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Dispersive liquid–liquid microextraction based on solidification of floating organic droplet followed by high-performance liquid chromatography with ultraviolet detection and liquid chromatography–tandem mass spectrometry for the determination of triclosan and 2,4-dichlorophenol in water samples

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ABSTRACT

A novel, simple and efficient dispersive liquid–liquid microextraction based on solidification of floating organic droplet (DLLME-SFO) technique coupled with high-performance liquid chromatography with ultraviolet detection (HPLC–UV) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed for the determination of triclosan and its degradation product 2,4-dichlorophenol in real water samples. The extraction solvent used in this work is of low density, low volatility, low toxicity and proper melting point around room temperature. The extractant droplets can be collected easily by solidifying it at a lower temperature. Parameters that affect the extraction efficiency, including type and volume of extraction solvent and dispersive solvent, salt effect, pH and extraction time, were investigated and optimized in a 5 mL sample system by HPLC–UV. Under the optimum conditions (extraction solvent: 12 μL of 1-dodecanol; dispersive solvent: 300 of μL acetonitrile; sample pH: 6.0; extraction time: 1 min), the limits of detection (LODs) of the pretreatment method combined with LC–MS/MS were in the range of 0.002–0.02 $\mu g\,L^{-1}$ which are lower than or comparable with other reported approaches applied to the determination of the same compounds. Wide linearities, good precisions and satisfactory relative recoveries were also obtained. The proposed technique was successfully applied to determine triclosan and 2,4-dichlorophenol in real water samples.

1. Introduction

Triclosan (5-chloro-2-[2,4-dichloro-phenoxy]-phenol, TCS) is a broad-spectrum antimicrobial agent, which is widely used in personal care products, such as hand cleansers, toothpastes, air fresheners and deodorants [1]. All these widespread applications might lead to the release of TCS into water environment. In fact, several authors have reported that TCS is a detectable contaminant in municipal biosolids, surface waters, wastewaters and domestic waters [2–5]. Although TCS is a lipophilic compound with low human toxicity [6], an *in vivo* study has showed that TCS has the capacity to affect thyroid hormone homeostasis in rats [7]. Besides, TCS is toxic to some aquatic species such as algae, daphnia and fish [8]. Furthermore, some experiments have demonstrated that under the UV light, sunlight or in the presence of low concentrations of free chlorine, aquatic TCS can be degraded and converted into dioxins and chlorine phenolic compounds such as 2,4-dichlorophenol (2,4-DCP), which is more toxic than TCS and is one of endocrine disrupters [9–12]. Most importantly, a number of studies have revealed that TCS blocks lipid biosynthesis by specifically inhibiting the enoyl–acyl carrier protein reductase and may induce bacterial resistance development [12–15]. Therefore, a rapid, sensitive and green method is required to determine TCS and 2,4-DCP in real water samples.

Generally, sample pretreatment procedures are very vital to improve the sensitivity and selectivity of analytical methods. Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are traditional preconcentration methods to extract TCS and 2,4-DCP from water samples [16–19]. However, LLE not only needs a great deal of deleterious organic solvent, but also is time-consuming and laboursome. SPE uses much less solvent than LLE, but is relatively expensive [20]. Recently, more efficient and miniature preparation techniques have been developed to detect TCS and 2,4-DCP in water samples, such as solid-phase microextraction (SPME) [21], stir-bar sorptive extraction (SBSE) [22–24], hollow-fiber liquid-phase microextraction (HF-LPME) [25,26] and dispersive liquid–liquid microextraction (DLLME) [4,5,27]. The major advantages of SPME are solvent-free and easily miniaturized [28], unfortunately, the

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most obvious shortcoming is that its fiber is fragile and expensive and has limited lifetime and sample carryover [29]. SBSE is also a solventless pretreatment method based on sorptive extraction. It has a higher recovery than SPME due to the use of a large amount of polydimethylsiloxane (PDMS) as extraction phase [23]. Nevertheless, it is still time-consuming, inconvenient and needs a special desorption device [30]. Although HF-LPME is simple, effective and consumes a small amount of organic solvent, long extraction time is often encountered [31]. A few years ago, a new liquid–liquid microextraction method named DLLME was introduced by Assadi and co-workers [20]. In DLLME, a mixture of extraction solvent (higher or lower density than water) [20,32] and water miscible dispersive solvent was rapidly injected into an aqueous sample. By the action of dispersive solvent, a stable cloudy solution consisting of fine droplets of the extractant dispersed entirely in the aqueous solution was formed, leading to a large contact area in the interface between extraction solvent and sample solution, thus, the extraction time of DLLME is very short. Owing to the outstanding merits of DLLME including simplicity, low cost, rapidity and high enrichment factor, this technique is widely accepted and successfully applied to the preconcentrations of different target compounds in aqueous samples [20,29,32–36]. Nevertheless, the extraction solvents, such as chloroform, dichloromethane, carbon tetrachloride and chlorobenzene, frequently used in DLLME, are extremely toxic and environment-unfriendly. Meanwhile, after centrifugation, the extractant is often evaporated to dryness with a mild nitrogen stream or a concentrator when it was analyzed by HPLC, which requires more time [34,35].

