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## **Dispersive liquid-liquid microextraction applied to the simultaneous derivatization and concentration of triclosan and methyltriclosan in water samples**

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### Abstract

A fast and novel sample preparation procedure for the determination of triclosan (TCS) and methyltriclosan (MTCS) in water samples is presented. Dispersive liquid-liquid microextraction, using a ternary mixture consisting of a disperser, an extractant and N-methyl-N-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) as derivatization reagent, was used for the simultaneous derivatization, case of TCS, and concentration of both species in different water samples. Analytes were determined by gas chromatography with tandem mass spectrometry (GC-MS/MS). Influence of different factors on the performance of the sample preparation process is thoroughly discussed. Under final working conditions, a mixture of 1 mL of methanol, 40  $\mu$ L of 1,1,1-trichloroethane and the same volume of MTBSTFA was added to 10 mL of water in a conical bottom glass tube. After centrifugation, the settled phase was injected directly in the chromatographic system. TCS was quantitatively extracted and converted into the corresponding *tert*-butyldimethylsilyl derivative, whereas for MTCS an extraction yield around 90% was attained. Limits of quantification between 2 and 5 ng L<sup>-1</sup> and reproducibility values below 10% were achieved; moreover, the performance of the extraction process was scarcely affected by the type of water sample. Globally, these values are comparable, or even better, to those reported for other approaches applied to the determination of same compounds, with the advantage of a shorter sample preparation step. Analysis of surface and wastewater samples confirmed the ubiquitous presence of TCS in the aquatic environment at levels from 20 to 700 ng L<sup>-1</sup>.

*Keywords:* Dispersive liquid-liquid microextraction; triclosan; silylation; water analysis; gas chromatography tandem mass spectrometry.

## 1. Introduction

Analytical and environmental aspects related to the so-called pharmaceutical and personal care products (PPCPs) are receiving an increasing attention in recent years. Among them, triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol], TCS, is one of the most concerning compounds. Although the acute toxicity of this bactericide is low [1], some data point to potential medium-term risks associated with its continuous release in the environment. On one hand, TCS has become an ubiquitous pollutant in waste and surface water [2,3], it presents relatively high concentrations ( $\mu\text{g g}^{-1}$  range) in bio-solids from sewage treatment plants (STPs) [4,5], it is bio-accumulated by several aquatic organisms [6,7], in vivo studies have proved its capability to modulate the levels of thyroxine in mammals [8], and it has been also detected in human fluids, such as breast milk [9]. On the other hand, once in the aquatic environment, TCS may evolve rendering persistent pollutants [10,11] and species with a higher bio-accumulative potential than the parent bactericide, such as methyltriclosan (MTCS), which has also been found in biota samples [1,12].

Solid-phase extraction (SPE) is the routine tool for the extraction of TCS and its by-products from water samples, previously to their determination using gas or liquid chromatography techniques [4,13,14]. In addition, other alternatives, based on microextraction processes, have been proposed. In a previous work, we optimised a solid-phase microextraction (SPME) method for the determination of TCS and MTCS in water samples [15]. Quintana et al. have also shown the suitability of stir-bar sorptive extraction (SBSE) for the concentration of TCS [16]. Hollow-fibre liquid-phase microextraction (HF-LPME), using n-dodecane immobilized in a porous polypropylene membrane, is also effective for the extraction of TCS from water samples, after in-situ acetylation with acetic anhydride [17]. All above microextraction approaches provide limits of quantification (LOQs) in the low  $\text{ng L}^{-1}$  range, they require a reduced sample intake and very low consumption of organic solvents; nevertheless, the extraction step is time-consuming since its kinetics is controlled by analytes diffusion from the bulk of the sample to the extraction media. Additionally, in gas chromatography (GC) determinations, TCS derivatization is advisable to improve the sensitivity of the method. In most applications, derivatization is carried out after the microextraction step, increasing even more the time required for sample preparation [15-16].

