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Original Research

Development of SPE/HPLC-DAD to Determine Residues of Selected Disinfectant Agents in Surface Water

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Abstract

The aim of our research was to develop a chromatographic method for simultaneous determination of five substances used as disinfectant agents.

Biphenyl-2-ol (BPh), chloramine T (ChT), 4-chloro-3-methylphenol (4C3MPh), triclosan (TCS), and triclocarban (TCC) were determined using high-performance liquid chromatography. Chromatographic analyses were carried out on a Develosil RP Aqueous AR-5 RP-30 column (250 mm × 4.6 mm, 5.0 μm particles) with gradient elution of mobile phase including methanol and water, and with a DAD detector. Satisfactory resolution of the determined substances was obtained in 10 minutes. The linearity ranges of the calibration curves (in matrix) of examined disinfectants ranged from 0.10 to 10.00 $\mu g \cdot mL^{-1}$ for ChT, 4C3MPh and TCC, and from 0.25 to 10.00 $\mu g \cdot mL^{-1}$ for BPh and TCS. The developed method was applied to determine of mentioned compounds in water samples.

Water samples were prepared for chromatography analyses by using C18 solid phase extraction discs. The recoveries of analyzed substances ranged from 82 to 101% (except for Chloramine T, which is unstable in water medium). Analyses were operated after extraction of analytes from 3 L of real water samples. The method detection limits in samples of water were: 1.6 $ng \cdot mL^{-1}$ for BPh, 0.6 $ng \cdot mL^{-1}$ for ChT, 1.4 $ng \cdot mL^{-1}$ for 4C3MPh, 1.9 $ng \cdot mL^{-1}$ for TCS, and 1.0 $ng \cdot mL^{-1}$ for TCC.

This method was successfully applied to analyze examined disinfectants in surface water samples collected from rivers and wastewater in Poland. Four of five compounds were detected in the tested samples in the concentration ranging from 5.05 to 30.36 $\mu g \cdot L^{-1}$.

Keywords: HPLC-DAD, SPE, disinfectant agents, water analysis

Introduction

Nowadays many different formulations are used for disinfection. These formulations consist of both inorganic and organic compounds. Disinfectants and antiseptics are used not only in hospitals, but also for other care applications in households and industries. The increase of consumption of disinfectants and antiseptics in all areas is caused by potential microbial contamination. Special attention should be directed to compounds that are dangerous for people's health and for the environment. The examples of such substances are chloramine T (ChT), 4-chloro-3-methylphenol (4C3MPh), biphenyl-2-ol (BPh), triclosan (TCS), and triclocarban (TCC), which are subjects of this study. These substances have been popularly used as antimicrobial and disinfectant components in medical

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products (hand disinfecting soaps, medical skin creams, dental products, surface disinfecting formulations, pharmaceutical preparations), many household cleansers, footwear, and so forth. The chemical structures of analytes are shown in Fig. 1.

Chloramine T is a highly toxic compound for aquatic organisms and should not be introduced to water, soil, and wastewater. Although its biodegradability is about 90%, chloramine T is acknowledged as dangerous for the environment, especially for water ecosystems.

Phenolic compounds reveal toxic effects on aquatic organisms [1-2]. Generally they can cause toxicity, persistence and bioaccumulation effects in the environment and may also be dangerous for human health [3]. 4C3MPh is one of the eleventh substituted phenols included in the Federal Register published by The Environmental Protection Agency in the United States (US-EPA) [3]. It is also one of 26 priority phenols (hazardous for people and the environment) included in European Union Directive No. 76/464/EEC [4].

Noteworthy substances are biphenyl-2-ol and triclosan. The toxicity studies of TCS have shown that this compound is associated with toxic effects [5], especially to aquatic flora. There is also a lack of data about toxicity and the occurrence of BPh in the environment, but this compound is similar to a xeno-estrogen [6]. Moreover, the chemical structures of both compounds are similar to highly toxic contaminants such as hydroxylated polychlorinated biphenyls or dioxins, so it is possible to convert TCS and BPh to these hazardous compounds under the influence of radiation or high temperature [7-8].

Triclocarban is commonly used (together with triclosan) as an antifungal and antibacterial agent. Although relatively little data exists about the toxicity of TCC, it has been found to impair reproduction in laboratory rats and that some of its degradation products are carcinogenic [9]. The potential toxicity and chronic hazard of triclocarban to aquatic organisms, especially fish, invertebrates, and plants is high [10]. For the sake of the aromatic nature and high chloride content, TCC have a significant resistance to biodegradation and tendency for environmental persistence [11].