Recently, dispersive liquid–liquid microextraction based on solidification of floating organic droplet (DLLME-SFO) has been developed as a novel sample preparation technique, which follows the same principle as the DLLME technique [37,38]. The main difference between DLLME-SFO and DLLME is that the extraction solvent used in the former is of low melting point and hypotoxicity. This method not only avoids the use of toxic organic solvent but also is easy to operate. After centrifugation and solidification, the solidified organic solvent can be effortlessly transferred into a microtube and used for instrument analysis after the solidified organic solvent melts. This convenient and inexpensive technique has been used for the extraction of polycyclic aromatic hydrocarbons, insecticides, organophosphorus pesticides and steroid hormone [31,38–40].

Until now, gas chromatography with electron-capture detection (GC–ECD) or coupled with mass spectrometry (GC–MS) and GC–MS/MS have been reported for the determination of TCS and 2,4-DCP in water samples [4,21]. Although TCS and 2,4-DCP can be detected at the ng L^{-1} level using these chromatographic methods, a tedious derivatization process is required before analysis. Thus, high-performance liquid chromatography with ultraviolet detection (HPLC–UV), liquid chromatography coupled with mass spectrometry (LC–MS) or tandem mass spectrometry (LC–MS/MS) would be a good alternative for the determination of TCS and 2,4-DCP because the intricate derivatization process is completely avoided.

The aim of this work is to develop the potential application of DLLME-SFO for the determination of TCS and 2,4-DCP in water samples by HPLC–UV and LC–MS/MS. Some experimental parameters that influenced the extraction efficiencies were optimized with HPLC–UV in details.

2. Experimental

2.1. Reagents and chemicals

Triclosan (99%) and 2,4-dichlorophenol (99%) were purchased from Alfa Aesar (Heysham, Britain) and Sigma–Aldrich (St. Louis, MO, USA), respectively (Table 1). Structures of the analytes are shown in Fig. 1. HPLC grade acetonitrile and methanol were obtained from Tedia Co. (Fairfield, OH, USA). HPLC grade ethanol and acetone were provided by Sinopharm Chemical Reagent (Shanghai, China). 1-Dodecanol, 1-undecanol, *n*-hexadecane and *n*-heptadecane were purchased from Aladdin Reagent (Shanghai, China). 2-Dodecanol was provided by Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent (Shanghai, China). Standard solutions (1000 mg L^{-1}) of TCS and 2,4-DCP were separately prepared by dissolving each compound in methanol. The daily standard working solutions of different concentrations were obtained by diluting the stock solutions with water. All solutions were kept at 4 °C in dark.

Table 1
pK_a and log $P_{octanol/water}$ values of TCS and 2,4-DCP.

Analyte	pK_a	log $P_{octanol/water}$	Reference
TCS	7.90	4.80	[41]
2,4-DCP	7.89	3.09	[42]

2.2. Instrumentation

2.2.1. HPLC–UV

Parameters optimization of DLLME-SFO was performed on a Waters HPLC system (Milford, MA, USA), which contains a 515 pump, a Rheodyne 7725i manual injector with a 5 μL injection loop, and a 2487 UV detector. An Elite Hypersil BDS C18 column (200 mm × 4.6 mm, 5 μm particle sizes) was applied to separation and a Millennim 32 software was employed to acquire and process chromatographic data. The mobile phase was a mixture of methanol/water (80:20, volume ratio) and the flow rate was 1.0 mL min^{-1}. The column temperature was controlled at 25 °C. The UV detector was set simultaneously at two different wavelengths of 280 and 287 nm for TCS and 2,4-DCP, respectively.

2.2.2. LC–MS/MS

LC–MS/MS analysis was performed on a Finnigan Surveyor Plus liquid chromatograph system coupled to a Thermo Scientific TSQ Quantum Ultra EMR system (San Jose, CA, USA) with an electrospray ionization (ESI) source. The analytes were separated on a Thermo Scientific RP18 column (50 mm × 2.1 mm, 5 μm particle size). The binary mobile phase composed of 90% methanol and 10% water (containing 0.2% ammonium acetate and 0.1% formic acid) was set at a constant flow rate of 200 μL min^{-1} and the column temperature was kept at 35 °C. A sample volume of 10 μL was injected with a Surveyor autosampler. LC–MS/MS parameters were as follows: ionization mode, negative mode; sheath gas pressure (N_2), 40 units; auxiliary gas pressure (N_2), 10 units; ion transfer tube temperature: 270 °C; collision gas pressure (Ar): 1.5 mTorr; spray voltage: 3000 V; Q1 resolution: 0.7 SRM; Q3 resolution: 0.7 SRM. A Xcalibur software was utilized to acquire and process chromato-

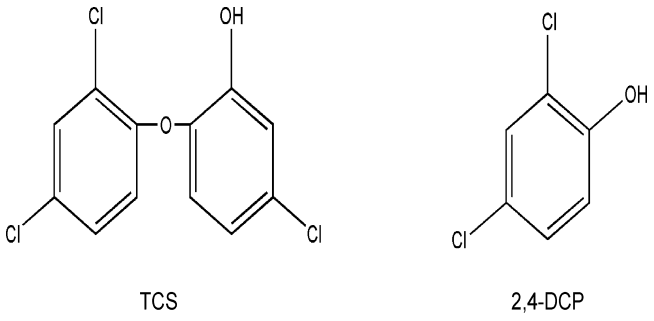

Fig. 1. Chemical structures of TCS and 2,4-DCP.

