Analytical Methods

PAPER

Determination of triclocarban in aquatic plants by using SPE combined with HPLC-ESI-MS/MS

Cite this: DOI: 10.1039/c3ay42304b

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Received 25th December 2013
Accepted 18th January 2014

DOI: 10.1039/c3ay42304b

www.rsc.org/methods

A specific, sensitive, and reliable analytical method involving homogenate extraction, solid phase extraction (SPE), and detection by high-performance liquid phase chromatography-electrospray ionization tandem mass spectrometry was developed in this study for the determination of triclocarban (TCC) in aquatic plants. Key factors that could affect the extraction and clean-up performance, including the extraction solvent and its volume, the homogenate extraction time, the SPE cartridge used, and the eluents and their volume, were examined and optimised. Under optimum conditions, the linearity of the method ranged from 0.2 ng g^{-1} to 200 ng g^{-1}, with correlation coefficients (r^2) >0.999. The limit of detection was 0.05 ng g^{-1}, based on the ratio of the chromatographic signal to baseline noise (S/N = 3). Spiked recoveries of TCC in real aquatic plant samples ranged from 91.8% to 106.1%. The matrix effect value was 101.90%, with a relative standard deviation of 5.1%. The proposed method was successfully applied to analyse TCC in aquatic plant samples collected from a natural water environment.

1. Introduction

Triclocarban (3,4,4′-trichlorocarbanilide, TCC) is a common antimicrobial and antibacterial agent used in many personal care products including antimicrobial soaps, antibacterial mouthwashes, and toothpastes. Widespread application and large consumption of TCC will lead to dispersion in the environment because of its incomplete removal during wastewater treatment. It has been one of the most frequently detected organic pollutants in aquatic environments,[1–4] and its concentrations have been reported to be as high as 5600 and 6750 ng L^{-1} in river water and wastewater, respectively.[5] The release of TCC into the environment can cause a number of environmental and human health problems. Recent studies have classified TCC as a new type of endocrine disruptor that works synergistically to amplify the expression of testosterone.[6,7] By studying the effects of TCC on thyroid hormone action and stress in frog and mammalian culture systems,[8] TCC was found to affect TH-responsive gene transcripts at maximum concentrations in mammalian cells. A study on genotoxicity shows that TCC can cause DNA damage in human liver cells.[9] Bioaccumulation studies have shown that TCC accumulates in algae,[10] snails,[11] fish,[12] amphibian larvae,[13] and worms.[14] Today, the potential risks associated with the release of TCC into the environment and its effects on human health have attracted increased attention worldwide. Therefore, a sensitive and efficient method for determining TCC in plant samples must be developed.

Despite their widespread application for over 50 years, analytical methods that may be used for the determination of TCC in environmental samples have emerged only in the last ten years, mainly for the analysis of water and soil samples.[13–16] Analytical methods for TCC determination in biological samples, especially aquatic plants, are limited.[10,17] Compared with TCC analysis in water or soil samples, determination of trace levels of TCC in plants presents greater challenges because plants contain large amounts of phytochromes and fatty or waxy materials that may induce severe matrix interference.[18] During chromatographic analysis, matrix effects may result in under- or over-estimation of the actual concentration. To overcome such effects, an appropriate enrichment and clean-up process must be applied to remove various co-extractives before instrumental analysis.

The present methods for TCC enrichment from aquatic plants focus on Soxhlet extraction[10] and homogenised tissue solvent extraction[17] combined with gel permeation chromatography purification, all of which are complex and time-consuming. Solid phase extraction (SPE) is not only a widely used enrichment technique but also an effective clean-up process that may be applied in the analysis of organic pollutants in environmental samples. Given the number of sorbents that may be applied in SPE and the many types of commercial SPE cartridges available in the market, SPE can meet nearly all of the demands of pre-concentration and clean-up in