Analyses of these compounds in surface water and wastewater requires the use of sensitive methods. To this purpose different analytical techniques were applied. The most common was gas chromatography mainly with MS detector [12-17] and liquid chromatography (both HPLC and UHPLC) with several types of detectors, generally spectrophotometric [12, 18-22] or mass spectrometer [9, 12, 14-17, 19-23] and rarely an electrochemical [18, 24] or fluorescent detector [25]. There are also several publications describing determination of these substances using capillary electrophoresis [4, 26], supercritical fluid chromatography [27-28] or ion chromatography [29-30]. Most of them are coupled with pre-concentration techniques – the most often applied is solid phase extraction (SPE).

A lot of methods have been found in literature for determination of 4C3MPh [1, 3-4, 6, 17-21, 24-25, 27-29], TCS [5-6, 9-10, 12, 14-16, 22-23, 30], and TCC [9-10, 15, 23] in water and wastewater, for BPh only several methods have been found [6, 12, 15]. There are few chromatographic methods for the determination of ChT [31]. Above articles concern determination of selected substances, but only individual substances or simultaneously with others (belonging to some group of chemical compounds or group of substance with specified functioning), however no articles describe a method for simultaneous determination of all five chemicals. In this paper a new HPLC-DAD method for the determination of disinfectant residues (simultaneously determination of ChT, 4C3MPh, BPh, TCS, and TCC) was elaborated and validated. There also are no publications describing a procedure (for example SPE) for preparing water samples for such analyses – application of this method to determine these compounds in surface water was also worked out in this study.

Going ahead in a future in the research area focusing on other chemicals and determining its residues in water, we should mention some publications. For example determination of pentachlorophenol (PCP) and carbaryl in water. At first, methods for its analyses were based on the formation of colored derivatives. Spectrometric methods are not very specific and have low sensitivity so that chromatographic methods are currently preferred. Quick and simple GC-MS

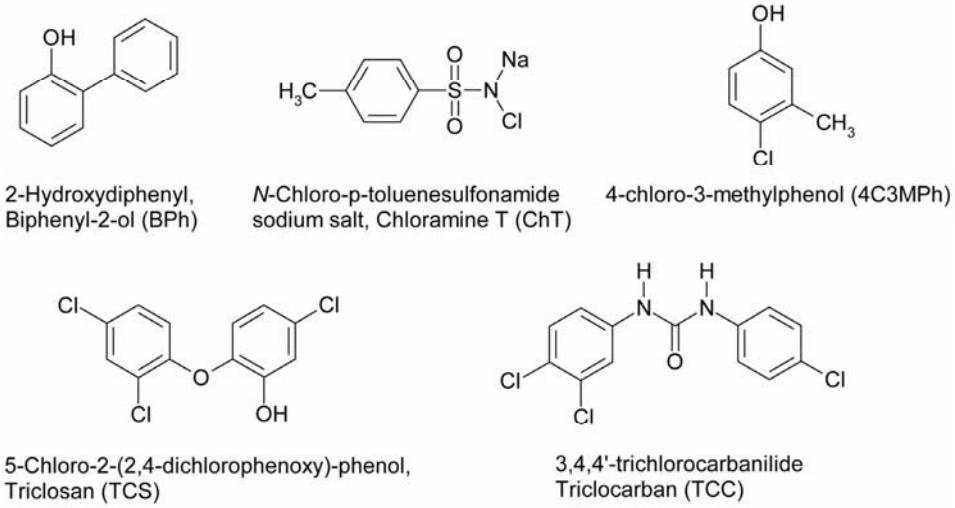

Fig. 1. Chemical structures of biphenyl-2-ol, chloramine T, 4-chloro-3-methylphenol, triclosan, and triclocarban.

and HPLC-DAD methods for simultaneously determining PCP and carbaryl in water without derivatization (to minimize analysis time and errors due to manipulation) have been developed [32, 33]. Additionally, methods based on solid-phase extraction (SPE) and microextraction (SPME) have been developed for the preparation of water samples. The main advantages of this method are that it avoids the use of organic solvents (like in liquid-liquid extraction procedures) and matrix effects do not interfere with the quantitation process [33].