Table 2
Physical properties of extraction solvents in the proposed DLLME-SFO method.

Extraction solvent	Boiling point (°C)	Melting point (°C)	Density (g mL^{-1})	Solubility in water	Reference
1-Undecanol	243	16	0.83	Immiscible	[44]
1-Dodecanol	259	22–24	0.83	Insoluble	[44]
2-Dodecanol	249	17–18	0.80	Insoluble	[38]
n-Hexadecane	287	18	0.77	Insoluble	[44]
n-Heptadecane	302	22	0.78	Insoluble	[44]

graphic data. The selected ion monitoring (SIM) mode was used for the determination of target compounds. The following *m*/*z* transitions were analyzed: *m*/*z* 160.8 ⇒ 125.1 (2,4-DCP), *m*/*z* 286.7 ⇒ 35.7 (TCS).

2.3. Extraction procedure

For DLLME-SFO, an 5 mL of aqueous solution (pH 6.0) containing TCS and 2,4-DCP was placed in a 10-mL glass tube. A mixed solution of 300 μL of acetonitrile (dispersive solvent) and 12 μL of 1-dodecanol (extraction solvent) was rapidly injected into the solution with a 500-μL syringe, and the mixture was shaken by a vortex mixer for 30 s. After centrifugation for 4 min at 3000 rpm, lots of fine solvent droplets of 1-dodecanol containing analytes were accumulated on the surface of the aqueous solution due to its lower density than water. The glass tube was immediately put into an ice box until the organic solvent was completely solidified. Then, the solidified solvent was transferred into a 200-μL microtube where it melted quickly at room temperature. It was found that a small amount of water accompanied the solidified solvent when we withdrew the latter from the glass tube. To avoid the interfere of the water, the melted solvent was centrifuged at 6000 rpm for 30 s using another centrifuge. Finally, for HPLC–UV analysis, about 10 μL of the extractant (the upper portion solvent in the microtube) was withdrawn out by a microsyringe and used for injection (the injection volume is 5 μL); for LC–MS/MS analysis, to satisfy the requirement of the minimum of sample volume in a sample tube, the extractant was diluted five times with methanol and 10 μL of diluted extractant was used for injection by the Surveyor autosampler.

2.4. Sample preparation

Three natural water samples were collected from South Lake, East Lake and the Yangtze River (Wuhan, China); tap water sample was sampled from our laboratory. All the water samples were separately filtered with 0.22 μm membrane filter which was provided by Tianjin Jinteng Experiment Equipment Co. Ltd. (Tianjin, China) and stored at 4 °C in dark.

2.5. Calculations of extraction recovery and relative recovery

The extraction recovery (ER) was defined as the ratio between the amount of the analyte in the floating phase (n_{flo}) and the initial amount of the analyte (n_0) within the sample.

$$ER\% = \frac{n_{flo}}{n_0} = \frac{C_{flo}V_{flo}}{C_0V_{aq}} \times 100$$

where C_{flo} and C_0 are the concentration of analyte in the floating phase and initial concentration of the analyte in the aqueous sample; V_{flo} and V_{aq} are the volumes of the floating phase and aqueous sample, respectively.

The relative recovery (RR) was obtained from the following equation [43]:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100$$

where C_{found}, C_{real}, and C_{added} are the total concentration of analyte after addition of known amount of standard in real sample, the original concentration of analyte in real sample and the concentration of known amount of standard which was spiked to the real sample, respectively.

3. Results and discussion

To obtain high extraction efficiency, several experimental parameters affecting the performance of DLLME-SFO, such as the type and volume of extraction and dispersive solvents, salt effect, pH and extraction time, were investigated by HPLC–UV using one variable at a time method as follows.

