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# Optimization of solid-phase microextraction conditions for the determination of triclosan and possible related compounds in water samples

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**Abstract**

A solid-phase microextraction (SPME) method for the determination of triclosan, methyl triclosan, 2,4-dichlorophenol and 2,3,4-trichlorophenol (considered as possible triclosan metabolites) in water samples was optimised. Analytes were first concentrated on a SPME fibre, directly exposed to the sample, and then triclosan and the two chlorinated phenols *on-fibre* silylated using *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA). Methyl triclosan remained unaffected during the derivatization step. Compounds were determined using gas chromatography in combination with mass spectrometry (GC–MS). Influence of different factors on the efficiency of extraction and derivatization steps was systematically investigated. Using a polyacrylate (PA) fibre quantification limits below 10 ng/l, and acceptable relative standard deviations, were obtained for all compounds after an extraction time of 30 min. *On-fibre* silylation was carried out in only 10 min. Moreover, the efficiency of the procedure was scarcely affected by the type of water sample. The method was applied to several samples of treated and raw wastewater, triclosan was found in all samples, at concentrations from 120 to 14,000 ng/l, and 2,4-dichlorophenol in most of them, at levels up to 2222 ng/l.


*Keywords:* Triclosan; SPME; *On-fibre* derivatization; GC–MS

## 1. Introduction

Triclosan, 2-(2,4-dichlorophenoxy)-5-chlorophenol, is an hydroxylated biphenyl ether with antimicrobial and bactericide properties included in the formulation of many personal care products (e.g. shampoos, soaps, creams and toothpastes), domestic and medical disinfectants, clothes and footwear [1]. Therefore, wastewater constitutes an important source of this compound in the environment [2,3]. Triclosan itself presents a low toxicity and moreover, it is removed to a considerable extent during conventional wastewater treatments [3]. However, different studies have suggested that under certain conditions, e.g. in presence of hypochlorite or due to photochemical reactions, the native specie can be converted into most toxic and persistent polar compounds such as chlorinated phenols [4,5] (mainly 2,4-dichloro and 2,3,4-trichlorophenol) polychlorinated biphenyl ethers [6,7] and dihydroxylated derivatives [8]; as well as, non-polar and bio-accumulative species such as methyl triclosan, already detected in tissues from aquatic organisms exposed to low levels of triclosan [9,10], and polychlorinated dibenzodioxins [11]. Those possible transformations, added to the continuous introduction of triclosan in the aquatic environment, have increased the interest in the occurrence, environmental fate and possible long-term effects associated to a continuous exposition to low levels of triclosan.

Analytical methods for the determination of triclosan, and related compounds, in water samples are based on a preconcentration step followed by the selective determination of the target compounds using mass spectrometry, normally in combination with gas chromatography. In this case, the derivatization of triclosan and its polar metabolites is advis-

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able to improve the sensitivity of the method. Diazomethane is one of the proposed derivatization reagents [3,6,12]; however, in this case, triclosan and methyl triclosan cannot be determined in the same analysis. Acetylation and silylation are alternative derivatization techniques, which allow to overcome this drawback [13–15]. Liquid–liquid extraction and solid-phase extraction (SPE) are normally employed as preconcentration techniques in order to achieve detection limits at the ng/l level [2,3,15,16]. Both require the concentration of large sample volumes and sometimes the evaporation of the final organic extract in order to reduce the detection limits of the method.

Solid-phase microextraction (SPME) is an alternative sample preparation technique for the extraction of organic compounds from water samples. It reduces the sample intake, avoids the use of organic solvents and minimizes the number of steps involved in the sample preparation. SPME is especially suitable in combination with gas chromatographic based techniques, since the extracted analytes are directly desorbed in the hot injector of the GC instrument. However, compounds containing polar groups in their structures (e.g. triclosan) should be derivatized to improve the quality of the GC separations. Derivatization can be performed in the aqueous sample or in the SPME fibre after the concentration step. The last option is preferred when water sensitive derivatization reagents, such as diazomethane or silylation species, are employed [17–19].

In this paper, a solid-phase microextraction procedure for the determination of triclosan, methyl triclosan, 2,4-dichlorophenol and 2,3,4-trichlorophenol in water samples is proposed. Analytes were first extracted from the sample using a SPME fibre and then *on-fibre* derivatized (except methyl triclosan) using *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA). Methyl triclosan and the *tert*.-butyldimethylsilyl derivatives of the rest of compounds were determined using GC–MS. Experimental parameters were optimised to achieve the maximum efficiency during the extraction and derivatization steps. The method was used to determine the presence and the levels of those compounds in wastewater samples.