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environmental monitoring. Sensitive and selective approaches for TCC determination have been established, including GC/MS,[6,19] GC/MS/MS,[20] LC/MS,[1,19] and LC/MS/MS.[22–24] To reduce complex derivatisation steps and possible interference of other compounds, HPLC-ESI-MS/MS was selected for the sensitive determination of TCC. This work aims at developing a convenient and reliable method for TCC determination in aquatic plants based on homogenate extraction, SPE clean-up, and HPLC-ESI-MS/MS determination. Key factors that could affect the extraction and clean-up performance, including the extraction solvent and its volume, the length of time of homogenate extraction, the SPE cartridge used, and the eluents and their volume, were investigated in detail. The developed method provides good sensitivity, recovery, and reproducibility and is thus a suitable method for the determination and confirmation of TCC in aquatic plants. Future applications of the proposed method may include monitoring systems for residue control programs.

2. Experimental

2.1 Reagents and chemicals

HPLC-grade methanol, *n*-hexane, dichloromethane, acetonitrile, and acetone were purchased from Tedia Company Inc. (Fairfield, OH, USA). TCC was purchased from Dajie Technical Co. Ltd. (Hunan, China). A standard stock solution of TCC (10 mg L^{-1}) was prepared in methanol and stored at 4 °C. Fresh working solutions were prepared daily by diluting the stock solution with a suitable solvent.

2.2 Sample preparation

Aquatic plant samples were handled using latex gloves, transported on ice to the laboratory, and refrigerated at 4 °C until analysis. Foreign particles were removed from the samples by hand-washing with deionised water, and plants were blot-dried with paper towels. Hereafter, the samples will be referred to as fresh weight and will be applied to all aquatic plant concentration references.

About 2.00 ± 0.02 g (fresh weight) of aquatic plants, prepared as described above, was placed in 50 mL polypropylene centrifuge tubes. After addition of 100 μL of 1.0 μg mL^{-1} TCC standard working solution and 20 mL of extraction solvent (20% acetone in dichloromethane), the tissues were homogenised and extracted using a high-shear dispersion homogeniser (FJ-200, Shanghai Specimen and Model Factory, China) at high speed for 1 min. The samples were vortexed for 2 min and then centrifuged for 5 min at 8000 rpm. Afterwards, all supernatants were transferred to a 100 mL pear-shaped flask, and an additional 20 mL of extraction solvent was added to the sample tube. The contents of the tube were mixed and centrifuged as above. The resulting supernatants were combined and evaporated to dryness using a rotary evaporator, and the residues obtained were dissolved in 2 mL of hexane.

Several SPE cartridges and operating conditions were systematically evaluated using plant extracts spiked with standards to develop the sample clean-up procedure. Three cartridges were tested including silica (Waters, USA), Oasis HLB (Waters, USA) and C18 (Oakville, ON, Canada). The silica cartridge was first activated with 5 mL of *n*-hexane. After sample loading, the cartridge was washed with 10 mL of a solution of 85 : 15 (v/v) *n*-hexane–dichloromethane. The eluent was then dried under N_2 gas and reconstituted in 1.0 mL of methanol for HPLC-ESI-MS/MS analysis. Similarly, the C18 and HLB cartridges were activated with 5 mL of methanol. After loading 1.0 mL of extracts re-dissolved in methanol, the cartridges were eluted with different solvents, including methanol, 50% acetonitrile in methanol, acetonitrile, and 50% acetone in methanol.

2.3 HPLC-ESI-MS/MS analysis

Chromatographic separations were performed with a 1200 Binary SL Rapid Resolution series pump (Agilent Technologies, Palo Alto, CA, USA). An Agilent Eclipse XDBC8 column (4.6 mm × 150 mm, 5 μm particle size) was held at 30 °C in an Agilent 1200 series SL column compartment. A sample volume of 10 μL was injected into an Agilent 1200 series SL autosampler using a binary mobile phase composed of 10% water and 90% methanol at a constant flow rate of 0.4 mL min^{-1}.