3.1. Optimization of various parameters

3.1.1. Selection of extraction and dispersive solvents

The selection of an appropriate extraction solvent is crucial in DLLME-SFO. It should have some properties: high affinity to analytes, low solubility in water, lower density than water, low volatility and proper melting point around room temperature (Table 2). In addition, it should not interfere with the peaks of analytes during chromatographic analysis. Based on the above requirements, five organic solvent candidates, including 1-undecanol, 1-dodecanol, 2-dodecanol, *n*-hexadecane and *n*-heptadecane were tested. In the cases of *n*-hexadecane and *n*-heptadecane as extraction solvents, the fine droplets were unable to accumulate together after centrifugation. Thus, they would not be ideal for our research scheme. Subsequently, 1-undecanol, 1-dodecanol and 2-dodecanol were used for further investigation. The results indicated that the three organic solvents exhibited similar extraction efficiencies (Fig. 2). However, both of 1-undecanol

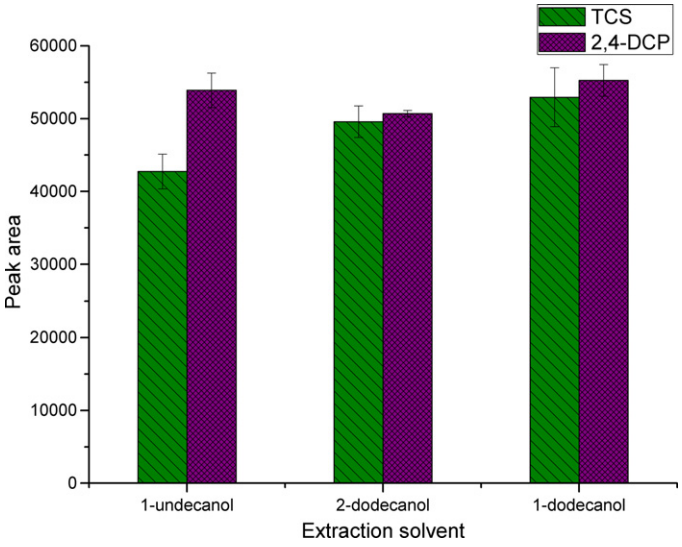

Fig. 2. Effect of extraction solvent type on extraction efficiency. Concentrations of TCS and 2,4-DCP are 50 μg L^{-1} and 60 μg L^{-1}, respectively. Sample volume: 5 mL, volume of extraction solvent: 12 μL, dispersive solvent: 200 μL of methanol, sample pH: 6.0, and extraction time: 1 min.

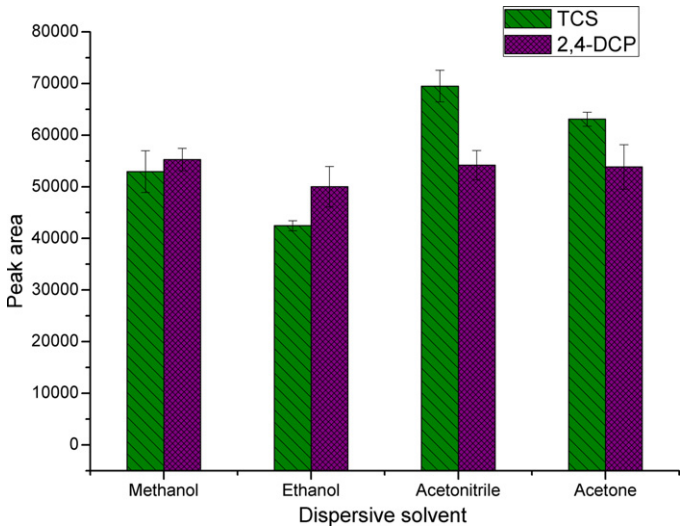

Fig. 3. Effect of dispersive solvent type on extraction efficiency. Concentrations of TCS and 2,4-DCP are 50 μg L^{-1} and 60 μg L^{-1}, respectively. Sample volume: 5 mL, extraction solvent: 12 μL of 1-dodecanol, volume of dispersive solvent: 200 μL, sample pH: 6.0, and extraction time: 1 min.

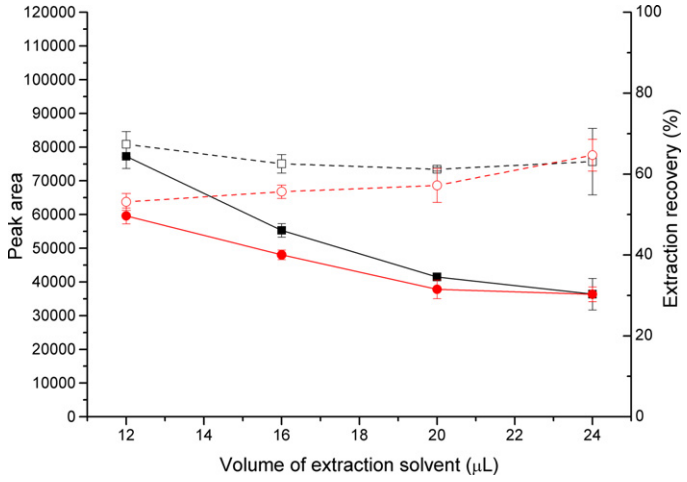

Fig. 4. Effect of volume of extraction solvent on peak area (solid line) and extraction recovery (dashed line). Filled square (■) and blank square (□): TCS; filled circle (●) and blank circle (○): 2,4-DCP. Concentrations of TCS and 2,4-DCP are 50 μg L^{-1} and 60 μg L^{-1}, respectively. Sample volume: 5 mL, extraction solvent: 1-dodecanol, dispersive solvent: 200 μL of acetonitrile, sample pH: 6.0, and extraction time: 1 min.

and 2-dodecanol had longer solidification time (>5 min) since the melting points of them were lower than that of 1-dodecanol. Moreover, the solidified solvents melted quickly because of the relatively low melting points, resulting in the difficulty in drawing them out. Therefore, 1-dodecanol was selected as the extraction solvent.