## 2. Experimental

### 2.1. Reagents and samples

Methanol grade HPLC was supplied by Merck (Darmstadt, Germany). Triclosan (TCS), 2,4-dichlorophenol (2,4-DCP), 2,3,4-trichlorophenol (2,3,4-TCP) and the derivatization reagent *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) were purchased from Aldrich (Milwaukee, WI, USA). Methyl triclosan (MTCS) was obtained from Toronto Research Chemicals (Ontario, Canada). Structures of the analytes are shown in Fig. 1. Individual solutions of these compounds and mixtures of them were prepared in methanol.

A manual SPME holder and fibres coated with different polymers: poly(dimethylsiloxane) (PDMS, 100 μm film thickness), polyacrylate (PA, 85 μm film thickness), Carboxen-PDMS (CAR-PDMS, 75 μm film thickness), poly(dimethylsiloxane–divinylbenzene) (PDMS-DVB, 65 μm film thickness) and Carbowax-DVB (CW-DVB, 75 μm film thickness), were obtained from Supelco (Bellefonte, PA, USA). Before their first use, each fibre was conditioned following the supplier specifications. SPE cartridges containing 60 mg of the OASIS HLB polymer were obtained from Waters (Milford, MA, USA).

Spiked and non-spiked samples (Milli-Q, river and wastewater) were used in this study. Discrete and flow proportional 12 h composite wastewater samples were collected in the influent and the effluent of an urban sewage plant equipped with primary and secondary treatments. This plant receives the combined wastewater from a 125,000 inhabitants city and also from a large hospital. Raw wastewater samples

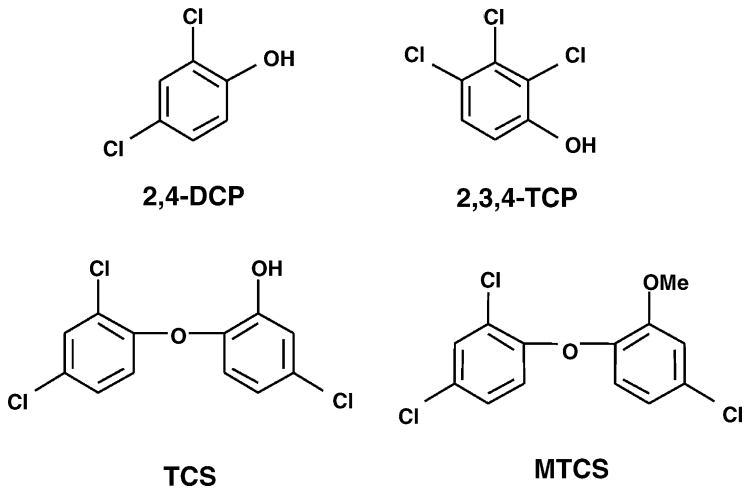


Fig. 1. Chemical structures of the selected analytes.

were also taken in the city and hospital sewers previously to their confluence. Samples were passed through glass fibre filters and then stored at 4 °C until being analysed.

### 2.2. Sample preparation

#### 2.2.1. SPME

Under optimal conditions, water samples were adjusted to pH 4.5 and placed on glass vials containing a magnetic stirrer. Vessels were filled completely with the sample and closed using a PTFE coated silicone rubber septum. Compounds were concentrated on a PA fibre directly exposed to the sample during 30 min, at room temperature (ca. 20 °C). In the case of spiked samples, the percentage of methanol in the SPME vessel was limited to a maximum of 1% in order to avoid changes in the yield of the extraction depending on the sample methanol content. Vials with capacities of 10, 22 and 110 ml were employed in this study. Once the extraction step was finished, the fibre was retracted into the SPME syringe and any possible drop of water attached to the needle removed with a soft tissue. Then, it was exposed to the headspace of a 1.5 ml GC autosampler vial containing 20 μl of MTBSTFA. *On-fibre* silylation of the analytes, except MTCS, was completed in 10 min at room temperature. PA fibres were desorbed at 280 °C for 3 min in the splitless mode.

#### 2.2.2. SPE

Solid-phase extraction was used to confirm the accuracy of results obtained for polluted samples using the SPME method. Extraction conditions were basically those given by Lee et al. [14]. The only modifications were that (1) ethyl acetate was employed to recover the compounds from the SPE cartridge (methanol is used in the original paper), and that (2) *tert.*-butyldimethylsilyl, instead of trimethylsilyl, derivatives of the extracted analytes (2,4-DCP; 2,3,4-TCP and TCS) were prepared.

