrol (DES) in water samples followed by ultraviolet visible spectrophotometry (UV-Vis) analysis. Several parameters affecting the extraction, including volume of salting-out solvent, the pH of sample solution and salting-out solvent, were optimized. The optimal salting-out parameters were obtained as follows: 2 mL of acetonitrile was added to 2 mL of sample solution with pH = 3 and then 5 mL salting-out solvent containing 5 mol/L sodium chloride at a pH of 2 was added to the solution for extraction. Under optimal SALLE-UV-Vis conditions, detection limits of 0.61 µg/mL and good linearity in the range from 2.0 µg/mL to 20 µg/mL with correlation coefficient $r = 0.9990$ was achieved. Satisfactory recoveries ranging from 81 to 102 % were obtained with lake and tap water samples.

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INTRODUCTION

Diethylstilbestrol (DES) has been used to treat various symptoms and have been used as growth promoters or oral contraceptives (Xu *et al.*, 2011). However, the illegal addition and abuse of DES has caused increasingly serious effects on the health of organisms because of its potential carcinogenic properties and other adverse effects (Vandenberg *et al.*, 2012). Also, many studies have confirmed the presence of DES at levels of toxicological concern in aquatic environments (Kuster *et al.*, 2004). Therefore, it is imperative to urgently develop simple, fast, and effective methods for monitoring the presence of and determining DES. Recently, several methods have been developed for the determination of DES including liquid chromatography (LC) (Xu *et al.*, 2011), gas chromatography (Vandenberg *et al.*, 2012), ultraviolet visible spectrophotometry (UV-Vis) (Kuster *et al.*, 2004) *etc.*. However, the concentration of DES in samples is very low and the matrix of some samples are complex.

In all the above work, many extraction methods such as liquid phase microextraction (Zou *et al.*, 2015), stir bar sorptive extraction (Hu *et al.*, 2013), molecularly imprinted solid phase microextraction (Lana *et al.*, 2014), membrane-assisted solvent extraction (Iparraguirrea *et al.*, 2014), solid phase extraction (Pérez-Fernández *et al.*, 2014) and dispersive solid phase extraction (Qiao *et al.*, 2014) have been used. Most of these methods are often complicated and time spending, e.g. the need of special instrument and the synthesis of the solid sorbent. Fortunately, salting-out assisted liquid-liquid extraction (SALLE) provides a sample preparation method which is a technique based on liquid liquid extraction in which an appropriate concentration of salt is added to achieve the separation of aqueous phase from the partially miscible organic phase (Qiao *et al.*, 2014) and simultaneously the target analytes are extracted into the separated upper organic phase. This method coupled sample clean-up (e.g. acetonitrile deproteinization) with enrichment (via salting-out extraction) has been reported for water (Liu *et al.*, 2010), biological (Wang *et al.*, 2011 and Zhao *et al.*, 2012), food (Razmara *et al.*, 2011 and Zhang *et al.*, 2011), meat (Tsai *et al.*, 2010) and drug (Noche *et al.*, 2011) sample extraction processes. In this study, we propose the use of SALLE for extraction of DES

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from two water samples, and ultraviolet visible spectrophotometry (UV-Vis) for determination of DES. To the best of our knowledge, this is the first demonstration for SALLE-UV-Vis for DES analysis.

MATERIALS AND METHODS

Reagents and Instruments

DES were purchased from Sigma-Aldrich (Steinheim, Germany), HPLC-grade methanol (MeOH) and acetonitrile (ACN) were provided by J&K Chemical (Beijing, China). NaCl, NaH_2PO_4 , H_3PO_4 , NaOH, and other affiliated chemicals were all obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solvents and chemicals were of analytical grade and used without further purification unless otherwise specified. A salting-out solution of 5 mol/L NaCl in 100 mmol/L phosphate buffer, and the pH was adjusted by 1 mol/L H_3PO_4 and NaOH. HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA), and was used throughout the work.

An Hitachi U-2910 UV-Vis spectrometry was provided by Hitachi Instrument Inc. (Hitachi, Japan). All the samples were passed through microporous nylon filters of 0.45 μm pore sizes in diameter (Pall Corporation, USA).

