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TECHNICAL NOTE

Rotating disk sorptive extraction of triclosan and methyl-triclosan from water samples

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Abstract A method of sample preparation based on use of rotating disk sorptive extraction (RDSE) has been developed for determination of triclosan (TCS) and methyl-triclosan (MTCS) in water samples. The sorptive and desorptive behavior of the analytes was studied by use of a rotating disk coated with polydimethylsiloxane (PDMS) on one of its surfaces. Chemical and extraction behavior were studied to establish the best conditions for extraction. The optimum conditions for both analytes were: sample volume 25 mL, pH4.5, NaCl concentration 6 % (*w*/*v*), disk rotational velocity 1,250 rpm, and extraction time 80 min. A desorption time of 30 min was used with 5 mL methanol. The detection limits for TCS and MTCS were 46 and 34 ngL^{-1}, respectively. Recovery was evaluated at two concentrations, 160 and 800 ngL^{-1}, and the values obtained were between 80 and 100 %. The method was applied to analysis of influent water at two treatment plants in Santiago, Chile.

Keywords Rotating disk sorptive extraction · Polydimethylsiloxane · Triclosan · Methyl-triclosan · Environment · Water

Introduction

Triclosan (TCS), 5-chloro-2-(2,4-dichlorophenoxy)phenol, is widely used in household products (e.g., hand soap, dish detergent, and cosmetics) as an antibacterial agent. Only a small number of its uses are regulated by the United States Environmental Protection Agency (US EPA) [1]. In Europe, however, commercialization of some biocides, including TCS, has been banned [2].

TCS is a low-polarity compound (log K_{ow} 4.8) that is slightly soluble in water (10 mgL^{-1} at 20 °C). Because of its properties and use in personal care products, this compound can be found in a variety of environmental compartments [3] and is classified as an emerging contaminant. TCS is present in aquatic plants, can bioaccumulate in fish and be absorbed by soil, and is also present in wastewater treatment plants [4].

Methyl-triclosan (MTCS) is a degradation product of TCS that is formed by biological methylation. MTCS is more lipophilic than TCS (log K_{ow} 5.4), suggesting greater bioaccumulation potential [5]. In view of the problems posed by emerging contaminants, particularly considering that both TCS and MTCS are endocrine disruptors [6, 7], it is necessary to develop reliable analytical methods able to detect these compounds at low concentrations in environmental samples. In this context, analytical methods have been developed for determination of TCS and MTCS. Modern methods for their determination in water samples are based on several sample-preparation techniques, including solid-phase microextraction (SPME) [8], solid-phase extraction (SPE) [9, 10], and stir-bar-sorptive extraction (SBSE) [11–13]. All of these techniques overcome the disadvantages of conventional extraction methods, particularly in terms of consumption of solvents and time. In our laboratory we have recently developed a rotating disk sorptive extraction (RDSE) technique, an alternative microextraction technique similar to SPME and SBSE. The rotating disk device has the advantage of providing a greater volume of the polydimethylsiloxane (PDMS) phase than in SPME, forming a larger PDMS surface area than that on the device used

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for SBSE [14–17]. Moreover, the disk used in RDSE can be rotated at a high velocity without risk of damaging the PDMS phase, which is never in contact with the bottom or walls of the vial containing the sample, unlike the device used in SBSE. This technical modification facilitates more efficient analyte mass transfer and faster extraction. Finally, the rotating disk device is much less costly than other techniques because it can easily be fabricated in the laboratory.

In this study, a method has been developed for simultaneous determination of TCS and MTCS from aqueous matrices. The analytes were extracted by RDSE and were then desorbed from the PDMS with methanol, derivatized, and detected by gas chromatography coupled to mass spectrometry (GC–MS).