### 2.3. Equipment

Analytes were determined by GC–MS. The employed system consist of a Varian Start 3400 CX gas chromatograph (Walnut Creek, CA, USA) equipped with a split–splitless injector and connected to an ion-trap mass spectrometer (Varian Saturn 3). Separations were carried out using a low bleed CPSIL8 type capillary column (30 m × 0.25 mm i.d., $d_f$: 0.25 μm) purchased from Varian. Helium (99.999%) was used as carrier gas at a constant head pressure of 60 kPa. The GC oven was programmed as follows: 3 min at 50 °C, 10 °C/min to 260 °C (held for 10 min). The GC–MS interface and the ion trap temperature were set at 260 and 220 °C, respectively. Mass spectra, in the electron impact mode (70 eV) were obtained in the range from 50 to 550 *m/z* units. SPME fibres were desorbed during 3 min, in the splitless mode, using the following temperatures: 250 °C for PDMS and PDMS-DVB, 280 °C for PA and CAR-PDMS, and 220 °C for CW-DVB. Compounds were quantified using peak areas obtained at the following *m/z* ratios 219 + 221 (2,4-DCP), 253 + 255 (2,3,4-TCP), 345 + 347 (TCS) and 302 + 304 (MTCS).

### 2.4. Quantification

Concentrations of the analytes in polluted samples were determined using the external standard calibration method. Slopes of calibration curves were obtained using ultrapure water samples spiked with the analytes at different concentration levels (at least four levels were considered). The obtained equations were employed to predict the concentration of the analytes in polluted samples.

## 3. Results and discussion

### 3.1. Preliminary experiments

The feasibility of the *on-fibre* silylation of 2,4-DCP, 2,3,4-TCP and TCS was firstly assessed using a PA fibre previously exposed to ultrapure water samples spiked with these compounds. Fifty microliters of MTBSTFA, 20 min and 40 °C were used as *on-fibre* silylation conditions [18]. GC–MS chromatograms showed three peaks for the *tert.*-butyldimethylsilyl derivatives of the analytes. The most intense signals in their MS spectra were 219 + 221 (2,4-DCP), 253 + 255 (2,3,4-TCP) and 345 + 347 (TCS), corresponding to the loss of the *tert.*-butyl moiety in the silylated compounds. Traces of native species were not observed in chromatograms monitored at *m/z* ratios: 162 + 164 (2,4-DCP), 196 + 198 (2,3,4-TCP) and 288 + 290 (TCS), which correspond to the base peak in MS spectra of the non-derivatized compounds. The variability of the extraction-derivatization procedure remained below 12% for all compounds ($n = 4$ replicates).

### 3.2. Optimization of microextraction conditions

Influence of different experimental parameters on the amount of TCS, MTCS, 2,4-DCP and 2,3,4-TCP concentrated on the SPME fibre was evaluated using spiked Milli-Q water samples (10 ng/ml). Derivatization conditions were those given in the above paragraph.

#### 3.2.1. Sampling mode and temperature

The effect of both parameters was investigated using a PA fibre and an extraction time of 20 min. Experiments were carried out in 22 ml vessels containing a magnetic stir bar and 15 ml of a spiked water sample adjusted to pH 2, using HCI 0.1 M, to insure that phenolic species are in the neutral form. Headspace extractions (HS) were carried out at room temperature and at 100 °C, whereas direct sampling was performed only at room temperature. For the less polar compound (MTCS), the highest yield was obtained in the HS mode at 100 °C, followed by direct exposure and HS at

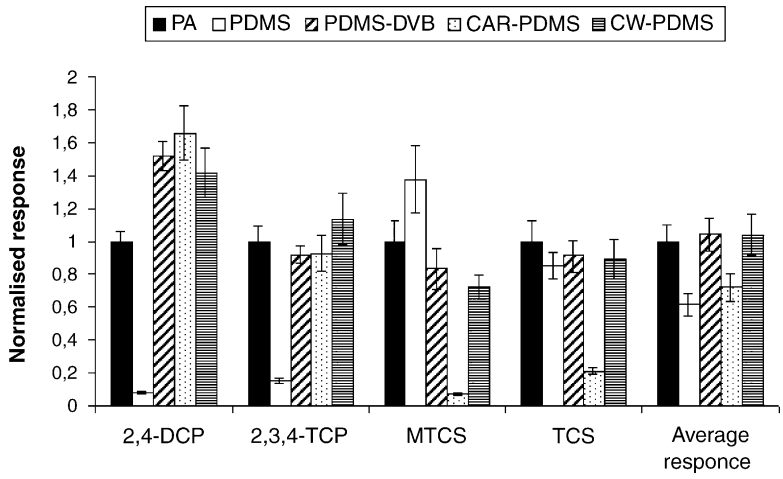


Fig. 2. Comparison of the extraction efficiency (direct sampling) using different SPME fibres. $N = 3$ replicates. Responses (peak areas) were normalised respect to the PA fibre.

room temperature, figure not shown. For the rest of species, the highest peak areas were obtained using direct sampling. As the goal of the study was the simultaneous determination of the four selected compounds direct sampling was used in further experiments.