One of the most recent modalities of microextraction is dispersive liquid-liquid microextraction (DLLME). This technique, introduced by Assadi et al. [18], uses a binary mixture of a water miscible solvent, named disperser, and a high density one with very low water solubility, referred as extractant, to extract and concentrate organic compounds from water samples. Acetone, methanol and acetonitrile are normally considered as dispersers, whereas, several high density, chlorinated solvents have been successfully tested as extractants. Fast addition of this extraction mixture to a water sample results in a cloudy state consisting of fine droplets of the extractant dispersed in the aqueous matrix. The large surface of contact between both phases leads to very fast, quasi-instantaneous, mass transference processes. After centrifugation of the sample, a drop of extractant settles in the bottom of the tube, usually providing high enrichment factors (EFs) as well as extraction yields. For the last two years, DLLME has been successfully applied to the concentration of different organic compounds in water samples [19-22]. In further works, a water compatible derivatization reagent has been also included in the extraction mixture [23,24], thus polar analytes are simultaneously derivatized and extracted improving the performance of their further determination by GC techniques. Additional improvements of the original idea from Assadi's group involve back extraction of ionisable analytes from the settled organic phase into an aqueous solution [25], and replacement of high density extractants by non-chlorinated solvents (e.g. dodecanol), with melting points close to room temperature, which are recovered after cooling down the extraction media [26].

The aim of this work is to evaluate the suitability of DLLME for the fast and sensitive determination of TCS and MTCS in water samples, integrating the derivatization of TCS in the extraction step. A ternary mixture consisting of a disperser, an extractant and N-methyl-N-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), as silylation reagent, was employed for this purpose. Although, as any other silylation reagent, MTBSTFA is unstable in aqueous solutions, it might be compatible

with the DLLME process if kinetics of TCS derivatization and extraction is faster than the decomposition of MTBSTFA. As far as we known, the combination of silylation reagents with DLLME has not been explored in the literature, yet. The influence of several experimental factors on the yield of the sample preparation process is thoroughly discussed and the performance of the developed method compared with that reported for other sample preparation techniques.

## **2. Experimental**

### **2.1. Reagents, standards and material**

HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Trace analysis quality acetone and chlorinated solvents: carbon tetrachloride ( $\text{CCl}_4$ ), chlorobenzene ( $\text{C}_6\text{H}_5\text{Cl}$ ) and 1,1,1-trichloroethane ( $\text{CH}_3\text{CCl}_3$ ) were obtained from Merck and Aldrich (Milwaukee, WI, USA). Sodium chloride, MTBSTFA and TCS were provided by Aldrich. MTCS was acquired from Toronto Research Chemicals (Toronto, Canada). Individual standard solutions and mixtures of both analytes were prepared in methanol. Further dilutions, used to spike water samples, were made in the same solvent. Calibration standards in the range from 2 to 1000  $\text{ng mL}^{-1}$  were prepared in each of the chlorinated solvents used in the DLLME process. Aliquots of these standards (0.5 mL) were mixed with 0.05 mL of MTBSTFA to convert TCS into the corresponding silyl derivative [27].

DLLME experiments were carried out in glass tubes (12 mL volume) with a conic bottom and a screw cap, furnished with a PTFE-lined septum, acquired from Afora (Barcelona, Spain).

Ultrapure (Milli-Q), tap, river and urban wastewater were employed in this study. Samples, except ultrapure water, were passed through glass fibre filters obtained from Millipore (Bedford, MA, USA). Tap water was obtained in the laboratory when needed, river and wastewater were stored in the dark, at 4 °C, for a maximum of 48 hours before analysis.

### **2.2. Sample preparation**

Sample preparation conditions were optimized with 10 mL aliquots of different water samples, spiked with TCS and MTCS at concentrations in the range from 0.01 to 2  $\text{ng mL}^{-1}$ . Under final working conditions, an extraction mixture, consisting of 1 mL of methanol, 40  $\mu\text{L}$  of  $\text{CH}_3\text{CCl}_3$  and the same volume of MTBSTFA, was added to water samples, placed in conical bottom glass tubes, with a gas tight syringe. This solution was shaken manually for 1 min and then centrifuged at 3500 rpm for 3 min. The aqueous supernatant was removed with a Pasteur pipette and the volume of the settled phase measured using a 50  $\mu\text{L}$  volume micro-syringe. An aliquot of this phase (total volume 39  $\mu\text{L}$ ) was poured into a 0.2 mL insert, placed at a 1.5 mL vial. Performance of the extraction process was evaluated on the basis of achieved enrichment factors (EFs) and extraction yields (recoveries). EFs were defined as the ratio between the concentration of each compound in the settled phase and that added to the water sample. Recoveries were calculated as the product of EFs by the ratio between the volumes of the sedimented phase and the water sample, multiplied by 100.