Mass spectrometry was performed on an Agilent 6410 triple-quadrupole mass spectrometer fitted with an ESI MS source and controlled using a Mass Hunter workstation. The ESI source conditions were established to obtain an average maximum intensity of the precursor ions. The MS conditions were maintained as follows: the nitrogen nebuliser pressure was set at 40 psi, and the nitrogen drying gas was set at 350 °C with a flow rate of 10 L min^{-1} high vacuum, 2.56 × 10^{-5}; and rough vacuum, 1.93. Direct injection of each compound in methanol was used to optimise the multiple reaction monitoring transitions. The optimal conditions are summarized in Table 1.

3. Results and discussion

3.1 Optimisation of sample extraction

To obtain the best TCC extraction performance in aquatic plants, several important parameters, including the extraction solvent, the extractant volume, and the extraction time, were optimised. Several experiments were designed and performed for this purpose.

3.1.1 Selection of the extraction solvent. The extraction solvent is a key parameter that affects the recovery of the target compound and the amount of remaining impurities in the extracted samples. Four extraction solvents, including 50% *n*-hexane in dichloromethane (A), dichloromethane (B), 20% acetone in dichloromethane (C), and 20% methanol in dichloromethane (D), were tested with spiked samples (50 ng g^{-1}) in a side-by-side comparison. The TCC averaged recoveries shown in Fig. 1 were 45.0%, 59.9%, 93.0% and 87.9% for solvents A, B, C, and D, respectively. The results indicate that amongst the solvents tested, solvent C has the highest extraction efficiency. Therefore, 20% acetone in dichloromethane was selected as the extraction solvent for sample preparation.

3.1.2 Effect of the extractant volume. Experiments to determine the optimal extractant volume were designed and

Table 1 Multiple reaction monitoring conditions for TCC

Compound	Time (min)	Div. valve[a]	Prec. ion[b]	Prod. ion[c]	(P1/P2) frag.[d]	Collision energy E_1/E_2 (eV)	ESI mode
	0	To waste	314.9	162/126	90	8/16	Negative
TCC	2	To MS	314.9	162/126	90	8/16	Negative

[a] Division valve. [b] Precursor ion. [c] Product ion. [d] Fragmentor.

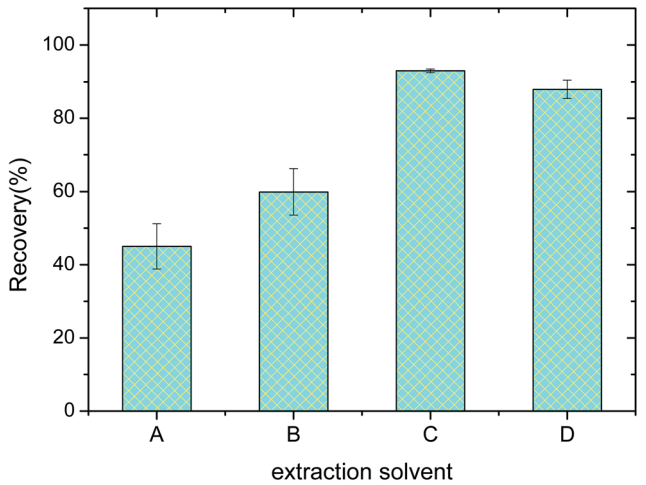

Fig. 1 Effect of the extraction solvent on the recovery of TCC. ((A) 50% *n*-hexane in dichloromethane; (B) dichloromethane; (C) 20% acetone in dichloromethane; and (D) 20% methanol in dichloromethane.)

performed by changing the volume of extraction solvent over the range of 10–30 mL. The recoveries of TCC in these experiments are shown in Fig. 2. The results showed that the TCC recovery increases from 89.3% to 93.0% as the volume of extraction solvent increases from 10 mL to 20 mL. However, extraction efficiencies remained nearly constant as the volume of extraction solvent continued to increase from 20 mL to 30 mL. Therefore, 20 mL of extraction solvent was adopted in subsequent experiments.

