Reverse-phase HPLC method for the simultaneous analysis of triclosan and triclocarban in surface waters
Irena Baranowska, Sylwia Magiera and Katarzyna Bortniczuk

ABSTRACT

A validated reverse-phase HPLC-DAD for the simultaneous determination of triclosan and triclocarban in surface water has been developed, because this method has not been used for determination of these disinfectant agents so far. An isocratic elution was achieved using a Develosil RP Aqueous AR-5 RP-30 column with a flow rate of 1.0 mL min⁻¹. The mobile phase consisted of mixed methanol and water in ratio of 90:10, v/v. DAD detector was used to monitor the analytes at 280 nm for triclosan and 265 nm for triclocarban. The time of analysis was 10 min and the retention time for triclosan and triclocarban was 5.81 min and 8.13 min, respectively. The solid phase extraction method was proposed for the preconcentration step. The extraction efficiencies were approximately 97% for triclosan and 87% for triclocarban. The linearity range for triclosan and triclocarban after pre-concentration were between 0.5–20 μg mL⁻¹ and 0.3–20 μg mL⁻¹, respectively. The LOD and LOQ of triclosan and triclocarban in real samples were 0.04 ng mL⁻¹ and 0.11 ng mL⁻¹, 0.17 ng mL⁻¹ and 0.50 ng mL⁻¹, respectively. The method has been sensitive and can be successfully applied to the fast and simultaneous determination of triclosan and triclocarban in surface waters.

Key words | HPLC, method validation, triclocarban, triclosan, water samples

INTRODUCTION

The number of chemicals which currently occur in the aquatic environment increased considerably in the last decades. In this regard, a diverse group of bioactive chemicals, receiving increased attention as potential environmental pollutants, includes active ingredients in personal care products (PPCPs) (Sanchez-Pedro et al. 2006). These compounds and their bioactive metabolites are continually introduced into the aquatic environment via a number of routes, but primarily through treated and untreated wastewater. Disinfectant agents, which are commonly added to cosmetics, detergents and other personal care products which prevent microbial growth are of the greatest interest in this respect. One of the most widespread disinfection substances contained in these products are triclosan and triclocarban.

Triclosan (TCS; Figure 1A) and triclocarban (TCC; Figure 1B) are antimicrobial agents which are widely used in households and personal care products such as toothpaste, mouthwash, cream, soap, deodorants, cosmetics and skin care lotions as well as other consumers goods (Ying et al. 2007; Chu & Metcalfe 2007).

Due to its similar structure to TCS and TCC the aforementioned substances might have a similar effect. TCS and TCC have a number properties which suggest their potential adverse environmental behavior. The above-mentioned chemical substances are toxic to humans and animals and they cause methemoglobinemia—a reduction in the birth rate as well as in the survival rate and body weight of the young. Their polychlorinated aromatic structure suggests on a potentially significant resistance to biotransformation and biodegradation (Halden & Paull 2005).

TCS or TCC were previously found in the aquatic environment (water and sediments) as the result of
wastewater emission from a specialty chemicals manufacturing plant, have been recently detected in urban wastewaters (Tixier et al. 2002).

Different methods have been reported in the literature for determination of TCS or TCC individually or combination with other disinfection substances. Various methods like GC with mass spectrometric detection have been reported for analysis of TCS in water samples (Adolfsson-Erici et al. 2002; Lindstorm et al. 2002; McAvoy et al. 2002; Singer et al. 2002; Bester 2005; Canosa et al. 2005; Mezcua et al. 2004; Sanchez-Pedro et al. 2006). Also, LC-UV (Federle et al. 2002; Piccoli et al. 2002; Tixier et al. 2002; Iwaki et al. 2008), LC-MS (Weigel et al. 2004) and LC-negative-ESI-MS/MS (Hua et al. 2005) methods have been reported for determination of TCS in waters, sludge and personal health care products. TCS has been quantified in urine in presence of other environmental phenolic compounds using on-line solid-phase extraction (SPE) coupled to high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), by Ye et al. (2005).

Halden & Paull (2004) have quantified TCC individually in aquatic environments using a validated LC/ESI/MS method. Piccoli et al. (2002) and Xie et al. (2008) have reported a method involving the analysis of TCC personal health care products and water sample, respectively, using HPLC with UV detection.