### 2.3. Instrumentation

In the earlier stages of this work analytes were determined using an Agilent (Wilmington, DE, USA) 6890 GC instrument furnished with a micro-electron-capture detection (micro-ECD) system. In further assays, a Varian (Walnut Creek, CA, USA) CP 3900 GC instrument connected to a Varian Saturn 2100 ion-trap mass spectrometry (MS) system was employed. In both instruments, analytes were separated with an Agilent HP-5 type capillary column (30 m x 0.25 mm I.D.,  $d_f$  0.25  $\mu\text{m}$ ) operated at a constant helium flow of 1.0 mL  $\text{min}^{-1}$ . The GC oven was programmed as follows: 50  $^{\circ}\text{C}$  (held for 1 min), at 10  $^{\circ}\text{C min}^{-1}$  to 270  $^{\circ}\text{C}$  (held for 10 min). Injections (1-2  $\mu\text{L}$  volume) were made in the splitless mode (splitless time 1 min) using an autosampler. The injector port was set at 280  $^{\circ}\text{C}$ .

Unless otherwise is stated, the GC-MS system was operated in the MS/MS mode, using optimal conditions reported elsewhere [27]. Product ions at 200+310 and 232+252+254  $m/z$  units were used for the quantification of TCS and MTCS, respectively. Their concentrations in the extracts from water samples were established by external calibration, comparing the responses obtained for these extracts and calibration standards prepared in the same solvent used as extractant in the DLLME process.

## 3. Results and discussion

### 3.1. Optimization of DLLME conditions

Preliminary extraction experiments were carried out with ultrapure water spiked with TCS and MTCS at 1 ng  $\text{mL}^{-1}$ . Ternary mixtures comprising 1 mL of methanol, 25  $\mu\text{L}$  of MTBSTFA and 20  $\mu\text{L}$  of different chlorinated solvents were evaluated as extraction solutions. Since MTBSTFA is slowly degraded in presence of methanol, these solutions were prepared immediately before being used. After addition of the extraction solution, samples were shaken manually for 1 min and then centrifuged for other 5 min.

#### 3.1.1. Extraction solvent

Yields and EFs of DLLME processes are mainly controlled by the characteristics of the extractant. On the basis of previous applications of this technique,  $\text{CCl}_4$ ,  $\text{CH}_2\text{Cl}_2$  and  $\text{C}_6\text{H}_5\text{Cl}$  were considered as extractants [28]. Under conditions described in the above paragraph, well-defined settled drops with volumes of 18  $\mu\text{L}$  ( $\text{CCl}_4$  and  $\text{CH}_2\text{Cl}_2$ ) and 21  $\mu\text{L}$  ( $\text{C}_6\text{H}_5\text{Cl}$ ) were obtained. For the three solvents, MTBSTFA (density 1.05 g  $\text{mL}^{-1}$ ) contributed significantly to increase the size of the settled drop; however, phases separation was not observed for binary mixtures of methanol and MTBSTFA, indicating its useless as extractant in DLLME. Chromatograms corresponding to extracts in  $\text{C}_6\text{H}_5\text{Cl}$  showed split peaks for MTCS and the silyl derivative of TCS. Although this problem can be alleviated using a different program of temperature in the GC oven than for the other two more volatile extractants, it was decided to discard the use of  $\text{C}_6\text{H}_5\text{Cl}$ . MTCS was recovered in a similar extension with  $\text{CCl}_4$  and  $\text{CH}_2\text{Cl}_2$ ; however, the latter solvent provided nearly twice higher responses for TCS, the most polar of the two considered compounds, Fig. 1. On the basis of this behaviour, and considering also its lower toxicity, trichloroethane was chosen as extractant. In all tested conditions, no signal was noticed for TCS as non-derivatized species, thus the efficiency of the silylation reaction was assumed to be quantitative.

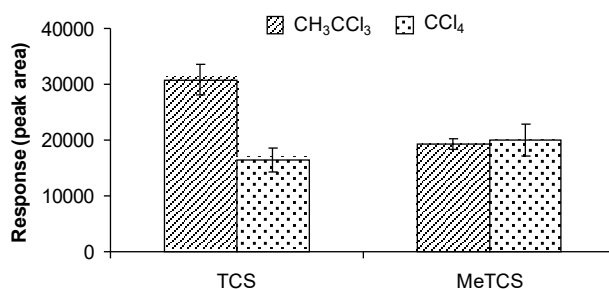


Fig. 1. Comparison of responses obtained using CCl<sub>4</sub> and CH<sub>3</sub>CCl<sub>3</sub> as extractants. Methanol, MTBSTFA, and extractant volumes were 1000, 25 and 20  $\mu$ L, respectively. N=4 replicates.