On the other hand, the dispersive solvent, which promotes the dispersion of 1-dodecanol into water, is an important component in the process of traditional DLLME. The dispersive solvent should be miscible both in the extraction solvent and water. To meet this requirement, methanol, acetonitrile, ethanol and acetone were studied. Acetonitrile was found to perform the best extraction efficiency (Fig. 3). This may be due to the synergic effect of good compatibility of acetonitrile with aqueous solution and low distributive ratio of analytes in the mixed solution of acetonitrile and water [45]. Hence, acetonitrile was chosen as the dispersive solvent for the following experiments.

3.1.2. Effect of volume of extraction solvent

The volume of extraction solvent usually has great influence on the extraction efficiency in DLLME-SFO. In this test, different amounts of extraction solvent (12, 16, 20 and 24 μL) were evaluated. As shown in Fig. 4, the peak areas decreased with the increase of 1-dodecanol. In fact, the extraction recoveries of analytes remain nearly the same due to the increase of the floating phase volume. However, when the volume of 1-dodecanol was below 12 μL, the solidified organic droplet was too little to draw out. So 12 μL of 1-dodecanol was used as the optimal volume.

3.1.3. Effect of volume of dispersive solvent

The influence of the volume of the dispersive solvent on extraction efficiency was investigated in the range of 100–500 μL, respectively. Fig. 5 depicts that the peak area of TCS increases slightly with the increase of acetonitrile. It is also observed that the peak area of 2,4-DCP keep flat when the dispersive solvent volume increased from 100 μL to 300 μL, and then decrease slowly with further increase of the dispersive solvent from 300 μL to 500 μL. Consequently, 300 μL of acetonitrile was selected as a compromise volume in the following studies.

3.1.4. Effect of salt concentration

In general, the addition of salt plays a vital role in conventional extraction process. Various experiments were performed by adding different amounts of NaCl (0–10%, w/v). It was found (Fig. 6) that salt concentrations had an opposite effect for the two compounds. For TCS, extraction efficiency was decreased with the addition of NaCl, this was expected as salting-in effect. In this case, the addition of salt led to the dissolution of more TCS in water, subsequently, the amount of TCS that can transfer into the floating phase decreased, resulting in a fall in the peak area of TCS. While for 2,4-DCP, the extraction efficiency was improved because of the salting-out effect. Therefore, no salt was added in the subsequent experiments.

3.1.5. Effect of pH

In most cases, the pH values of samples can influence the ratios of ionic to molecular forms of the analytes. To increase the extraction efficiency of 2,4-DCP in DLLME, it is necessary to acidify

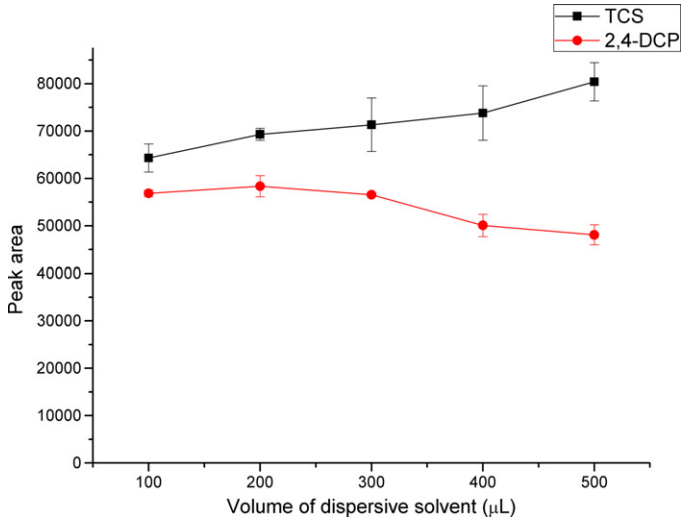

Fig. 5. Effect of volume of dispersive solvent on extraction efficiency. Concentrations of TCS and 2,4-DCP are 50 μg L^{-1} and 60 μg L^{-1}, respectively. Sample volume: 5 mL, extraction solvent: 12 μL of 1-dodecanol, dispersive solvent: acetonitrile, sample pH: 6.0, and extraction time: 1 min.