Nevertheless, to our knowledge, no article related to the method of HPLC, in which the simultaneous separation of TCS and TCC was conducted satisfactorily, has ever been described in literature. In one publication the method of the determination of TCS and TCC using a liquid chromatography with electrospray ionization and tandem mass spectrometry (LC–ESI-MS/MS) have been reported, however, without chromatographic separation (Chu & Metcalfe 2007).

The aim of the present work was to develop an accurate, specific, precise and sensitive HPLC method for the simultaneous determination of TCS and TCC from surface water. Taking the availability of apparatus into account the proposed chromatography system may be highly applicable in many laboratories which lack sophisticated analytical instruments such as LC–ESI-MS/MS. This fact is of the paramount importance, due to the large demand for the methods enabling the simultaneous determination of these two popular disinfectant agents, which contaminate water ecosystems.

### EXPERIMENTAL

#### Chemicals and reagents

Triclosan (TCS; purity, 97.0%) and triclocarban (TCC; purity, 99%) were bought from Sigma-Aldrich (St. Louis, MO). Acetonitrile, water, methanol for HPLC analysis (HPLC grade) were obtained from Merck (Darmstadt, Germany). Methanol (MeOH) was purchased from POCH S.A. (Gliwice, Poland).

The analytical standard stock solution of TCS and TCC was prepared by dissolving 10 mg of the solid standard substance in 10 mL of methanol. The working solutions were prepared by dilution of the stock standard solutions. Additional mixed standard stock solutions of TCS and TCC were prepared using methanol as a solvent. All solutions were stored in a refrigerator at 4°C.

#### Water samples

The water samples were collected from different rivers and lakes in the south of Poland in Spring 2009. Three samples of water were taken from all regions. The samples were stored in 5 L containers in the dark at 4°C until analysis. Water was collected according to the PN-74/C-04620.00 standard and transported to the laboratory as fast as
possible. The water samples were filtered using a Bakerbond nylon filter with 0.20 μm pores.

Solid phase extraction (SPE) of water samples were performed by using of Bakerbond spe-12G system (J.T. Baker Inc., Philipsburg, USA). The cartridges (C18; 6 mL, 500 mg) were conditioned with 5 mL of methanol and 4 mL of water at a flow rate 2 mL min⁻¹. After adding 1 L of the water sample, the columns were dried under vacuum for 10 minutes. TCS and TCC were eluted with 7 mL methanol and the solvent evaporated to dryness and the residue dissolved in 1 mL methanol. An aliquot of 20 μL of the sample was injected into HPLC for analysis. From each sample of water three samples were prepared and all samples were measured three times.

Instrumentation and chromatographic conditions

Chromatography was performed on Merck (Merck Hitachi, Germany) chromatographic system equipped with a Model L-6200A Intelligent Pump with dynamic mixing chamber and Model L-4500A diode array detector. Samples were injected through a Rheodyne injector valve with fixed loop at 20 μL. Data acquisition and integration was performed using System Manager HSM D-7000, Version 2.1 (Merck-Hitachi, Germany). The values were collected from the computer integrator with 0.001-min accuracy for retention time and 0.001 absorbance scale.

HPLC analysis was performed by isocratic elution in reverse-phase mode. The chromatographic separation was performed using a Develosil RP Aqueous AR5 RP-30 (250 × 4.6 mm, particle size 5.8 μm) column, equipped with a pre-column Develosil RP Aqueous AR RP-30 (Nomura Chemical Co., LTD., Japan). Separation was achieved using a mobile phase consisting of mixed methanol and water in the ratio of 90:10, v/v pumped at a flow rate 1 mL min⁻¹. The analytes were monitored using a DAD detector at a wavelength of 280 nm for TCS and 265 nm for TCC. The column was maintained at ambient temperature.

Method validation

The method was validated with respect to parameters including linearity, limit of detection (LOD), limit of quantification (LOQ), specificity and recovery. Precision and accuracy of the method were carried out using three different concentrations of binary mixture. For the validation of the HPLC method surface water as the matrix was used. The mixed standards stock solution containing the TCS and TCC was spiked into water samples collected from a river which did not contain target compounds. The water samples was perpetrated according to the procedure described in the “Water sample” section.