Preparation of standard and sample

Standard stock solutions containing 1000 $\mu\text{g}/\text{mL}$ of DES was prepared by dissolving the required amounts of the standard in MeOH. They were stored in a refrigerator at 4 $^\circ\text{C}$. Working solutions were prepared from the stock solutions by dilution with appropriate amounts of Milli-Q water. Lake water was collected from an artificial lake located in Longhua District of Haikou City (China). Tap water was obtained in the laboratory when needed. All the water samples were passed through microporous nylon filters with the pore sizes of 0.45 μm in diameter. The samples were kept under refrigeration at 4 $^\circ\text{C}$ in the dark. Several aliquots from 2 mL filtered water samples were spiked with DES standard with different concentrations and followed by the SALLE procedure.

SALLE procedure

Briefly, 2.00 mL of water sample was placed in a 15 mL of screw-cap glass test tube and spiked with DES at 10 $\mu\text{g}/\text{mL}$. ACN (2 mL) was added into the sample solution. After the mixture was ultrasonicated for 1 min, 5 mL salting-out solution was added into the mixture and vortexed for 5 min. In this step, a two-phase solution was formed and the DES in the water samples were extracted into the upper organic phase (ACN phase). Then, the supernatant was collected for UV-Vis analysis which λ_{max} was set at 230 nm.

RESULTS AND DISCUSSION

SALLE condition optimization

Effect of sample pH

DES is weakly diprotic acidic compounds, and its $\text{pK}_{\text{a},1}$ and $\text{pK}_{\text{a},2}$ values are 7.23 and 10.14 (Wen *et al.*, 2013). Therefore the extraction solution pH has great impact on ionization

degree of DES. When pH was low, the neutral species of DES was so nonpolar that their solubility in water is extremely low, the DES was easy to be extracted into the ACN phase, then the value of A increased. As shown in Figure 1, the absorbance (A) of DES was the biggest when pH was 3. When pH = 2, the value of A was smaller than when pH = 3. This might be because the acidity caused adverse effect on extraction. The value of A were more or less when pH were 4 and 6. When pH was 5, the extraction efficiency was the lowest. So pH 3 was selected as the optimum pH value of sample solution.

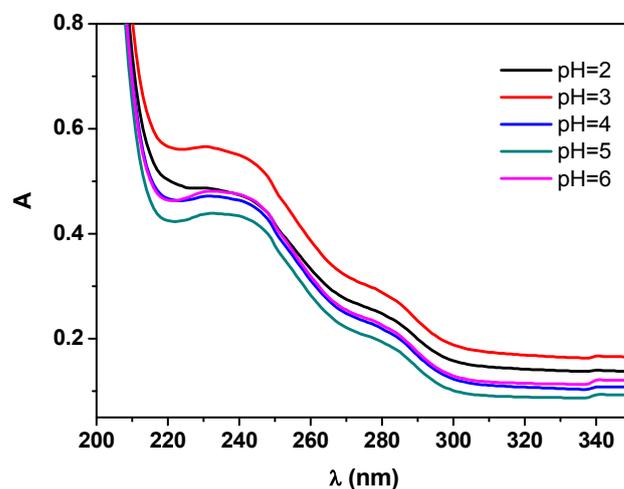


Figure 1. Effect of sample pH on extraction efficiency. SALLE conditions: sample volume, 2 mL; salting-out solution, 5 mol/L NaCl, 5 mL and pH 2; extraction solvent, ACN, 2 mL.

Effect of salting-out solution volume

As salting-out solution volume has a critical effect on extraction efficiency, its volume was optimized in the range from 1 to 5 mL. The value of A increased with salting-out solution volume increasing in the range of 1 to 5 mL, as shown in Figure 2. This may be because increasing the volume of salting-out solution decreased the volume of the ACN phase and therefore resulted in higher concentrations of DES in the organic phase, then the value of A is bigger. So, 5 mL was adopted in the following studies.

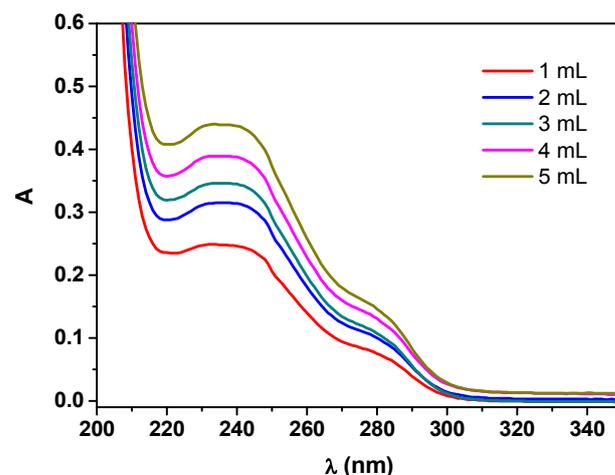


Figure 2. Effect of sample pH on extraction efficiency. SALLE conditions: sample volume, 2 mL pH = 3; salting-out solution, 5 mol/L NaCl, and pH 2; extraction solvent, ACN, 2 mL.