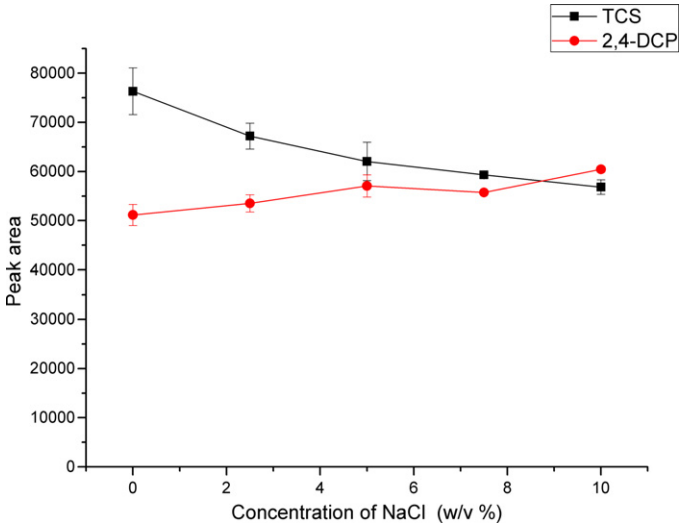

Fig. 6. Effect of salt addition on extraction efficiency. Concentrations of TCS and 2,4-DCP are 50 μg L^{-1} and 60 μg L^{-1}, respectively. Sample volume: 5 mL, extraction solvent: 12 μL of 1-dodecanol, dispersive solvent: 300 μL of acetonitrile, sample pH: 6.0, and extraction time: 1 min.

the sample [27]. Moreover, under alkaline conditions, the floating phase cannot aggregate to form the fine droplets of 1-dodecanol after centrifugation. Considering the above aspects, the various pH values in the range from 1 to 7 were optimized. The results illustrated in Fig. 7, shows that the maximal peak areas are acquired at pH 6.0 for both the two compounds.

3.1.6. Effect of extraction time

The extraction time is defined as an interval from the injection of the mixture of extraction and dispersive solvents to the start of centrifugation in the DLLME procedure [20]. After the addition of the mixture of 1-dodecanol and acetonitrile, the sample solution was shaken by a vortex mixer for 30 s. In this research, a series of extraction times (0.5, 1, 5, 10, 15, 20, and 25 min) were examined. The results demonstrated that the extraction time had no significant effect on extraction efficiency. It attributes to the large contact area in the interface between extraction solvent and aqueous solution.

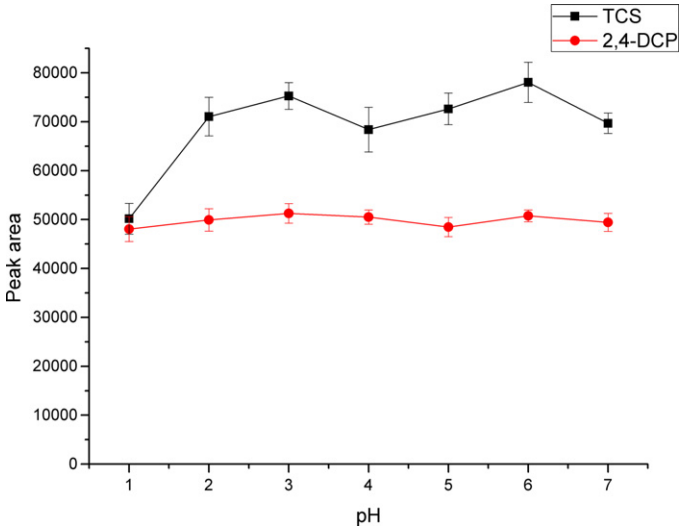

Fig. 7. Effect of pH on extraction efficiency. Concentrations of TCS and 2,4-DCP are 50 μg L^{-1} and 60 μg L^{-1}, respectively. Sample volume: 5 mL, extraction solvent: 12 μL of 1-dodecanol, dispersive solvent: 300 μL of acetonitrile, and extraction time: 1 min.

Table 3
Linearity, limit of detection and reproducibility of the DLLME-SFO method combined with HPLC–UV.

Compound	Linearity (μg L^{-1})	r^2	LOD (μg L^{-1})	RSD (%)
TCS	0.5–500	0.9998	0.10	4.4
2,4-DCP	0.8–800	0.9996	0.48	4.1

Thereby, the transference of the analytes from aqueous phase to extractant phase is very rapid. For the sake of convenient operation, 1 min was selected for the extraction time.

In sum, the optimal conditions in a 5 mL of sample volume were as follows: 12 μL of 1-dodecanol was served as extraction solvent and 300 μL of acetonitrile was used as dispersive solvent, the pH value of the sample was adjusted to 6.0, no salt was added and the extraction time was only 1 min.

3.2. Quantitative aspects

3.2.1. HPLC–UV

Under the optimal conditions, a good performance was acquired for the quantitative analyses of the two target analytes by HPLC–UV. The results are shown in Table 3. Good linearities were obtained with the correlation coefficients 0.9996 and 0.9998, for TCS and 2,4-DCP, respectively. The limits of detection (LODs), on the basis of signal to noise ratio (S/N) of 3, were 0.10 μg L^{-1} for TCS and 0.48 μg L^{-1} for 2,4-DCP. The relative standard deviation (RSD, $n = 5$) values were 4.4% for TCS and 4.1% for 2,4-DCP by five replicated extraction of spiked samples with 50 μg L^{-1} TCS and 60 μg L^{-1} 2,4-DCP.