Linearity of the method was studied by injecting six concentrations of standard TCS and TCC mixtures prepared in water matrix in the range of 0.3–40.0 μg mL⁻¹ and 0.1–40 μg mL⁻¹ of TCS and TCC, respectively, in six times, into the HPLC system and chromatographed under the conditions described in chromatographic condition. Calibration graph was constructed by plotting peak area versus concentration for each analyte and was fitted by linear regression equation.

The LOD and LOQ were calculated using Equations (1) and (2), respectively. The theoretically determined values of detection and quantitation limits were cross checked by actual analysis of these concentrations using developed method.

LOD = S.D. × 3.3/slope of calibration curve (1)

LOQ = S.D. × 10/slope of calibration curve (2)

where S.D. is the standard deviation of the responses of minimum diluted concentration.

For method precision, binary mixtures containing three different concentrations (10, 5 and 1 μg mL⁻¹) were prepared. Six injections of three different concentrations of mixture of TCS and TCC were given on the same day for intra-day precision. Six injections of three different concentrations of binary mixture of disinfection substances were given after five days for inter-day precision. The intra and inter-day variation was expressed in terms of standard deviation (S.D.) and % relative standard deviation (% R.S.D.). Accuracy was determined by the comparison of the mean result of six analyses to the nominal concentration. The result is expressed as the ratio (expressed in percentage) of the concentration calculated by the regression analysis to the nominal concentration.

The selectivity of developed HPLC method was evaluated through possible interference which may be...
expected to be present. The matrix influence between water samples of different origins before and after spiking TCS and TCC was tested.

For recovery assays the standard addition method was used. Water samples were analyzed for six times after addition of known amount of standard solutions. The concentrations were calculated from the calibration curves.

RESULTS AND DISCUSSION

Chromatographic method development

The various combinations of isocratic or gradient mobile phases and stationary phases ranging from reverse phase to normal phase failed to yield a reasonable separation between TCS and TCC. One of the most significant features of all HPLC methods is the fact that they were developed for determining only one of those two compounds. In this study to analyse TCS and TCC together in their combined, isocratic reverse phase HPLC in combination with DAD detector was developed and optimized. To optimize the HPLC conditions for the separation of TCS and TCC, the type of column, mobile phase composition, the wavelength of detection, the flow rate and the column temperature were scrutinized.

Different chromatographic columns were used for the separation of TCS and TCC. When experiments were performed with C18 column, with different parameters (length, diameter, particle size) and from different manufacturers, the analytes were eluted in the same retention time (cannot separate). The inability to resolve the components of interest on the C18 column led to the examination of the other stationary phases with increased non-polarity. Develosil RP Aqueous AR-5 RP-30 (250 × 4.6 mm, 5.8 μm) column gave good resolution and good asymmetry. This column displayed high sensitivity, the best separation, peak symmetry and reproducibility of the analyzed research compounds. Consequently, the average retention times were 5.81 min for TCS and 8.13 min for TCC.

By changing the percentages of methanol the polarity of a mobile phase was reduced, which improved the separation of TCS and TCC, additionally, shortened the elution time of TCS and TCC. The amount of 90% of methanol was selected as the ratio of the organic modifier for the baseline separation of TCS and TCC as it gave the best resolution (Figure 2). The retention time of TCS and TCC for the methanol–water (90:10, v/v) compositions was less than 10 min at a flow rate of 1 mL min⁻¹. The resolution between TCS and TCC peak and peak shapes was excellent.

The effect of column temperature was studied at different temperatures (20, 25, 35, 40°C). The column temperature was optimized at 25°C ± 2, which gave the best resolution. The sensitivity of HPLC method that uses DAD detection depends upon proper selection of detection wavelength. The optimum wavelength for detecting the analytes with the adequate sensitivity of TCS and TCC were at 280 and 265 nm, respectively.

Design of sample preparation procedure

In this study, the optimization of extraction methods for the TCS and TCC in water was conducted by the comparison of different SPE cartridges (HLB, SDB, C8,
C18, NEXUS, ENV, PPL), different methods of conditioned and eluted. The better clean-up method was discovered by using C18 cartridges which effectively eliminated the interfering peaks and resulted in the high recoveries of both analytes. Finally, the C18 column was used for extraction of TCS and TCC from water samples. The C18 column was conditioned by methanol and water. No washing solution was applied in this method. After loading the sample, the SPE cartridge was dried and then eluted with methanol. The developed method finally gave chromatograms without interfering peaks at the retention time of TCS and TCC. Moreover, the high recoveries of the analytes (98.90% for TCS and 87.37% for TCC) were obtained and the recoveries were very repeatable. The developed HPLC method shows, that TCC is more non-polar than TCS (t = 5.81 min; log $K_{ow}$ = 4.5 for TCS; t = 8.13 min; log $K_{ow}$ = 6.0; for TCC). TCC likely extracts stronger to solid phase (C18), compared to TCS and thus may be less elution.