### 3.2.2. Fibre selection

Normalised peak areas for each one of the considered compounds, as well as the average normalised response for all of them, with five different fibres are shown in Fig. 2. The PA fibre was taken as reference. Extractions were performed at room temperature during 30 min. The non-polar PDMS fibre showed a low affinity for the phenolic species (2,4-DCP and 2,3,4-TCP) and the CAR-PDMS one by MTCS and TCS; globally, similar results were obtained for the other three fibres, Fig. 2. Two of them (the single adsorbent coated PA fibre and the bipolar sorbent coated PDMS-DVB one) were considered in further optimisation experiments.

### 3.2.3. Stirring, sample pH and salt (sodium chloride) addition

Their influence on the yield of the microextraction, for PA and PDMS-DVB fibres, was investigated using experimental factorial designs at two levels ($2^3$) with two central points. In all experiments, the microextraction time was fixed to 30 min. Sample pH was always maintained below the p$K_a$ values of the phenolic species. Low and high levels for the three factors are given in Table 1. Data (peak areas) obtained in the 10 experiments involved in each factorial design were evaluated using the software package Statgraphics Plus (Manugistics, Rockville, MD, USA). Standardized coefficients of the main effects for each experimental factor are also given in Table 1. These coefficients estimate the effect of a given factor on the yield of the microextraction for each analyte. The absolute value is proportional to the influence of the corresponding factor on the SPME step. A positive sign corresponds to an increase in the yield of the extraction when the factor changes from the low to the high value, whereas a negative sign indicates the opposite behaviour. The observed tendency was similar for both fibres: stirring was the most important factor with a positive effect on the yield of the extraction, especially for the higher molecular weight compounds: TCS and MTCS, which probably have the lower diffusion coefficients in the water sample. The addition of sodium chloride produced a decrease in the yield of the extraction for most of the compounds. In some cases (TCS and MTCS using the PMDS-DVB fibre, and MTCS with the PA fibre), this diminution was statistically significant at the 95% confidence level. Finally, sample pH played a negative but non-significant effect on the efficiency of the extraction. Magnetic stirring (500 rpm) and pH 4.5 were fixed as working conditions, whereas the effect of the ionic strength was evaluated in more detail. At low

Table 1
Standardized coefficients of the main effects for factors considered in the optimisation of the microextraction step (direct sampling) using PA and PDMS-DVB fibres

| Factor | Level | | PA | | | | PDMS-DVB | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Low | High | 2,4-DCP | 2,3,4-TCP | MTCS | TCS | 2,4-DCP | 2,3,4-TCP | MTCS | TCS |
| Stirring | No | Yes | 2.1 | 2.3 | 12.0[a] | 9.5[a] | 3.2[a] | 2.3 | 8.7[a] | 26.0[a] |
| NaCl (g/ml) | 0 | 0.2 | 0.02 | −0.7 | −9.1[a] | −1.8 | −0.5 | −0.7 | −6.5[a] | −5.5[a] |
| pH | 3 | 6 | −0.8 | −0.4 | −2.5 | −0.9 | −0.9 | −0.9 | −0.6 | −1.2 |

[a] Significant factors at the 95% confidence level.