3.1.3 Effect of the homogenate extraction time. To examine the effect of the homogenate extraction time on TCC recovery, experiments in which the extraction time was varied from 0.5 min to 4.0 min were performed. The experimental results are shown in Fig. 3. When the extraction time was beyond 1.0 min, the TCC extraction efficiencies remained constant. The TCC recoveries between 90.7% and 93.0% were found, which may be deemed satisfactory for the determination of TCC in aquatic plants. To save operation time, an extraction time of 1.0 min was adopted in subsequent experiments.

3.2 SPE optimisation

Some studies show that ESI is susceptible to matrix components,[25,26] which may result in signal suppression or isobaric interference and decrease in assay sensitivity. To reduce matrix effects, an additional clean-up step is necessary. SPE has gained popularity as a technique for extracting polar to medium-polarity analytes from aqueous environmental samples. In this work, SPE was used as a clean-up step for extracts prepared from aquatic plant samples. Several parameters, such as the SPE cartridge used and the eluent and its volume, influence clean-up efficiency.

3.2.1 Selection of SPE cartridges. TCC recoveries are based on the premise of good separation effects between TCC and phytochrome to eliminate interference from the aquatic plant

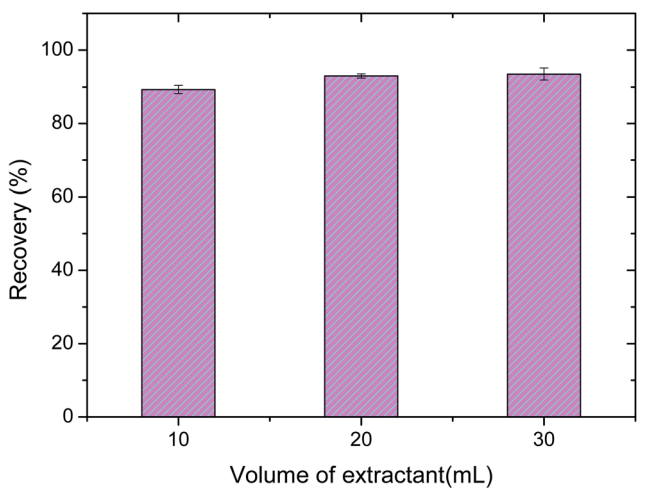

Fig. 2 Effect of the volume of extraction solvent on the recovery of TCC.

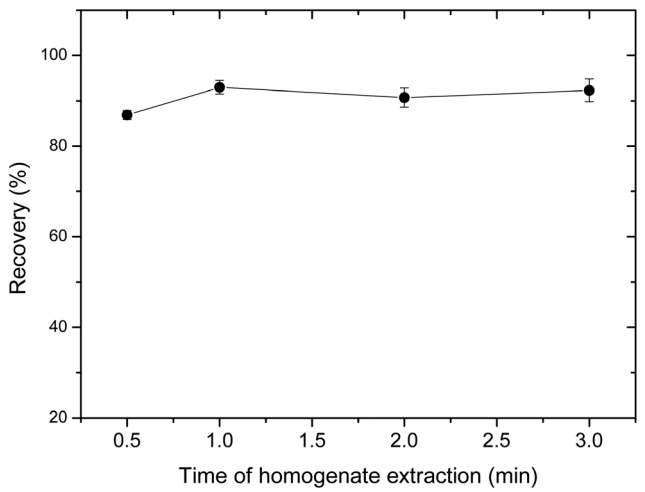

Fig. 3 Effect of the time of homogenate extraction on the recovery of TCC.

matrix. The SPE sorbent is a key parameter that affects the separation of TCC and phytochrome. We initially tested several SPE sorbents, including the Oasis HLB cartridge and C18 cartridge. The Oasis HLB cartridge has been previously used to clean up plant material extracts[27,28] and municipal biosolids[21] for the determination of TCC. However, we found that the TCC recovery using this cartridge was unsatisfactory (below 70%). In comparison, TCC and some of the phytochrome were washed away by using the C18 cartridge. Good separation of TCC and phytochrome and high TCC recovery were achieved with a silica cartridge. Thus, a silica cartridge was selected for TCC clean-up.