3.2.2. LC–MS/MS

Under the optimized DLLME-SFO and LC–MS/MS conditions, the validation procedure for the developed method was carried out with spiked ultrapure water. The results are listed in Table 4. Good linearities in the ranges of 0.02–10 μg L^{-1} for TCS and 0.05–50 μg L^{-1} for 2,4-DCP were observed. The LODs were 0.002 μg L^{-1} and 0.02 μg L^{-1} for TCS and 2,4-DCP, respectively. The RSD ($n = 5$) values were 6.2% for TCS and 8.5% for 2,4-DCP by five replicated extraction of spiked samples with 2 μg L^{-1} TCS and 2,4-DCP.

3.3. Comparison of DLLME-SFO with other microextraction techniques

The analytical performance of the presented method was compared with other microextraction methods such as SPME, SBSE, HF-LPME and DLLME reported recently. The respective LOD, RSD, sample volume and sample preparation time of each method are summarized in Table 5. As shown in the table, the sample preparation time of DLLME-SFO method is much shorter than other extraction techniques (SPME, SBSE and HF-LPME). The RSDs for the DLLME-SFO are lower than or the same as other techniques. In terms of the sensitivity, DLLME-SFO combined with LC–MS/MS using only 5.0 mL of water sample has lower LODs than other methods except SPME. However, SPME linked to GC–MS demanded a derivatization process which led to sample loss and needed more

Table 4
Linearity, limit of detection and reproducibility of the DLLME-SFO method combined with LC–MS/MS.

Compound	Linearity (μg L^{-1})	r^2	LOD (μg L^{-1})	RSD (%)
TCS	0.02–10	0.9960	0.002	6.2
2,4-DCP	0.05–50	0.9983	0.02	8.5

Table 5
Comparison of the proposed method with other methods.

Compounds	Extraction method	Detection method	LOD (ng L^{-1})	RSD (%)	Sample volume (mL)	Sample preparation time (min)	Reference
TCS, 2,4-DCP	SPME with derivatization	GC–MS	2[a,c] 4–7[b,c]	8.7–17.5[a] 7.2–17.2[b]	15	40	[21]
TCS	SBSE	GC–MS	5	4.0–7.0	10	120	[22]
TCS	HF-LPME with *in situ* derivatization	GC–MS	20	6.9	10	20	[25]
2,4-DCP	HF-LPME	HPLC–UV	400	3.1	10	20	[26]
TCS	DLLME with derivatization	GC–MS/MS	2[c]	3.6-9.5	10	5	[4]
2,4-DCP	DLLME	HPLC–DAD	100	5.4	5	15	[27]
TCS, 2,4-DCP	DLLME-SFO	HPLC–UV	100[a] 500[b]	4.1–4.4	5	8	This work
TCS, 2,4-DCP	DLLME-SFO	LC–MS/MS	2[a,d] 20[b,d]	6.2-8.5	5	8	This work

[a] TCS.
[b] 2,4-DCP.
[c] Limit of quantification (LOQ).
[d] Diluted five times.

Table 6
The relative recoveries of the method by HPLC–UV.

Compound	Spiked ($\mu g\,L^{-1}$)	Tap water		Yangtze River		East Lake		South Lake	
		Measured ($\mu g\,L^{-1}$)	RR (%)	Measured ($\mu g\,L^{-1}$)	RR (%)	Measured ($\mu g\,L^{-1}$)	RR (%)	Measured ($\mu g\,L^{-1}$)	RR (%)
TCS	0	N.D.		N.D.		N.D.		N.D.	
	5.00	5.47	108	5.42	107	5.27	105	5.60	110
	20.00	16.90	85	18.16	91	16.94	85	17.55	88
2,4-DCP	0	N.D.		N.D.		N.D.		N.D.	
	5.00	4.38	89	4.14	85	4.41	90	5.41	107
	20.00	18.98	95	19.58	98	18.46	93	17.54	88

N.D.: not detected; RR: relative recovery.

pretreatment time. All these results indicate that DLLME-SFO is a fast, repeatable, sensitive and simple technique.

3.4. Analysis of environmental real samples

To assess the applicability of the proposed method, four real water samples were analyzed. To examine possible matrix effects, these water samples were spiked with TCS at 5 $\mu g\,L^{-1}$, 2,4-DCP at 20 $\mu g\,L^{-1}$ for HPLC–UV method. For LC–MS/MS analysis, TCS was spiked at 0.5 $\mu g\,L^{-1}$ and 2,4-DCP at 2 $\mu g\,L^{-1}$. The results are shown in Tables 6 and 7. It was found that satisfactory relative recoveries for both target compounds were obtained in the range of 83–119%, which indicated that the proposed method was reliable for the determination of trace amount of TCS and 2,4-DCP in various real water samples.