### 3.1.2. Type and volume of disperser

In addition to methanol, acetone and acetonitrile were tested as alternative dispersers. Considering 1 mL of the first solvent, the yield of the DLLME process remained around 70% for MTCS and close to 80% for TCS. The same volume of acetone and acetonitrile provided recoveries between 55 and 60%, with an equal size for the settled phase (18  $\mu$ L), thus methanol was retained as disperser.

Table 1 summarizes extraction yields and EFs corresponding to three different volumes of this disperser: 0.5, 1 and 1.5 mL. Between 0.5 and 1 mL of methanol, the recoveries of the process remained unmodified; however, for 1.5 mL the efficiency of the extraction was reduced considerably, as a consequence of the increase in the solubility of the analytes in the water sample. Achieved EFs were dependant of two factors: (1) the yield of the extraction and (2) the size of the settled phase, which underwent a significant reduction with the increase in the volume of disperser. The net result was a slight increment in the EFs with the volume of disperser. Taking into account data in Table 1, 1 mL of methanol was maintained as working value for the volume of disperser. Although, under these conditions, EFs were slightly lower (around 5%) than those achieved with 1.5 mL, the size of the settled phase was nearly twice larger and thus, it resulted easier to handle.

	0.5 mL MeOH (20 $\mu$ L) <sup>a</sup>		1.0 mL MeOH (18 $\mu$ L) <sup>a</sup>		1.5 mL MeOH (11 $\mu$ L) <sup>a</sup>	
	Recovery $\pm$ SD	EFs	Recovery $\pm$ SD	EFs	Recovery $\pm$ SD	EFs
TCS	78.7 $\pm$ 6.6	394	79.3 $\pm$ 6.8	466	53.4 $\pm$ 9.3	485
MTCS	73.8 $\pm$ 7.0	368	72.9 $\pm$ 8.2	429	48.4 $\pm$ 6.5	440

Table 1. Extraction yields (recoveries, %) and enrichment factors (EFs) for different volumes of methanol. CH<sub>3</sub>CCl<sub>3</sub> and MTBSTFA volumes were 20 and 25  $\mu$ L, respectively. N=5 replicates. <sup>a</sup> Volume of the settled phase.

### 3.1.3. Ionic strength and sample pH

The effect of the ionic strength on the performance (yields and EFs) of the extraction was assessed with samples containing four different concentrations of sodium chloride. For MTCS, the yield of the DLLME decreased around 25% when the concentration of NaCl in the sample increased from 0 to 10%. In case of TCS, this diminution accounted for a 15%. This trend matches with that reported for SPME studies [15] and it can be explained due to the higher viscosity of the sample,

which slows down mass transference kinetics for non-polar compounds. As the size of the sedimented phase increased also with the ionic strength of the water sample (from 18 to 22  $\mu\text{L}$ ), a noticeable reduction in the EFs of the method was observed, Fig. 2.

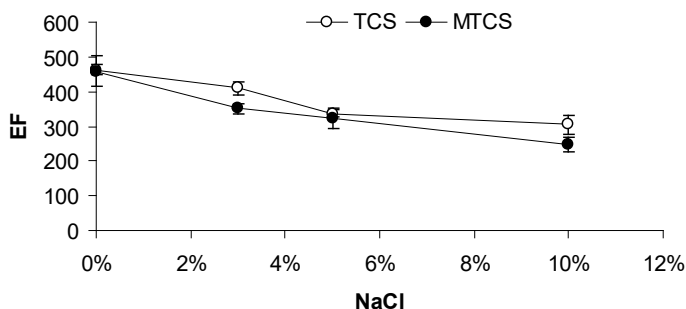


Fig. 2. EFs as function of the concentration of NaCl added to the samples, n=3 replicates.