3.2.2 Selection of the eluent. Plants have complex environmental matrices, and extracts from this material contain many co-extractives that may interfere with LC-MS/MS analysis. To promote further separation of TCC and phytochrome, three eluents, including 65% *n*-hexane in dichloromethane, 75% *n*-hexane in dichloromethane, and 85% *n*-hexane in dichloromethane, were tested. We found that TCC can be washed out by a defined amount of all three solvents before phytochrome desorption but TCC recoveries varied amongst the solvents (Fig. 4). The highest TCC recovery and best repetitiveness were obtained using 85% *n*-hexane in dichloromethane. Therefore, 85% *n*-hexane in dichloromethane was chosen as the eluent for the SPE procedure.

3.2.3 Selection of the eluent volume. The effects of the eluent volume on TCC recovery were investigated. Experiments in which the volumes of eluent varied over the range of 0–14.0 mL were designed and performed, and the results are shown in Fig. 5. The TCC recovery increased as the eluent volume increased from 0 mL to 10.0 mL. When the eluent volume was over 10.0 mL, the TCC recovery remained nearly constant. To retain less impurities and achieve better recoveries, the eluent was collected from 2.0 mL to 10.0 mL in all subsequent experiments.

3.3 HPLC-MS/MS analysis

There have been several methods reported in the literature for the TCC analysis in various sample matrices, including GC-MS[6,19] and LC-MS/MS techniques.[22,23] HPLC-MS/MS is a convenient analytical method because no derivatization step is required. In this work, high performance liquid chromatography with electrospray (negative ion mode) ionization and tandem mass spectrometry was used for the determination of TCC. For TCC detection, a transition of 314.9 → 162 was selected. The greatest sensitivity was observed when monitoring the *m*/*z* 314.9 ions that represent a minor chlorine isotope. The multiple reaction monitoring (MRM) chromatograms of TCC are shown in Fig. 6.

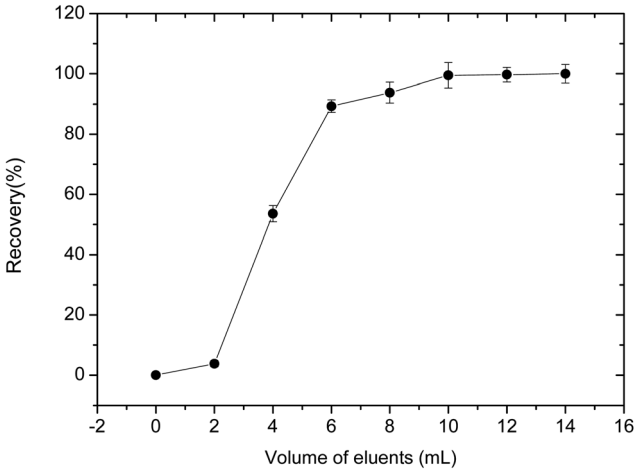

Fig. 5 Effect of the volume of eluent on the recovery of TCC.

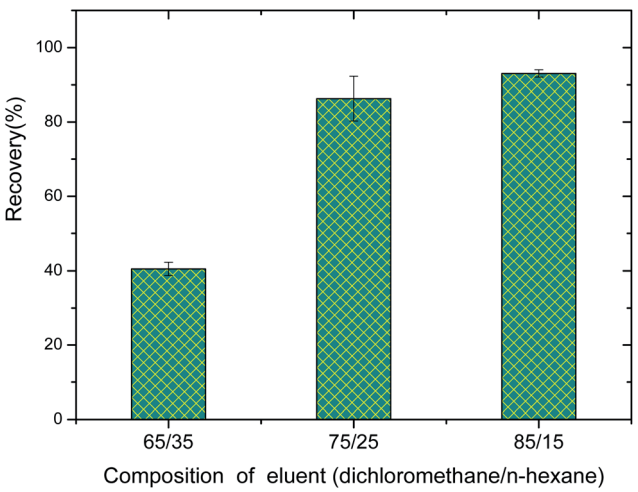

Fig. 4 Effect of the eluent on the recovery of TCC.