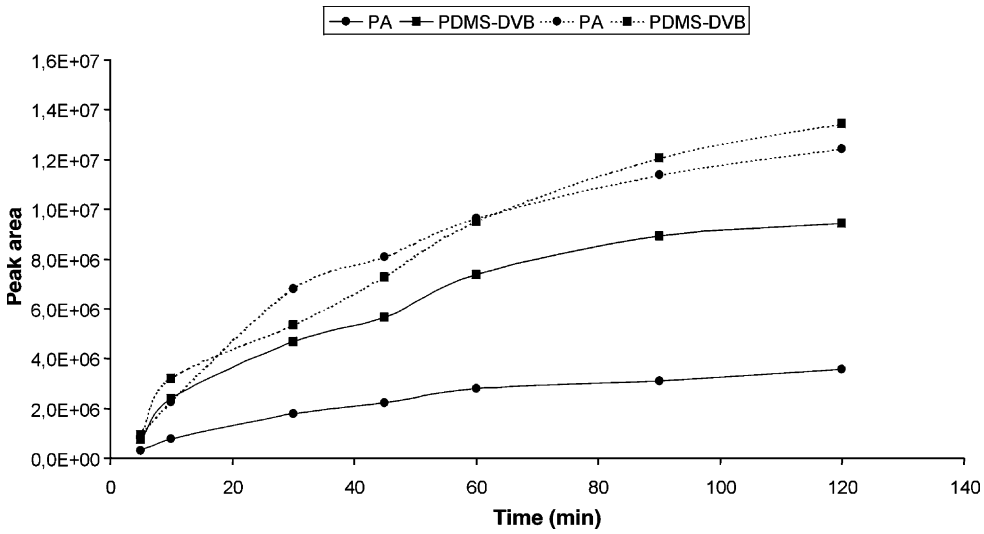


Fig. 3. Time course of the SPME for 2,4-DCP (solid line) and TCS (dotted line) using PA and PDMS-DVB fibres. Direct extraction at room temperature using spiked water samples adjusted at pH 4.5.

sodium chloride concentrations (0.025–0.050 g/ml) a slight increase in the efficiency of the extraction was observed; however, as the effect was non-significant in further experiments no salt was added to the samples.

### 3.2.4. Extraction time

Influence of extraction time on the responses obtained for 2,4-DCP and TCS, using PA and PDMS-DVB fibres, are shown in Fig. 3. As illustrated, none of both compounds reached the equilibrium after an exposure time of 2 h. A similar behaviour was observed for 2,3,4-TCP and MTCS, figure not shown. In agreement with the results presented in Fig. 2, 2,4-DCP showed a higher affinity for the PDMS-DVB fibre than for the PA one. For the rest of analytes, similar yields were obtained with both fibres using exposition times up to 2 h. In spite of these slow extraction rates, the extraction time was limited to 30 min to speed up sample preparation. Obviously, the corresponding diminution in the sensitivity of the method was assumed.

### 3.2.5. Memory effects

The presence of residual compounds (derivatized or not) in both fibres, after a desorption step of 3 min using those temperatures indicated in the experimental section, was evaluated by exposing them to the headspace of a vessel containing a fresh solution of MTBSTFA. Carry over effects were not noticed for 2,4-DCP and 2,3,4-TCP with any of both fibres. Peak areas of MTCS and TCS in the second desorption represented between 0.5 and 1.5% of those in the first one. The highest carry over was observed for TCS with the PDMS-DVB fibre. In order to avoid contamination problems between consecutive samples, fibres were additionally desorbed during 3 min at 280 °C (PA) and 250 °C (PDMS-DVB), after the chromatographic injection.

### 3.2.6. Sample volume

Vessels with capacities of 10, 22 and 110 ml were employed in this study. In all cases, the headspace volume over the sample was reduced to the minimum in order to avoid the dilution of the analytes in this phase. Using 10 and 22 ml vessels, similar results were obtained for all compounds with both fibres; however, for the 110 ml vials a slight decrease was observed in the responses of TCS and MTCS using PA and PDMS-DVB fibres. They are the heaviest compounds for which the influence of stirring was most relevant. It was assumed that a decrease in the stirring efficiency, when the largest vessels are used, is responsible for this slight diminution in the yield of the SPME for both compounds. The 22-ml volume vessels were used in further experiments.

## 3.3. On-fibre derivatization

The effects of time, temperature and volume of MTBSTFA on the peak areas of the silylated compounds was investigated using experimental factorial designs at two levels for PA and PDMS-DVB fibres. Low and high values for the three variables (20–100 μl MTBSTFA, 40–70 °C, and 10–40 min) were selected on the basis of previously reported results for the *on-fibre* silylation of anti-inflammatory drugs containing carboxylic groups [18]. In this case, the higher the temperature the lower were the peak areas. This behaviour pattern was observed for all compounds (including MTCS which does not undergo any derivatization reaction). It is believed that at high-temperatures analytes may be partially desorbed from the fibre during the derivatization step, especially when long derivatization times are used. The tendency was the same for all species and both fibres, but the most significant negative effect of the temperature was observed for 2,4-DCP and 2,3,4-TCP using the PA fibre, figure not shown. The vol-

Table 2
Linearity, quantification limits and relative standard deviations (R.S.D.) of the proposed method using PA and PDMS-DVB fibres

| Compound | Linearity | | | | Q.L (S/N = 10) (ng/l) | | RSD (%), $n = 3$ replicates | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PA | | PDMS-DVB | | PA | PDMS-DVB | PA | | PDMS-DVB | | |
| | $R^2$ | Slope ($10^5$) | $R^2$ | Slope ($10^5$) | | | 15[a] | 70[a] | 0[a] | 40[a] | 60[a] |
| 2,4-DCP | 0.9999 | 18 | 0.9994 | 41 | 7 | 4 | 7.2 | 9.5 | 8.0 | 18.2 | 17.2 |
| 2,3,4-TCP | 0.9961 | 41 | 0.9998 | 47 | 2 | 2 | 7.6 | 10.3 | 7.8 | 15.8 | 17.4 |
| MTCS | 0.9990 | 80 | 0.9999 | 83 | 2 | 2 | 9.2 | 6.8 | 6.4 | 23.3 | 21.1 |
| TCS | 0.9999 | 84 | 0.9999 | 87 | 2 | 2 | 8.7 | 8.0 | 5.9 | 21.3 | 17.5 |