TCS presents a pKa of 7.8 units, thus the pH of water samples might affect to the efficiency of derivatization and/or extraction steps, leading to variations in the yield of the method. To evaluate this possibility, aliquots of ultrapure water were adjusted at three different pHs (6,7 and 8 units) with a 0.1 M phosphate buffer solution. Lower and higher values were not tested since they are unusual in real-life water samples. As the volume of the extract remained unaffected by the pH of the sample, peak areas were compared directly to detect possible changes in the yield of the sample preparation. No significant differences were noticed in the responses measured neither for TCS nor for MTCS (Fig. 3), consequently, in further experiments water samples were processed as received, without pH adjustment.

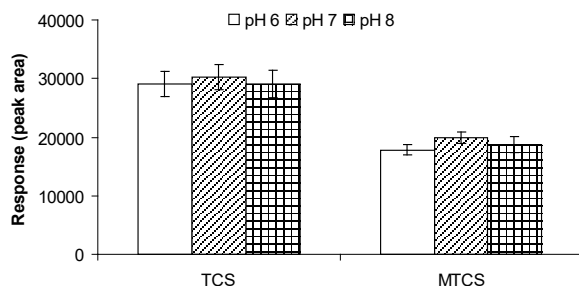


Fig. 3. Effect of sample pH on the performance of the DLLME process, n=4 replicates.

#### 3.1.4. Trichloroethane and MTBSTFA volumes

Previous DLLME experiments were carried out with 10 mL of ultrapure water. For this matrix, using 1 mL of disperser, 20  $\mu\text{L}$  of extractant and 25  $\mu\text{L}$  of MTBSTFA, the yield of the extraction remained around 75%, equivalent to an EF around 440 times, for both compounds, Table 1. Higher volumes of  $\text{CH}_2\text{Cl}_2$  led to lower EFs. Raising the volume of MTBSTFA resulted in a similar trend, data not shown. When raw wastewater samples were processed under above conditions, a whitish interface appeared between the sample and the settled extract, making extremely difficult to recover the latter. In order to overcome this drawback, the volume of extractant was doubled. Fig. 4 presents the responses (peak areas)

obtained for 10 mL aliquots of a spiked ( $2 \text{ ng mL}^{-1}$ ) raw wastewater sample using  $40 \text{ }\mu\text{L}$  of  $\text{CH}_3\text{CCl}_3$  and variable volumes of MTBSTFA. The response for MTCS decreased steady with the silylation reagent due to its contribution to the size of the sedimented phase, which increased from  $32$  to  $41 \text{ }\mu\text{L}$ . In case of TCS, the maximum response was noticed for  $30 \text{ }\mu\text{L}$  of MTBSTFA. For lower volumes the compound was not completely derivatized, whereas for larger amounts its concentration in the settled organic phase was diluted. In order to guarantee phase separation, as well as the quantitative derivatization of TCS, for any type of water sample,  $1 \text{ mL}$  of methanol containing  $40 \text{ }\mu\text{L}$  of  $\text{CH}_3\text{CCl}_3$  and the same volume of MTBSTFA was selected as extraction mixture. Under these conditions, the volume of the settled phase ( $39 \pm 1 \text{ }\mu\text{L}$ ) was large enough to be poured into a GC vial insert, making possible to use of an autosampler to automate the injection of extracts.

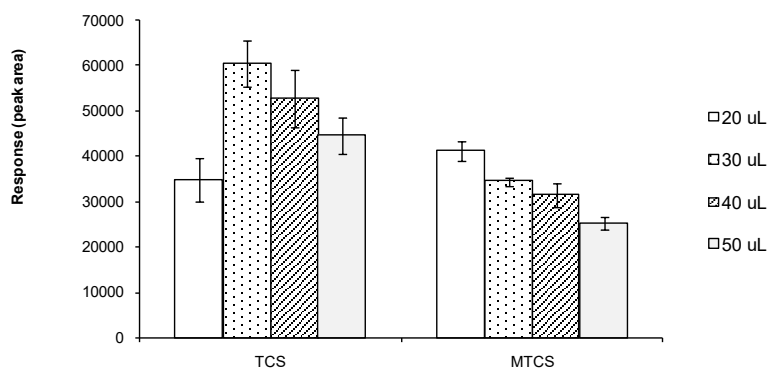


Fig. 4. Influence of the volume of MTBSTFA on the responses obtained for a spiked ( $2 \text{ ng mL}^{-1}$ ) raw wastewater sample. Methanol and  $\text{CH}_3\text{CCl}_3$  volumes were  $1 \text{ mL}$  and  $40 \text{ }\mu\text{L}$ ,  $n=4$  replicates.