Experimental

Reagents

Nano-pure water from a Barnstead water system (Dubuque, IA, USA) was used throughout the work. The analytes TCS and MTCS (both 99.5 % purity) were purchased from Dr Ehrenstorfer (Augsburg, Germany). A standard stock solution of the analytes was prepared in methanol (GC–MS/pesticides grade analysis; Fisher Scientific, Fair Lawn, NJ, USA). Hexachlorobenzene (HCB, 99.5 % purity), used as internal standard, was purchased from Dr Ehrenstorfer. Labeled $^{13}C_{12}$ triclosan (50 mgL^{-1}), purchased from Wellington Laboratories (Ontario, Canada), was used as a surrogate standard in analysis of real water samples. Nitrogen 5.0 and helium 5.0 were purchased from Linde (Santiago, Chile) and were used for final extract evaporation and as chromatographic carrier gas, respectively. Ethyl acetate, acetone (both HPLC-grade, 99.8 % purity) and sodium chloride (99.5 % purity) were purchased from Merck (Darmstadt, Germany). The PDMS phase was prepared from a Sylgard 184 silicone elastomer kit (Dow Corning, MI, USA) in accordance with the manufacturer's recommendations. Citrate buffer (disodium citrate, Merck; 0.1 molL^{-1}) was adjusted to pH4.0 with hydrochloric acid (Merck). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), from Sigma–Aldrich (Milwaukee, WI, USA), was used as derivatizing agent.

Instruments and software

A Thermo Scientific gas chromatograph, Focus model (Milan, Italy) coupled to a Thermo Fisher Scientific model ISQ (Austin, TX, USA) mass-selective detector was used for final determinations. The fused silica capillary column used was a Restek (Bellefonte, PA, USA) RTX-5MS (30 m×0.25 mm i.d.; 0.25 μm film thickness) coated with 5 % phenyl–95 % methylpolysiloxane. Two microliters of sample extract was injected into the gas chromatograph using the splitless mode. The injector temperature was 250 °C. The initial column temperature was 100 °C (1 min) and was increased to 300 °C at 10 ° min^{-1}. A constant flow of 1.0 mLmin^{-1} helium was used as carrier gas. The solvent delay was 7 min. A dwell time of 0.1 s was used for each *m*/*z* value. The MS transfer line was maintained at 250 °C and quantification was based on calibration with the standard analyte using the mass spectrometer in selective ion monitoring (SIM) mode. Table 1 shows the ions monitored for the analytes and the internal and surrogate standards.

The beaker containing the sample and the rotating disk was placed on an MR 300 magnetic stirrer (Heidolph Instruments, Germany).

Statistical software (Statgraphics Centurion XV for Windows; Manugistics, Rockville, MD, USA) was used to build the experimental design and to analyze experimental results by analysis of variance (ANOVA).

Preparation of the rotating disks

The extraction device used in this study (Fig. 1) was a Teflon disk (1.5 cm diameter) into which a miniature magnetic stirring bar (Teflon-coated Micro Stir bar from VWR International) was embedded. A film of PDMS was subsequently attached to one side of the disk with double-sided adhesive tape.

The PDMS films were prepared as follows. The base-to-catalyst mix ratio was 10:1 (*w*/*w*), and the cure time at room temperature was 48 h. Before curing, the gel solution was poured into circular molds (1 mm deep×1.5 cm diameter), and films were stored in a vacuum desiccator for 48 h for PDMS gelation.

Method validation

Linearity

Calibration curves were obtained by processing eight standard solutions in duplicate. The concentration ranges

Table 1 GC–MS data

Analyte	Retention time (min)	Target and qualifier ion *m*/*z* values[a]
TCS	18.26	*345*, 347, 200
HCB	12.20	*284*, 286, 282
MTCS	16.24	*302*, 304, 252
$^{13}C_{12}$ TCS	18.27	*357*, 359, 361

[a]Values in italics are target ions

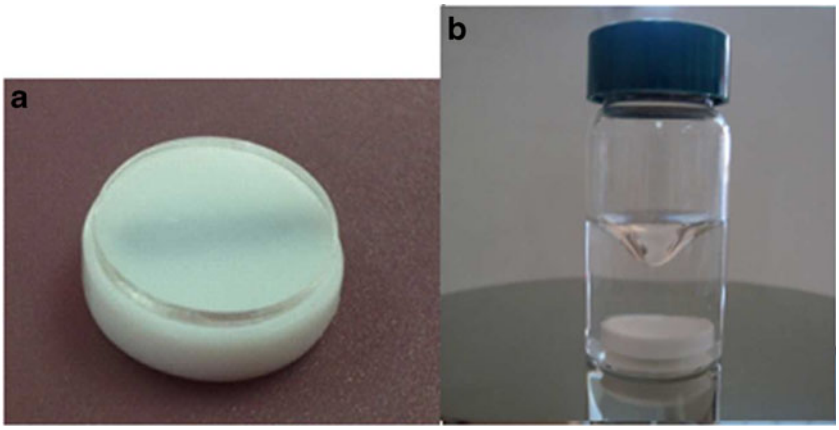

Fig. 1 **a** Rotating device used in RDSE, coated with PDMS as sorbent phase. **b** RDSE process in the extraction vial

studied were 160–2000 ng L^{-1} for TCS and 120–2000 ng L^{-1} for MTCS.