3.4 Method validation

Linearity of calibration, sensitivity, precision, high recovery, and lower matrix effects are essential factors that define a quantitative analytical method.

3.4.1 Linearity and sensitivity. Under the optimum conditions determined above, the linear range and limit of detection (LOD) were obtained to evaluate the sensitivity of the proposed method. Linearity was tested by varying the concentrations of TCC from 1.0 ng g^{-1} to 200 ng g^{-1}. The calculated curve illustrated in Table 2 displays a good linear relationship between the

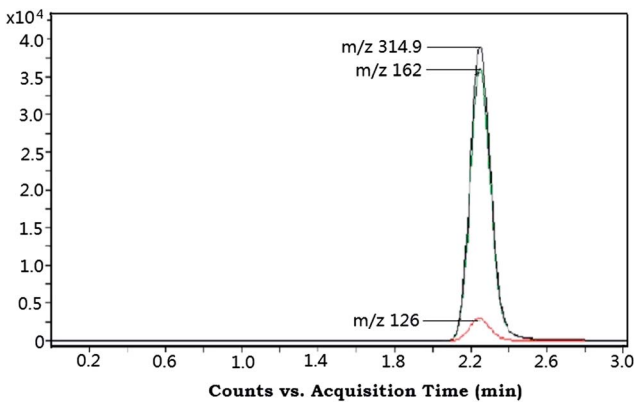

Fig. 6 The MRM chromatograms of TCC in the reference standard solution.

Table 2 Calibration curve and relevant RSD of TCC

Concentration (ng mL^{-1})	1.0	5.0	50.0	100.0	200.0
Mean responses ($n = 4$)	4305	22 070	238 703	483 300	955 829
RSD (%) ($n = 4$)	2.1	1.7	1.4	1.6	1.9
Calibration curve	$y = 4756x - 509$, $r^2 = 0.9994$				

peak area and concentration of TCC. The correlation coefficient (r^2) was found to be 0.9994. The sensitivity was evaluated by determining LODs and the limit of quantitation (LOQ). According to the FDA guide on Analytical Procedures and Methods Validation,[29] the LOD was defined as the concentration with a signal-to-noise ratio (S/N) of 3. The LOQ was defined as the lowest concentration that could be determined with 80–120% accuracy and not higher than 20% precision values, and the analyte response should be at least five times compared with the blank response. In this work, the LOD was found to be 0.05 ng g^{-1}, and the LOQ was 0.17 ng g^{-1}.

3.4.2 Accuracy and precision. Accuracy is expressed as recovery, which was calculated as follows:

$$R(\%) = 100 \times \frac{C_m - C_b}{C_s} \quad (1)$$

where R is the recovery, C_b and C_s are the blank concentrations of TCC in aquatic plants and spiked TCC concentrations, and C_m is the measured concentration with the developed method. Accuracy was measured using six determinations at three concentrations (1.0, 15.0, and 50.0 ng g^{-1}), and the standard deviation was calculated. The precision of the method was assessed by determining the intra- and inter-day relative standard deviation (RSD). Both recoveries and RSD of the method were tested with spiked aquatic plant samples at three different concentrations. The intra-day RSD was determined by replicate analyses ($n = 4$) performed on the same day. Inter-day RSD was determined by replicate analyses performed on four different days. The mean recoveries and intra- and inter-day RSDs of the method are shown in Table 3. The mean recoveries ranged between 91.8% and 106.1%, with an intra-day RSD of less than 9.6% and an inter-day RSD of less than 15.1%. These results indicate that the proposed method has good accuracy and precision.