### 3.1.5. Extraction and centrifugation time

After adding the extraction mixture to the sample, vessels were closed, manually shaken for  $1 \text{ min}$  and then allowed to stand for different times before being centrifuged. Fig. 5 plots the EFs achieved for different extraction times. The horizontal line in the graph represents the ratio between sample and extract volumes ( $10 \text{ mL}$  and  $39 \text{ }\mu\text{L}$ ), which corresponds to the maximum concentration factor (256 times) assuming a 100% yield for the DLLME process. Taking into account the variability of the results, it can be concluded that (1) extraction time did not affect the yield of the method, and (2) the average EFs for TCS and MTCS were 256 and 231 times, respectively (equivalent to absolute extraction yields of 100% and 90%). Both results (very fast extraction kinetics and high extraction yields) are in agreement with those obtained in previous applications of this microextraction technique to other organic compounds [18-22].

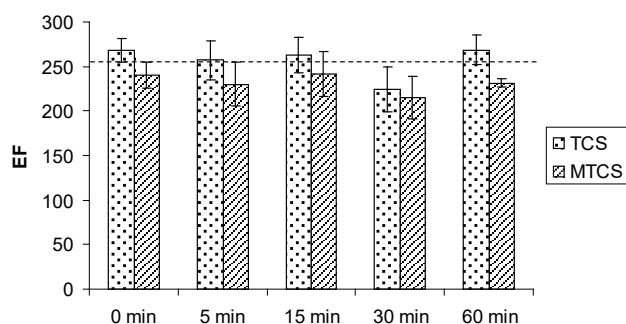


Fig. 5. EFs for different extraction times, n=4 replicates. The horizontal line corresponds to the maximum EF value.

As regards centrifugation time, neither the size of the settled phase, nor the concentration of the analytes in the extract, changed when samples were centrifuged different times between 3 and 20 min, figure not shown. Thus, the centrifugation step was limited to 3 min in order to improve the productivity (sample throughput) of the method. Under final working conditions, less than 15 min passed since the extraction mixture is added to the sample until the final extract is transferred to the autosampler of the GC system. Therefore, using a four positions centrifuge, each sample is concentrated in less than 5 min.

### 3.2. Method performance

Performance of the optimised DLLME method was evaluated by GC-MS/MS. Linearity was assessed using samples fortified at eight different concentration levels in the range from 10 to 2000 ng L<sup>-1</sup>. Correlation coefficients (R<sup>2</sup>) for the obtained graphs were equal or higher than 0.999, Table 2. Precision was examined with samples spiked at different concentrations within the above range. Relative standard deviations (RSDs) obtained under repeatability (intra-day precision) and reproducibility (inter-day precision) conditions remained under 10%, Table 2. Procedural blanks showed the absence of contamination problems; thus, achieved LOQs were controlled by the EFs of the DLLME process and the sensitivity of the GC-MS/MS system. Values of 5 and 2 ng L<sup>-1</sup> were attained for MTCS and TCS, respectively, Table 2. For the parent bactericide, this LOQ (2 ng L<sup>-1</sup>) is low enough to monitor its presence in waste and surface water samples [4,5,13-16,29]. The LOQ of MTCS is lower than its highest levels reported in treated wastewater (up to 100 ng L<sup>-1</sup>) [7,30]; however, it is unsuitable for surface water analysis, where the compound normally remains below the 1 ng L<sup>-1</sup> level [12, 29]. As far as we known, such low values have been attained only using GC in combination with magnetic sector mass spectrometers [29, 30].

Compound	Linearity R <sup>2</sup>	Repeatability <sup>a</sup> (RSD %)			Reproducibility <sup>b</sup> (RSD %)	LOQs ng L <sup>-1</sup>
		0.05 ng mL <sup>-1</sup>	0.2 ng mL <sup>-1</sup>	1 ng mL <sup>-1</sup>		
TCS	0.9990	9.5	3.2	3.6	5.7	2

MTCS	0.9996	6.6	6.8	8.6	7.7	5
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Table 2. Linearity, precision and limits of quantification (LOQs) for the optimized DLLME method. <sup>a</sup>n=4 replicates in the same day; <sup>b</sup>n=12 replicates in 3 consecutive days.