Effect of salting-out solution pH

Like sample pH, the salting-out solution pH also has great impact on ionization degree of DES. As shown in Figure 3, the value of A was the biggest when the salting-out solution pH was 2 and bigger evidently than other pH. Consequently, the optimum salting-out solution pH was set at pH 2.

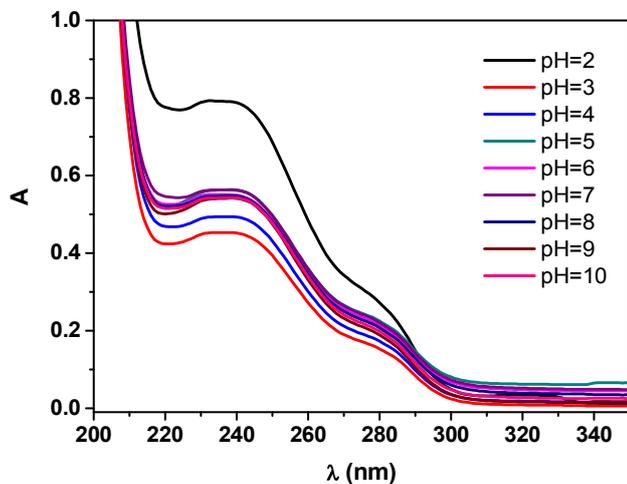


Figure 3. Effect of salting-out solution pH on extraction efficiency. SALLE conditions: sample volume, 2 mL pH = 3; salting-out solution, 5 mol/L NaCl, 5 mL; extraction solvent, ACN, 2 mL

So, the optimized conditions for extraction of DES and ideal SALLE efficiency were attained as follows: salting-out solution, 5 mol/L NaCl in 100 mmol/L phosphate buffer, 5 mL and pH 2; extraction solvent, ACN, 2 mL; and sample, pH 3.

Analytical figures of merit of the SALLE-HPLC-UV method

The performance of the SALLE-UV-Vis method under optimal conditions was investigated. Linear correlation coefficients (r) assessed at six different concentrations were obtained between A and the corresponding concentrations of DES in the range of 2.0–20 $\mu\text{g/mL}$, as shown in Table 1. LOD for the DES, calculated as the analyte concentration for which the value of A was three times the background noise ($3S/N$), was 0.61 $\mu\text{g/mL}$ for the UV-Vis analysis.

Table 1. Linear range, slope, intercept, correlation coefficients, and LOD for DES

Analyte	Linear range ($\mu\text{g/mL}$)	Linear equation	Correlation coefficient (r)	LOD ($\mu\text{g/mL}$)
DES	2.0-20	$A = 0.041c + 0.0401$	0.9990	0.61

The method was validated for reproducibility of the A of the analyses. The relative standard deviation (RSD) values of A for Intra-day ($n=6$) and Inter-day ($n=6$) are shown in Table 2.

Table 2. Reproducibility of SALLE-UV-Vis method

Standard	Intra-day ($n=5$)	Inter-day ($n=5$)
	(RSD,%)	(RSD,%)
	A	A
DES	0.26	0.11

Determination of DES in water samples

The developed SALLE-UV-Vis method was further applied to tap and lake water samples in order to check its practicality. The DES were not detected in the water samples. Recoveries were calculated for the spiked water samples with 2.5, 5 and 10 $\mu\text{g/mL}$ standards, respectively. The results are listed in Table 3. Satisfactory recoveries were obtained, ranged from 81% to 102%. This validated the SALLE-UV-Vis greatly applicable for the selective extraction, and accurate quantitation of trace DES in water samples.

Table 3. Recovery of DES from tap and lake water samples

Analyte	Added ($\mu\text{g/mL}$)	Recovery (%)	
		Tap water	Lake water
DES	2.5	81	86
	5	92	90
	10	102	97

Conclusions

In conclusion, a good, easy, and efficient method for the determination of DES in water samples was developed. The developed SALLE-UV-Vis offered wide linear range, good quantitative ability, and high precision. Also, compared with the classic extraction methods based on LLE and SPE, which often required large volume of sample and organic solvent, this method was demonstrated to be a simple, fast, cost effective, and eco-friendly option for determination of DES in water samples.

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