[a] Previous extraction cycles.

ume of MTBSTFA and the derivatization time did not show significant effects for any compound with both fibres. They were fixed to 20 μl and 10 min, respectively. Additional experiments were carried out comparing the efficiency of *on-fibre* derivatization at room temperature and 40 °C. As similar results were obtained in both cases (figure not shown) by convenience the derivatization step was performed without temperature control. Under optimal conditions, traces of non-derivatized analytes were not observed in the chromatograms.

### 3.4. Performance of the method

The linearity of the method was evaluated using water samples spiked with the selected compounds at seven different concentration levels from 30 to 10,000 ng/l. Correlation coefficients ($R^2$) from 0.996 to 0.999 were obtained for all compounds with both fibres. Slopes of the addition curves, using PA and PDMS-DVB fibres, were similar for all compounds with the exception of 2,4-DCP, Table 2. Obtained limits of quantification (defined for a ratio S/N 10) ranged from 2 to 7 ng/l depending on the compound and the fibre. In the case of wastewater samples these values should be multiplied by a factor of two to compensate for a higher baseline noise. These limits of quantification are similar to those reported (from 5 to 20 ng/l) after solid-phase extraction of one-liter samples, derivatization of the analytes and GC–MS determination [3,14,20]. Relative standard deviations of the extraction–derivatization process, using samples spiked at 200 ng/l, ranged from 7 to 10% using a PA fibre. For the PDMS-DVB one, similar values were achieved for new fibres; however, a significant lost of repeatability was observed with the increase in the number of extraction-derivatization cycles. The reason of the behaviour is not clear, maybe partial blockage of pores in the surface of the DVB polymer, with co-extracted non-volatile compounds, could explain the increase in the variability of the results for this fibre. Apparently, stability problems, such as partial or total stripping of the coated phase from the silica core, were not observed for any of both fibres (PA and PDMS-DVB) even after 100 extraction cycles.

Table 4
Accuracy of the method for a raw wastewater sample. Concentrations in ng/l

| Compound | Non-spiked sample (average ± S.D.) | | Spiked sample (average ± S.D.) | |
|---|---|---|---|---|
| | SPME | SPE (reference [14]) | Added conc. | Found conc. (SPME) |
| 2,4-DCP | 183 ± 18 | 163 ± 17 | 941 | 1164 ± 70 |
| 2,3,4-TCP | n.d. | n.d. | 986 | 964 ± 48 |
| TCS | 729 ± 43 | 690 ± 35 | 989 | 1734 ± 62 |
| MTCS | n.d. | n.d. | 940 | 982 ± 53 |

n.d.: below the limit of quantification.

Matrix effects were investigated using Milli-Q, river and wastewater (raw and treated). Samples were adjusted at pH 4.5, filtered and spiked with the selected compounds at the 3 ng/ml level. In the case of wastewater, non-spiked samples were also analysed and obtained peak areas subtracted from those corresponding to the spiked samples. Each sample was processed in triplicate. In view of the obtained results, Table 3, and considering the precision of the proposed method, Table 2, it can be stated that, for both fibres, the yield of the extraction it is scarcely affected by the type of water sample and thus by matrix effects.

Table 3
Influence of the water sample type on the yield of the microextraction using PA and PDMS-DVB fibres

| Compound | Normalised responses with their relative standard deviation (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PA fibre | | | | PDMS-DVB fibre | | | |
| | Milli-Q | River | Effluent | Influent | Milli-Q | River | Effluent | Influent |
| 2,4-DCP | 100 (11) | 102(4) | 101 (7) | 98 (10) | 100 (13) | 100 (18) | 94 (5) | 98 (12) |
| 2,3,4-TCP | 100 (8) | 109(9) | 98 (9) | 96 (9) | 100 (8) | 104 (12) | 107 (9) | 113 (6) |
| MTCS | 100 (8) | 73(1) | 77 (7) | 93 (1) | 100 (11) | 99 (3) | 107 (16) | 101 (10) |
| TCS | 100 (10) | 88(10) | 96 (8) | 100 (5) | 100 (9) | 102 (8) | 100 (8) | 104 (5) |

Average peak areas ($n = 3$) normalised to ultrapure water; effluent: treated wastewater; influent: non-treated wastewater.