Table 3 summarizes some data related to the performance of different sample preparation methods applied to the determination of TCS and/or MTCS in water samples. As the rest of microextraction techniques (SPME, HF-LPME and SBSE), DLLME requires lower sample volumes than SPE based procedures. Its precision is similar to that attained with the rest of techniques and equal or lower LOQs, except in case of methods using high-resolution mass spectrometry (HRMS) detection, are obtained. Without any doubt, the major advantage of DLLME is the high sample throughput.

Analytes	Extraction method	Determination technique	Sample vol. (mL)	LOQs (ng L <sup>-1</sup> )	Time (min) <sup>a</sup>	Precision RSD (%)	Ref.
TCS	HF-LPME	GC-MS	10	60 <sup>b</sup>	20	7	[17]
TCS, MTCS	SPME	GC-MS	22	2	40	8-9	[15]
TCS	SBSE	GC-MS	15	90 <sup>b</sup>	240	20	[16]
TCS, MTCS	SPE	GC-HRMS	1000	1 <sup>b</sup>	40	n.a.	[29]
TCS, MTCS	SPE	GC-MS	1000	5-10	30	3-8	[7]
TCS	SPE	GC-MS	1000	30 <sup>b</sup>	40	<7	[14]
TCS, MTCS	DLLME	GC-MS/MS	10	2-5	5	3-10	This work

Table 3. Performance of different methods applied to the determination of TCS and MTCS in water samples. <sup>a</sup>Estimated sample preparation time per sample; <sup>b</sup>Reported limits of detection were multiplied by a factor of 3; n.a. not available

Table 4 shows the effect of the type of matrix on the performance of the method. Each sample was divided in two fractions and one of them spiked with target species at different concentrations. Sodium thiosulphate (20 mg L<sup>-1</sup>) was added to tap water to prevent the decomposition of TCS in presence of free chlorine [31]. Spiked and non-spiked aliquots of each sample were extracted in triplicate and responses measured for TCS and MTCS normalized to those corresponding to ultrapure water with the same addition level. Obtained results (Table 4) indicated that, the efficiency of DLLME process was affected in a minor extension by the nature of water samples. Even in case of wastewater, the decrease in the efficiency of the extraction accounted for less than 20 % when compared with ultrapure water. These results indicate to the possibility of using external calibration, instead of the time-consuming standard addition protocol, as quantification technique.

	Relative recovery (% ± SD)			
	Tap water (0.2 ng mL <sup>-1</sup> ) <sup>a</sup>	River water (0.2 ng mL <sup>-1</sup> ) <sup>a</sup>	Treated wastewater (1 ng mL <sup>-1</sup> ) <sup>a</sup>	Raw wastewater (2 ng mL <sup>-1</sup> ) <sup>a</sup>
TCS	103.3 ± 2.3	102.8 ± 2.1	95.5 ± 9.6	93.4 ± 7.0

MTCS	100.9 ± 2.5	105.4 ± 2.2	81.7 ± 12.5	87.3 ± 6.1
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Table 4. Relative extraction efficiencies for different water samples. Normalized values to those achieved for ultrapure water with the same addition level, n=4 replicates. <sup>a</sup> Added concentration

### 3.3. Real samples analysis

The optimized method was applied to grab samples of river and wastewater obtained from an urban STP equipped with primary and activated sludge treatments. Samples were passed through glass fibre filters and stored at 4°C, for a maximum of 48 hours, before analysis. TCS was found in all samples at concentrations from 40 ng L<sup>-1</sup> up to more than 700 ng L<sup>-1</sup>, depending on the type of water. MTCS remained under the quantification limit of the method (5 ng L<sup>-1</sup>) in sample code 6 and could not be detected in the rest. Globally, these values are similar to those reported in previous works for river and wastewater samples in Europe [5,16,29]. As a further assessment of the accuracy of the method, two samples (codes 3 and 6) were spiked with TCS and MTCS. Measured concentrations were in reasonable agreement (recoveries from 95 to 112 %) with the added amount plus the native levels of TCS and MTCS in both samples, Fig. 6.