Accuracy

Accuracy was estimated by measurement of recovery. Four samples of river water enriched with TCS and MTCS at two concentrations, 160 and 800 ng L^{-1}, were analyzed and recovery was calculated. Accuracy for wastewater samples was also assessed by comparison of results with those obtained by use of solid-phase microextraction (SPME) [8].

Precision

The precision of the method was determined as the repeatability of results from the recovery experiments, and was expressed as relative standard deviation (RSD, %; n=4). Repeatability was also determined for wastewater samples (n=3).

Selectivity

The selectivity of the GC–MS procedure was based on monitoring the appropriate ion (m/z) for each analyte (Table 1).

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ were estimated as the analyte concentrations for which the peak signals were, respectively, three and ten times the background noise from the chromatogram.

Confirmation criteria

To confirm a finding as unequivocally positive, a maximum ratio tolerance of ±25 % was accepted between the intensities of the target and qualifier ions. Obviously, the same retention time in sample and standard was also required to confirm a positive.

General procedure

A standard or a water sample (25 mL) was poured into a beaker and the pH was adjusted to 4.0 with 0.1 mol L^{-1} citrate buffer. Surrogate standard ($^{13}C_{12}$-TCS) at 5 μg L^{-1} and NaCl at 20 % (w/v) were added. The rotating disk coated with the PDMS phase was placed inside the beaker, and the disk was rotated at 1,250 rpm for 80 min at room temperature. After extraction, the disk was placed in a 10 mL beaker containing 5 mL methanol as a desorbing solvent, and was stirred for 30 min at 1,250 rpm. The methanol extract containing the concentrated analyte was then evaporated to dryness under a stream of N_2 and the residue was re-dissolved in 1 mL ethyl acetate. MTBSTFA (50 μL) was added to this extract (500 μL) and derivatization was performed for 45 min at 80 °C. Before injection, 10 μL 5 mg L^{-1} HCB was added as internal standard, and the analytes were determined by GC–MS.

In the study of the conditions affecting extraction, the concentration of the analytes was 1,000 ng L^{-1} and the signals were not normalized with reference to surrogate standards.

Results and discussion

The effect of pH on TCS and MTCS response was studied between pH 1 and 14 (Fig. 2). Sample pH affects the

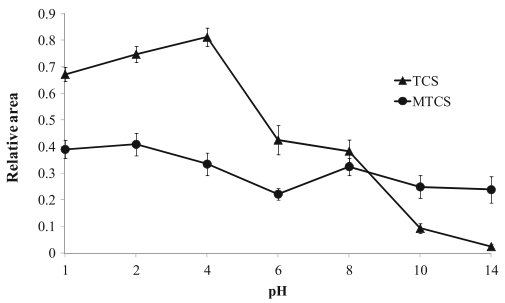

Fig. 2 Effect of pH on extraction of TCS and MTCS by RDSE

dissociation of TCS (pK_a=7.9); extraction in the neutral form is favored between pH 1 and 4. MTCS is present in the neutral form irrespective of pH, and its extraction efficiency remains almost constant throughout the entire pH range. Consequently, optimization studies were performed at a fixed pH of 4.0.

Optimization of the RDSE process

It is well known that use of matrix modifiers in microextraction techniques can enhance the efficiency of extraction of some analytes, depending on their polarity. Methanol is one of the most commonly used organic modifiers, particularly for extraction of apolar analytes (i.e. those with log K_{ow}>5.0), because this solvent prevents adsorption of these analytes on the walls of the vial [18]. NaCl is also used, because of its the salting out effect, changing the ionic strength of the sample. For semi-polar and polar analytes, addition of salt increases extraction efficiency, by making the analytes insoluble in water and increasing their affinity for the organic phase.