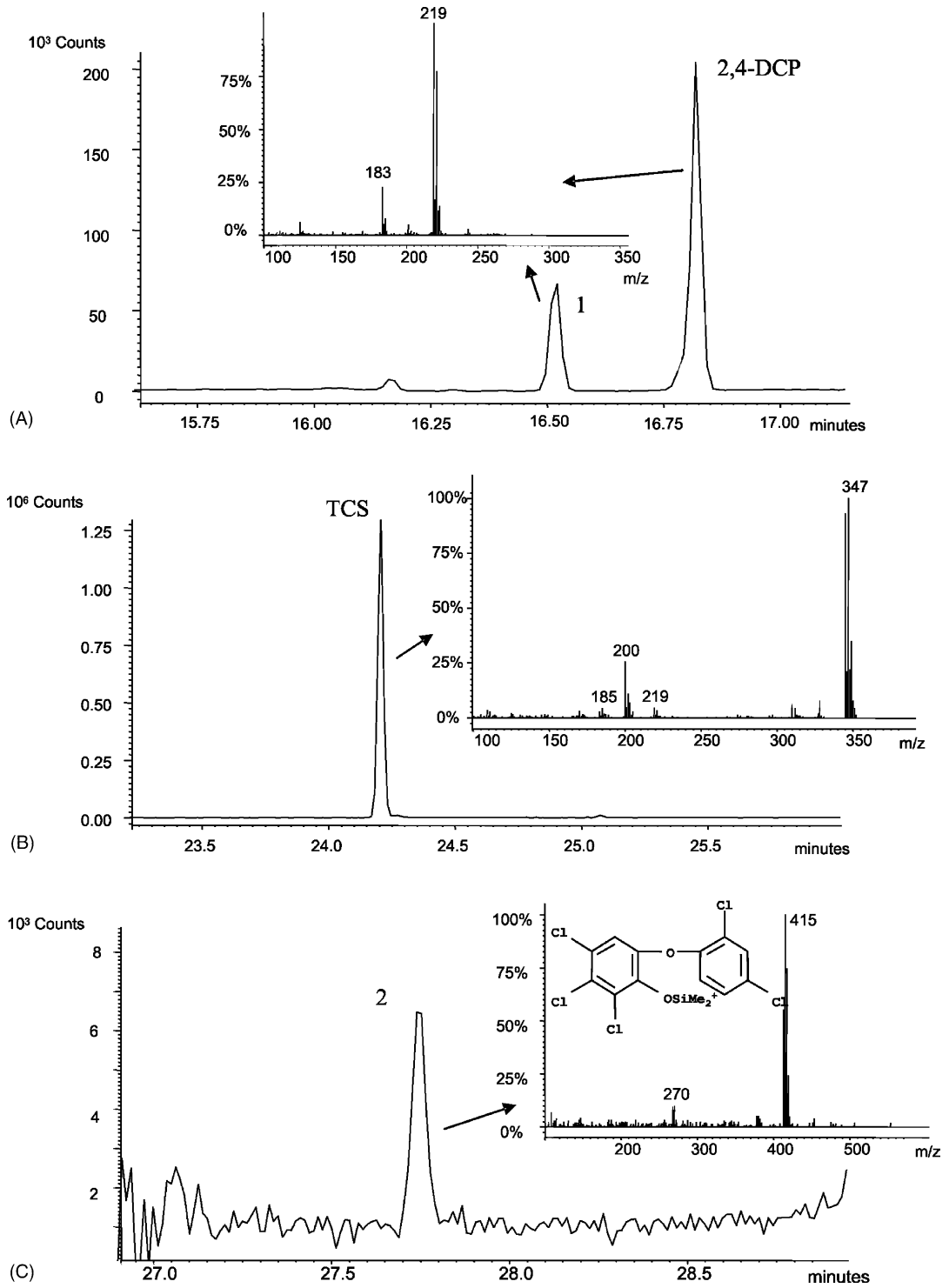


Fig. 4. GC–MS chromatogram for wastewater sample (no. 8). Signals at *m*/*z* 219 + 221 (A); 345 + 347 (B); and 413 + 415 + 417 (C). According to their MS spectra, peaks 1 and 2 could correspond to a dichlorophenol isomer and a pentachlorinated phenoxy-phenol, respectively.