3.4.3 Matrix effects. Given its selectivity and sensitivity, HPLC-MS/MS is an excellent choice for bioanalytical analyses. However, one of the greatest drawbacks of LC-ESI-MS/MS is suppression or enhancement of signals by co-extractives from the sample matrix.[25,26] Matrix effects can be minimised by efficient sample clean-up to remove co-extracted materials.

In this work, the clean-up process by SPE greatly reduced the presence of interference in the sample. To evaluate matrix effects during TCC determination in aquatic plant samples, a standard addition method was performed.[30] Six aquatic plant samples were spiked with 50 ng g^{-1} TCC and prepared for analysis according to the methods described above. Each sample was then divided into two subsamples, A and B. About 100 μL of subsample A was spiked with 100 μL of a standard solution containing 100 ng mL^{-1} TCC. Then, about 100 μL of subsample B was diluted with 100 μL of methanol. Subsamples A and B were used to determine matrix effects by comparing the increases in the responses of TCC between the two subsamples (A and B) with the responses of the analytes in an external standard.

The ME value was calculated as follows:

$$\mathrm{ME}(\%) = 100 \times \frac{A - B}{S} \quad (2)$$

where ME is the matrix effect and A, B, and S are the average peak areas of TCC in subsamples A and B, and the analytical standard (S), respectively. The matrix effect value was 102.7%, with an RSD of 4.6%. This finding indicates that the effects of the matrix on the signals are not serious after sample clean-up by SPE.

Table 3 Full recoveries of TCC and relative standard deviation (RSD) from aquatic plant samples

		Intra-day detection ($n = 4$)			Inter-day detection ($n = 4$)		
Blank conc. (ng g^{-1})	Spiked conc. (ng g^{-1})	Measured conc. (mean ± S.D.) (ng g^{-1})	Recovery (%)	RSD (%)	Measured conc. (mean ± S.D.) (ng g^{-1})	Recovery (%)	RSD (%)
5.1	1.0	6.16 ± 0.60	106.1	9.6	6.11 ± 0.91	101.0	15.1
5.5	15.0	19.58 ± 1.02	93.5	5.1	18.87 ± 0.93	91.2	4.8
5.1	50.0	51.05 ± 1.45	91.8	2.9	52.14 ± 2.98	94.1	5.9

Table 4 Analytical results of TCC in real aquatic plant samples

Sampling sites	Aquatic species	Mean concentration (ng g^{-1}) ($n = 4$)	RSD (%) ($n = 4$)
Jiazi lake	*Elodea canadensis Michx*	35.5	7.7
	Elodea densa (Planch.) Casp.	45.7	6.8
Daming lake	*Vallisneria natans*	70.3	8.4
Qushuiting Rivulet	*Hornwort*	19.5	9.3
	Elodea canadensis Michx	45.2	3.3

3.5 Real sample analysis

To assess the applicability of the proposed method in actual determination of aquatic plants, the contents of TCC in four aquatic plants, including *Elodea canadensis Michx*, *E. densa* (Planch.) Casp., *Vallisneria natans*, and *hornwort*, collected from three different natural water environments, were analysed. All samples were processed and analysed according to the method described above by replicate analyses ($n = 4$). TCC was detected in all five aquatic plant samples at levels ranging from 19.5 ng g^{-1} to 70.3 ng g^{-1}, as shown in Table 4.

4. Conclusions

The combination of homogenate extraction and SPE clean-up followed by HPLC/MS/MS determination yields a specific, sensitive, and reliable analytical method for TCC determination in aquatic plants. The optimised method showed wide linearity, good repeatability, and satisfactory accuracy. Experimental results showed that signal suppression resulting matrix effects from aquatic plants are not a serious problem for the target compound when an effective SPE clean-up step is performed. These facts demonstrate that the proposed method has great potential for the determination of TCC in various aquatic plant samples. In addition to this application, the current method may be extended for the isolation and determination of other personal care products in aquatic plants.

Acknowledgements

The authors are thankful for the financial support by the National Natural Science Foundation of China (no. 21077069 and 21007035).

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