A factor study was therefore conducted considering the concentrations of NaCl and MeOH and the volume of the sample to establish the effect of these factors on extraction of TCS and MTCS, using an extraction time of 120 min. For both NaCl and MeOH, a minimum level of 0 % (*w*/*v* and *v*/*v*, respectively) and a maximum level of 20 % were defined. The sample volume studied was between 10 and 40 mL. These levels were selected on the basis of previous studies in which other, similar, microextraction techniques were used [14, 15, 19].

A factorial design of 2^K levels was applied, where K is the number of factors. To obtain experimental conditions suitable for extraction of both compounds, we used the desirability function to simplify the responses. In this analysis, each individual response "*i*" is associated with its own partial desirability function (d*i*). This value varies from 0 to 1 according to the closeness of the response to its target value. The n individual desirability functions are then combined as geometric means to obtain the overall desirability function (D=($d1$, $d2$, $d3$, … ,dn)/n). The model obtained from regression of experimental global desirability factors was validated by ANOVA.

In this optimization, the chromatographic response for each analyte was normalized by the volume of the sample, on the basis that if the concentration of the analyte is kept constant its mass increases as a function of sample volume. Under these conditions, when the volume of sample is increased from 10 to 40 mL, extraction efficiency is reduced, because contact between the analyte and the solid phase surface is favored when sample volume is reduced.

The desirability function increases with decreasing methanol concentration and increasing NaCl concentration (Fig. 3); however, only the concentration of NaCl was significant (p<0.05) with regard to analyte recovery. Therefore, the optimum conditions for rotating disk sorbent extraction are: 20 % NaCl, 0 % MeOH, and 10 mL sample.

Although the optimum sample volume was 10 mL, for subsequent experiments, a volume of 25 mL was used, because preconcentration factors are higher for larger sample volumes.

Under the experimental conditions established, the time taken for extraction to reach equilibrium was studied (Fig. 4). The samples were extracted by RDSE for different times ranging from 15 to 240 min. Extraction time affects the amount of analyte concentrated in the PDMS phase, as shown in Fig. 4. Extraction yield increases with extraction time until equilibrium is reached after approximately 60–80 min.

Analytical features of the method

Table 2 shows the analytical features of the method. Values significantly lower than the LOQ for TCS and MTCS (between 1 and 20 ngL^{-1}) have been obtained by use of SPME [8] and SBSE [11]. In those techniques the high sensitivity achieved is a result of the use of thermal desorption (TD), which enables determination of extremely low concentrations, because all the analyte extracted is desorbed and introduced into the analytical instrument for analysis. In this study, because we were

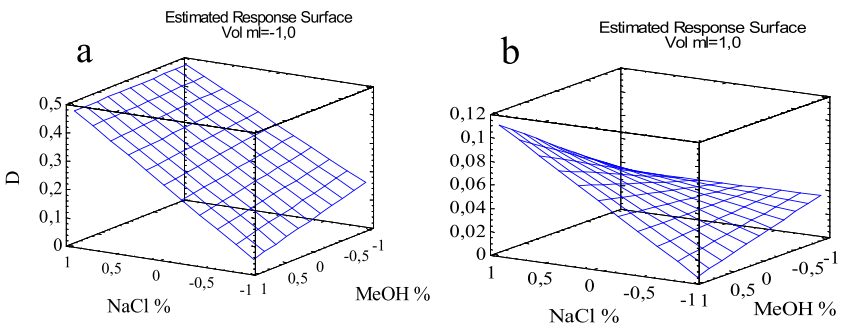

Fig. 3 Response surfaces for desirability: (**a**) sample volume= 10 mL; (**b**) sample volume= 40 mL

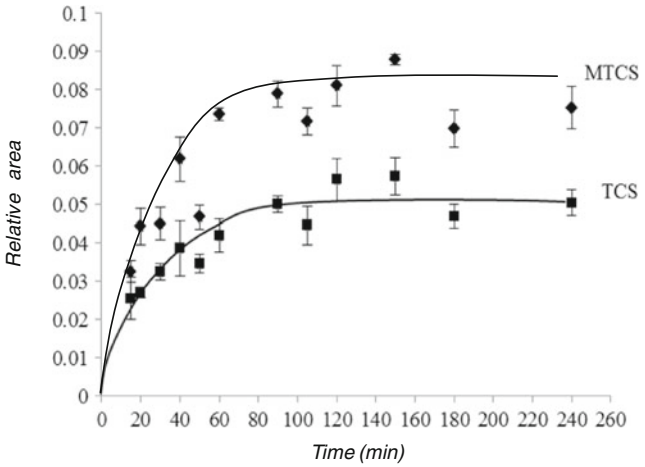

Fig. 4 Time profiles for extraction of TCS and MTCS

unable to use thermal desorption because of a lack of suitable instrumentation, we left open the possibility of working with low or high sample volumes, taking into account that we plan to address thermal desorption in future studies.