Sample code	Sample type	Concentration (ng L <sup>-1</sup> ) ± SD	
		TCS	MTCS
1	Treated wastewater	74 ± 12	n.d.
2	River water	41 ± 4	n.d.
3	River water	26 ± 4	n.d.
3	River water spiked at 100 ng L <sup>-1</sup>	139 ± 11	112 ± 7
4	River water	105 ± 5	n.d.
5	Raw wastewater	728 ± 87	n.d.
6	Treated wastewater	104 ± 14	< 5
6	Treated wastewater spiked at 400 ng L <sup>-1</sup>	484 ± 32	385 ± 8

Table 5. Levels of TCS and MTCS in water samples. n.d. not detected

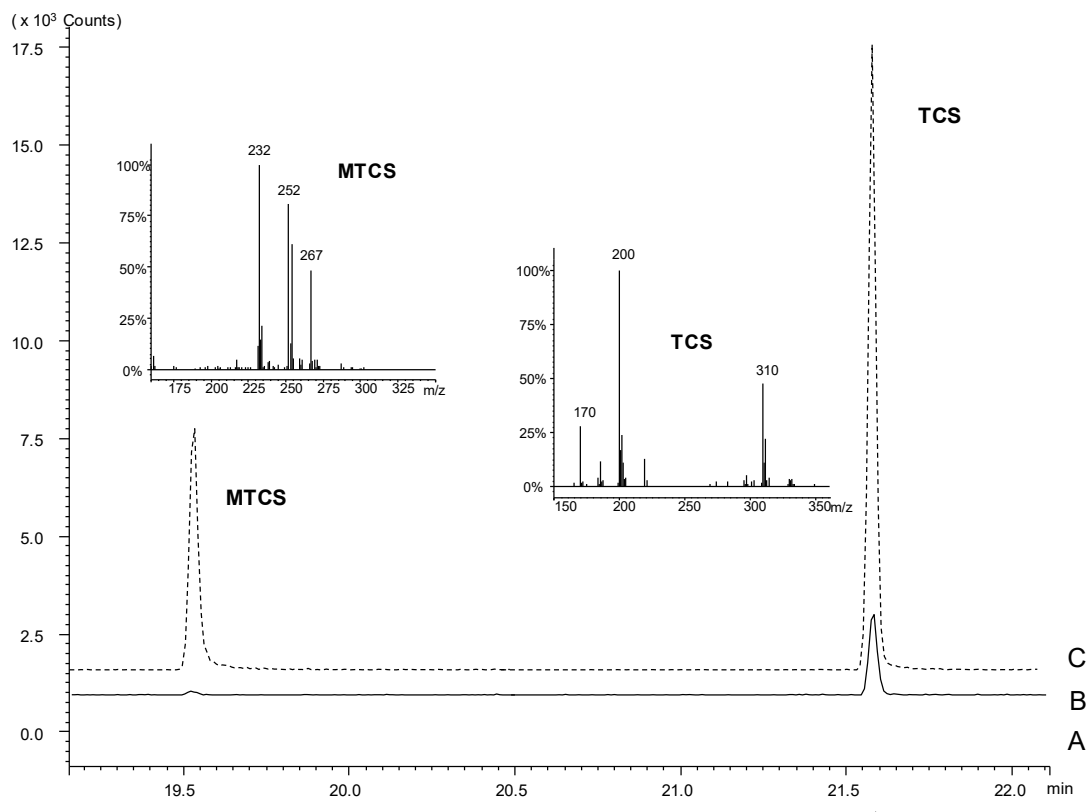


Fig. 6. GC-MS/MS chromatograms for a procedural blank (A) and sample code 6, table 5, before (B) and after (C) addition of TCS and MTCS at 400 ng L<sup>-1</sup>.

#### 4. Conclusions

Fast mass transference kinetics of DLLME allowed integrating a derivatization step, using a water sensitive silylation reagent, in the extraction of TCS and MTCS from water samples, providing extraction efficiencies higher than 90% for both species and EFs close to 250 times. The performance of the proposed method is mainly controlled by the nature and volume of disperser, extractant and silylation reagent, as well as the ionic strength of the sample. Precision, LOQs and linearity obtained under optimized conditions are suitable for the determination of TCS in surface and wastewater samples. In case of MTCS, a further improvement in sensitivity is advisable to measure concentrations of this pollutant below the 5 ng L<sup>-1</sup> level. For the best of our knowledge, this work constitutes the first application of DLLME, which incorporates a silylation reaction in the sample preparation scheme. This approach could be of interest for the determination of other phenolic and acidic PPCPs, whose ability to react with MTBSTFA, in organic media, has been previously demonstrated [16].

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