**Analytical method validation**

The LOD and LOQ of TCS and TCC are 0.04 ng mL$^{-1}$ and 0.11 ng mL$^{-1}$; 0.17 ng mL$^{-1}$ and 0.50 ng mL$^{-1}$, respectively. The linear regression parameters for calibration curve after pre-concentration are listed in Table 1.

Table 2 gives the intra-day and inter-day precision and accuracy values of the measured concentrations of TCS and TCC calculated from linearity plots. The measurement of concentration at three different levels of binary mixtures of disinfection substances showed low values of % R.S.D. (<3.0%), which suggested an excellent precision of the method.

Also all these mean intra-day accuracies were in the range of 2.58% to 2.80% for TCS and −0.81% to 2.59% for TCC. When the accuracies from the individual daily validation runs were combined, the overall mean accuracies, measures of inter-day accuracy, were less than 3.12% for both compounds.

The influence of different ratio between TCS and TCC on the accuracy of the determination was also studied. Different ratio between TCS and TCC has no influence on the accuracy of the determination. The results were presented in Table 3. The chromatogram shown in Figure 3 demonstrates that there was acceptable resolution of the TSC from TCC, allowing the resultant peak to be easily quantified and having no influence on decrease of accuracy.

**Determination of TCS and TCC in surface waters**

The developed method with SPE extraction and HPLC analysis was applied for the analysis of surface water samples. In this research, different water samples collected from different sources of Poland were analyzed. All samples were filtered through a 0.22 μm filter membrane and stored at 4°C before being used. The solid phase extraction method with C18 column was proposed for the preconcentration step and for eliminated interfering materials from the samples.

In a number of samples taken from rivers and lakes TCS and TCC were not detected. In four water samples the presence of analyzed compounds was ascertained. As a result, TCS and TCC were detected in surface waters with a concentration up to 18.9 ng mL$^{-1}$ and 0.9 ng mL$^{-1}$, respectively. The peak of the TCS and TCC identification of the analytes was based on the comparison of retention time and on the standard addition method. Additionally, the application of the DAD detector enabled the identification of the TCS and TCC by comparison of the absorption spectra.

**Table 1 | Retention time and calibration curve parameters of examined compounds (n = 6)**

<table>
<thead>
<tr>
<th></th>
<th>Retention time of standard (SD) (min)</th>
<th>Linear dynamic range (μg mL$^{-1}$)</th>
<th>Slope (a) (S$a$)</th>
<th>Intercept (b) (S$b^*$)</th>
<th>$S_{xy}$</th>
<th>Correlation coefficient ($r^2$)</th>
<th>LOD (ng mL$^{-1}$)</th>
<th>LOQ (ng mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCS</td>
<td>5.80 (0.01)</td>
<td>0.5–20.0</td>
<td>8,074 (92)</td>
<td>2,038 (957)</td>
<td>1,648</td>
<td>0.9994</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>TCC</td>
<td>8.13 (0.02)</td>
<td>0.3–20.0</td>
<td>72,043 (376)</td>
<td>8,552 (3,670)</td>
<td>7,203</td>
<td>0.9998</td>
<td>0.17</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Standard deviation of slope.
†Standard deviation of intercept.
‡Residual standard deviation of regression coefficient.
The SPE procedure, that was used in our research, effectively removed the interfering polar organic material from the water samples. The developed method finally gave chromatograms without interfering peaks at the retention time from 4 min to 10 min, where the peaks for analyzed compounds (TCS: 5.78 min and TCC: 8.15 min) were observed (Figure 3). Regardless of the fact, if TCS and TCC were in analyzed water or not, near the retention time of examined compounds any signals which can be from the excipients were not observed. The results prove selectivity of the developed method.