However, when liquid desorption was used in SBSE, the LOD achieved was 90 ngL^{-1} [13], similar to the value obtained in this study. Moreover, SBSE methods only determine TCS with an extraction time between 2 and 4 h, compared with 60–80 min in this RDSE study. As stated above, the disk used in this study had a large contact surface area that accelerates the extraction process, and it can be stirred at higher rotating velocities without deterioration of the phase.

The RSDs were lower than 10 %, which indicates that the precision of the method is very close to that obtained in other studies. For example, with SPE–GC–MS, the RSD determined in the range 10 to 10,000 ngL^{-1} was below 8 % [20]. With SBSE–GC–MS, the RDS is 20 % [13], and when liquid–liquid extraction is used with GC–MS, RSD values were between 6 and 12 % [10, 21]. However, recovery of the method for TCS was >95 %, and for MTCS recovery was between 80 and 87 %. Thus, in this study the reliability of the method based on RDSE for the simultaneous extraction of the analytes from real samples is ensured.

Table 2 Analytical features of the method

Analyte	LOD (ngL^{-1})	LOQ (ngL^{-1})	Recovery (%, n=4)		Linearity, R^2
			160 ngL^{-1}	800 ngL^{-1}	
TCS	46	152	102±8	97±6	0.9994
MTCS	34	114	88±9	81±8	0.9995

LOD limit of detection; *LOQ* limit of quantification

Analysis of real samples

To assess the applicability of the RDSE method, samples of untreated wastewater, obtained directly from the influent of two treatment plants in the Metropolitan Region of Santiago (Chile) were analyzed. We expected to find substantial concentrations of TCS. The samples were collected in plastic containers and stored at 4 °C until the time of analysis. A surrogate standard ($^{13}C_{12}$-TCS) was added to real samples for quality-control purposes.

The results obtained from rotating disk extraction are presented in Table 3. To validate extraction by RDSE, samples were also analyzed by SPME. Both methods were compared using the Tukey test, and results were statistically equivalent (95 % confidence level). In addition, two samples were spiked with TCS and MTCS, and the recoveries obtained were in a range 70 to 100 %.

Both analytes were found in the samples by RDSE and SPME, but MTCS was below the limit of quantification of the methods. The concentration of TCS was similar to those previously reported for wastewater samples in Canada [20], Germany [21], Switzerland [22], and Spain [23].

Conclusions

A microextraction technique was developed for simultaneous extraction of TCS and MTCS by RDSE. The factors that affect extraction of the compounds were optimized; it was found that extraction efficiency depends on the volume of sample extracted and its ionic strength. The proposed method has the advantage of largely minimizing the volume of solvent used for sample preparation, and ngL^{-1} detection limits were achieved. This method of extraction was faster than its SBSE counterpart.

Relative standard deviations were <10 %. The linearity of the method was also acceptable in the concentration range studied (r=0.9997) and recovery was between 80 and 100 %. Because of these features, rotating disk extraction could be used for real samples. There are few references in the literature about simultaneous extraction and analysis of TCS and MTCS in water samples, so this study is presented as an option to be considered for future analysis.

Table 3 Results from determination of TCS and MTCS in real water samples (n=3)

Sample	RDSE (μgL^{-1})		SPME (μgL^{-1})	
	TCS	MTCS	TCS	MTCS
Treatment Plant 1	2.7±0.4	<LOQ	1.91±0.09	<LOQ
Treatment Plant 2	1.8±0.6	<LOQ	1.8±0.1	<LOQ

Significant concentrations of TCS were found in wastewaters; this is indicative of the widespread use of TCS as an antimicrobial agent in a variety of commercial products.

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