Accuracy was evaluated comparing the results obtained for a grab raw wastewater sample with the optimised SPME method and with a classic approach based on exhaustive extraction of the compounds using a SPE cartridge [14]. A good agreement was found between the concentrations of 2,4-DCP and TCS measured with both procedures. MTCS and 2,3,4-TCP were not detected in the same using any of both procedures, Table 4. In addition to these results, an aliquot of the same sample was spiked with the four considered compounds (ca. 1 ng/ml), stored overnight at 4 °C, and processed with the

Table 5
Levels of TCS and 2,4-DCP in sewage water samples

| Sample code | Sample type | Sampling mode | Fibre | Conc. (ng/l) ± S.D. | |
|---|---|---|---|---|---|
| | | | | 2,4-DCP | TCS |
| 1 | Plant influent | Discrete | PA | 144 ± 11 | 242 ± 18 |
| 1 | Plant influent | Discrete | PDMS-DVB | 151 ± 17 | 229 ± 40 |
| 2 | Plant influent | Composite | PA | n.d. | 433 ± 52 |
| 3 | Plant effluent | Composite | PA | n.d. | 209 ± 22 |
| 4 | Plant influent | Composite | PA | 54 ± 5 | 966 ± 164 |
| 5 | Plant effluent | Composite | PA | 348 ± 27 | 321 ± 40 |
| 6 | Hospital sewer | Discrete | PDMS-DVB | 842 ± 250 | 2000 ± 440 |
| 7 | City sewer | Composite | PA | 35 ± 3 | 121 ± 12 |
| 8 | Hospital sewer | Composite | PA | 1223 ± 75 | 4148 ± 423 |
| 9 | City sewer | Composite | PA | 160 ± 9 | 382 ± 26 |
| 10 | Hospital sewer | Composite | PA | 2222 ± 277 | 13944 ± 1760 |

n.d.: below the limit of quantification.

SPME method. Obtained results represented between 95 and 105% of spiked plus native concentrations.

### 3.5. Application to real samples

The proposed method was applied to the analysis of several wastewater samples. MTCS and 2,3,4-TCP were not detected in any sample. TCS was found in all of them (from 120 to more than 14,000 ng/l), and 2,4-DCP in eight of the ten considered samples (at concentrations from 35 to 2222 ng/l), Table 5. Sample no. 1 was processed using PA and PDMS-DVB fibres, in agreement with the results observed during optimisation experiments, the best precision was obtained with the first one. Pairs 2–3 and 4–5 correspond to composite water samples from the influent and the effluent of a sewage plant, collected in two different dates. A significant removal of TCS in the plant was observed comparing the results obtained for both pairs of samples; however, it is smaller than efficiencies around 95% given for other plants with similar characteristics [3,7]. TCS concentrations in the inlet of the plant are lower than those reported for several plants in USA (from 4 to 17 ng/ml) [7] and, much lower than levels up to 500 ng/ml found in a plant located in the south of Spain [21]; the concentrations of TCS in the effluent are similar to those reported for several plants in Switzerland [3]. 2,4-DCP was only found in one of the two pairs of samples (sample code 4-5). Surprisingly, higher levels were measured in the effluent than in the influent of the plant.

Levels of TCS and 2,4-DCP in a discrete wastewater sample from the hospital sewer (no. 6) were higher than in those samples taken in the inlet of the sewage plant. In order to assess if the concentrations of both compounds in wastewater from the hospital are significant different than in domestic sewage; two pairs of composite samples (7–8 and 9–10) were taken in the city and hospital sewers before their confluence. Obtained results clearly confirmed the higher levels of TCS and 2,4-DCP in the hospital wastewater, Table 5. In addition to these compounds, another dichlorophenol isomer and traces of a pentachlorinated compound, which could correspond to the *tert*.-butyldimethylsilyl derivative of 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol, a potential metabolite of TCS [5,7], were also found in composite samples from the hospital sewer, Fig. 4.

## 4. Conclusions

A precise, sensitive and solvent free SPME method for the determination of TCS, MTCS and two possible related chlorinated phenols in water samples has been developed. The *on-fibre* silylation of the phenolic compounds (2,4-DCP, 2,3,4-TCP and TCS) can be performed in only 10 min with a small consumption of silylation reagent and without need of temperature control. Quantification limits below 10 ng/l can be achieved after a extraction step of 30 min. The use of PA fibres is preferred to PDMS-DVB ones since a better repeatability was obtained. Preliminary results, using GC–MS detection, have demonstrated the applicability of the proposed method to the analysis of real samples; and also, the presence of TCS, and the endocrine disrupter 2,4-DCP, in wastewater samples. Systematic studies and monitoring campaigns are necessary to investigate the main sources, occurrence, fate and the possible correlation between the presence of triclosan and other chlorinated compounds, such as 2,4-DCP, in real wastewater samples.

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