Unfortunately, no target compounds were found in all four real water samples collected by DLLME-SFO combined with HPLC–UV, it was possibly due to the low sensitivity of UV detector. When DLLME-SFO combined with LC–MS/MS was employed, TCS was detected in all the natural samples. The concentrations of TCS in East Lake (0.038 $\mu g\,L^{-1}$) and South Lake (0.031 $\mu g\,L^{-1}$) were higher than that of the Yangtze River (0.026 $\mu g\,L^{-1}$). No 2,4-DCP was found at levels above the method detection limits in any real water samples collected. The possible reason was that the yield of 2,4-DCP from TCS was low or it could be further degraded into others under the natural conditions. A typical chromatogram of the extracted target compounds from blank (a) and spiked (b) the Yangtze River samples using the DLLME-SFO combined with LC–MS/MS are shown in Fig. 8.

TCS as a broad-spectrum antimicrobial, its general existence in those water environments may cause aquatic bacterial mutations resulting in the produce of the drug-resistant super bacteria, which resist a sweeping array of antibiotics, raising alarms to public health system. So, TCS contamination may become a serious environmental problem or even an enormous social trouble.

Both East Lake and South Lake are internal lakes of Wuhan city, which has a population of nine million. The high concentrations of TCS found in these two lakes indicated that residents in these areas may discharge domestic wastewater into these lakes directly or indirectly, which should attract attention of the Wuhan Environmental Protection Bureaus. Wuhan city is located in the middle and lower reaches of the Yangtze River, so the existence of TCS in the Yangtze River along Wuhan city may imply random domestic wastewater discharge from upstream regions or Wuhan city and its surrounding areas. Furthermore, due to the huge bulk of Yangtze River, the quantity of TCS seems to be an incredible number. Therefore, Chinese State Environmental Protection Administration should pay much attention to this serious problem.

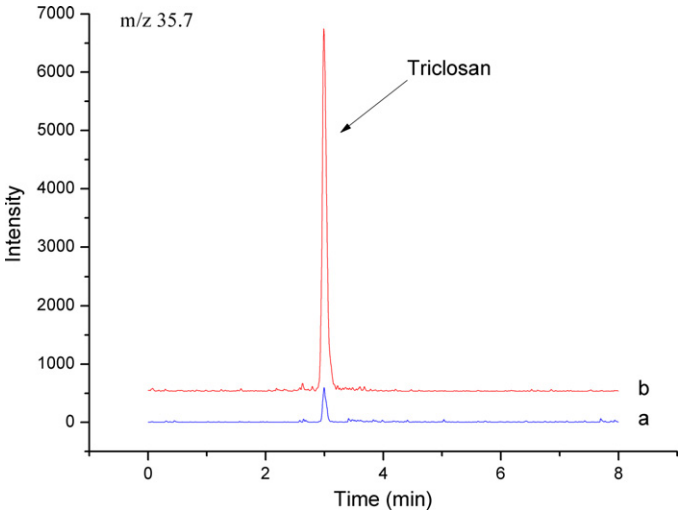

Fig. 8. Typical SIM chromatograms of triclosan in blank (a) and spiked (b, 0.5 μg/L) the Yangtze River samples using the DLLME-SFO method combined with LC–MS/MS.

Table 7
The relative recoveries of the method by LC–MS/MS.

Compound	Spiked ($\mu g\,L^{-1}$)	Tap water		Yangtze River		East Lake		South Lake	
		Measured ($\mu g\,L^{-1}$)	RR (%)	Measured ($\mu g\,L^{-1}$)	RR (%)	Measured ($\mu g\,L^{-1}$)	RR (%)	Measured ($\mu g\,L^{-1}$)	RR (%)
TCS	0	N.D.		0.026		0.038		0.031	
	0.50	0.43	87	0.49	93	0.46	84	0.53	99
	2.00	2.10	105	2.24	111	2.36	116	2.32	114
2,4-DCP	0	N.D.		N.D.		N.D.		N.D.	
	0.50	0.48	95	0.41	83	0.43	86	0.45	90
	2.00	2.11	105	2.37	119	2.38	119	2.29	115

N.D.: not detected; RR: relative recovery.

4. Conclusions

In this study, a novel, simple, and sensitive DLLME-SFO technique coupled with HPLC–UV and LC–MS/MS was developed for the determination of TCS and one of its degradation product 2,4-DCP. Compared with other methods, DLLME-SFO combined with HPLC–UV or LC–MS/MS avoids derivatization process and can be performed with a much shorter extraction time. Additionally, the method requires only small volume of low toxicity extraction solvent.

To the best of our knowledge, this was the first time that the DLLME-SFO was applied for the determination of TCS and 2,4-DCP in real water samples, which displayed wide linearities, good precisions, and satisfactory relative recoveries. Especially, the LODs of DLLME-SFO–LC–MS/MS were in the range of 2–20 $ng\,L^{-1}$ which are lower than or comparable with other reported approaches applied to the determination of the same compounds. We are convinced that the technique possesses a great potential in the rapid preconcentration and analysis of different types of organic compounds from environmental samples.

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