However signals from 1 min to 4 min, where excipients for matrix of surface water were presented, were clearly visible. These non-polar components were eluted with TCS and TCC. The identification of these compounds was not carried out, because it is the large group of analyst (for example: saturated and unsaturated aliphatic hydrocarbons, aromatic hydrocarbons, ethylbenzene and xylene isomers (BTEX) or perchloroethene (PCE)). To have all excipients from water determined, it is necessary to develop new chromatographic conditions. This information can be classified as analytical screening speciation and Polish

### Table 2 | Intra-day and inter-day precision and accuracy for examined compounds (n = 6)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration added (µg mL⁻¹)</th>
<th>Concentration found (µg mL⁻¹)</th>
<th>S.D.² (µg mL⁻¹)</th>
<th>R.S.D.³ (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCS</td>
<td>10</td>
<td>10.25</td>
<td>0.17</td>
<td>1.72</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.12</td>
<td>0.04</td>
<td>0.86</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.03</td>
<td>0.01</td>
<td>0.57</td>
<td>2.80</td>
</tr>
<tr>
<td>TCC</td>
<td>10</td>
<td>9.92</td>
<td>0.05</td>
<td>0.47</td>
<td>−0.81</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.09</td>
<td>0.06</td>
<td>1.28</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.02</td>
<td>0.03</td>
<td>2.83</td>
<td>2.59</td>
</tr>
<tr>
<td>Inter-day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCS</td>
<td>10</td>
<td>10.31</td>
<td>0.11</td>
<td>1.13</td>
<td>3.07</td>
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<tr>
<td></td>
<td>5</td>
<td>5.16</td>
<td>0.03</td>
<td>0.65</td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>1.01</td>
<td>0.02</td>
<td>1.68</td>
<td>1.15</td>
</tr>
<tr>
<td>TCC</td>
<td>10</td>
<td>10.00</td>
<td>0.25</td>
<td>2.55</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.01</td>
<td>0.05</td>
<td>0.93</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.00</td>
<td>0.02</td>
<td>1.59</td>
<td>−0.23</td>
</tr>
</tbody>
</table>

²Standard deviation.
³Relative standard deviation.

### Table 3 | Influence different ratio between TCS and TCC on the accuracy (n = 6)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration added (µg mL⁻¹)</th>
<th>Concentration found (µg mL⁻¹)</th>
<th>S.D.² (µg mL⁻¹)</th>
<th>R.S.D.³ (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCS</td>
<td>10</td>
<td>10.13</td>
<td>0.05</td>
<td>0.54</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.04</td>
<td>0.02</td>
<td>2.23</td>
<td>3.21</td>
</tr>
<tr>
<td>TCS</td>
<td>1</td>
<td>0.99</td>
<td>0.01</td>
<td>1.14</td>
<td>−0.50</td>
</tr>
<tr>
<td>TCC</td>
<td>10</td>
<td>10.02</td>
<td>0.04</td>
<td>0.36</td>
<td>2.12</td>
</tr>
<tr>
<td>TCS</td>
<td>5</td>
<td>5.05</td>
<td>0.13</td>
<td>2.66</td>
<td>1.06</td>
</tr>
<tr>
<td>TCC</td>
<td>1</td>
<td>1.01</td>
<td>0.01</td>
<td>1.50</td>
<td>1.15</td>
</tr>
</tbody>
</table>

²Standard deviation.
³Relative standard deviation.
water was studied from this point of view (Kot & Namieśnik 2000; Wasik et al. 2001; Kuczyńska et al. 2004; Polkowska et al. 2005).

Fortunately, the components presented in sample did not influence on determination of desired analyts TCS and TCC. TCS and TCC exhibited symmetric peak shape and could be well resolved from each other. The development of analytical method for simultaneous determination of TCS and TCC has been taken for granted, because any article related to the method of HPLC has ever been described in literature so far.

CONCLUSIONS

A validated isocratic reverse-phase HPLC method has been developed for the simultaneous determination of TCS and TCC in water. The following study presents the first report on the simultaneous determination of TCS and TCC in surface water using HPLC. This method is sensitive, simple, accurate, precise and possesses good linearity. A high percentage of recovery by SPE shows that the method can be successfully used for the determination of analyzed compounds in water samples. In conclusion, the SPE method using RP-HPLC, developed in this study, can be used to quantify TCS and TCC in binary combination without interference from aqueous samples and can be applied in laboratories monitoring aqueous environment.

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