Determination of polycyclic aromatic hydrocarbons (PAHs) in water provides some important information about techniques, allowing for preparation of water samples. Solid phase extraction (SPE), stir bar sorptive extraction (SBSE), and solid phase microextraction (SPME) are the most often applied methods [34-36].

The following problem is water contamination due to the wide variety of pesticides used in agricultural practices. Monitoring the trace levels of pesticides in waters is very important for health protection and environmental control. The 98/83 European Directive requires the measurement of pesticide residues at a target concentration of 1.0 $mg \cdot L^{-1}$ in surface water and 0.1 $mg \cdot L^{-1}$ in drinking water. In order to reach such levels of detection, very efficient extraction techniques are necessary. The classical approach for pesticide extraction from waters is liquid-liquid extraction (LLE). This technique is time consuming and requires large volumes of expensive and toxic solvents. A substantial improvement for aqueous sample preparation techniques has resulted from the development of solid-phase extraction (SPE), using bonded silica sorbents. SPE technique gives more advantages: shorter time of analysis, lower cost, and the consumption of very low volumes of organic solvents. Nowadays extraction methods are improved with new technologies in order to reduce time required for sample extraction and preparation. Solid phase microextraction (SPME) has become popular for the analysis of organic compounds. It combines sampling and pre-concentration in one step. SPME has been applied extensively to determine pesticide residues in water samples. Stir bar sorptive extraction (SBSE) is a technique that also appears as a convenient and efficient extraction method. Single drop microextraction (SDME) has been developed recently as an alternative extraction technique for determination of residues in water. SDME provides analyte extraction in a single drop of organic solvent. The small volumes of organic solvent (from 0.5 to 2.5 mL) are used and when extraction is finished, the single drop of organic solvent is injected into the GC. SDME avoids the problems of solvent evaporation (this is a serious problem in LLE and SPE) as well as fiber degradation (like in SPME). SDME technique is also fast, inexpensive and uses simple equipment [37-40].

Techniques of sample preparation presented in [32 to 40] are good examples of determinations of organic compound residues in water. Different methods (for instance: SPE, SPME, SBSE, and SDME), their applications, advantages, and disadvantages were presented. It is helpful to select a method proper to specific determination.

Experimental Procedures

Chemicals

Chloramine T (N-chloro-p-toluenesulfonamide sodium salt; CAS 149358-73-6), 4-chloro-3-methylphenol (2-chloro-5-hydroxytoluene; CAS 59-50-7), biphenyl-2-ol (2-hydroxydiphenyl; CAS 90-43-7), triclosan (5-Chloro-2-(2,4-dichlorophenoxy)-phenol; CAS 3380-34-5), and triclocarban (3,4,4'-trichlorocarbanilide; CAS 101-20-2) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The reagents for mobile phase (methanol and water) were of HPLC grade and were obtained from Merck (Darmstadt, Germany). Other reagents (methanol for stock solutions and sulfuric acid for acidification) were of analytical grade and were obtained from POCH (Gliwice, Poland). Stock solutions were prepared by dissolving 10.0 mg (weighed with accuracy to 0.1 mg) of substances in 10.0 mL of methanol. In order to obtain an intermediate standard solution for quantitative analysis the stock solution was properly diluted in methanol before analysis. All solutions were stored in dark glass bottles at 4ºC . The standard solutions in the concentration 1 $mg \cdot mL^{-1}$ were stable for 3 months. Intermediate solutions were prepared directly before analysis.

Instrumentation

HPLC experiments were performed using a La Chrom ELITE Hitachi chromatograph equipped with a L-2130 pump and a L-2455 DAD detector (Merck, Germany). Chromatographic analyses were carried out on a Develosil RP Aqueous AR-5 RP-30 column (250 × 4.6 mm, 5.0 μm) (Nomura Chemical, Japan). The analytical column was guarded with the pre-column Develosil RP Aqueous AR RP-30 (10 × 4.0 mm, 5.0 μm) (Nomura Chemical, Japan). Separations were carried out with gradient elution of mobile phase containing methanol (A) and water (B) at 20-22ºC. Gradient conditions were 0 min 57% A, 2 min 90% A, 3 min 100% A, 6 min 100% A, 10 min 57% A with a flow rate of 0.9 $mL \cdot min^{-1}$.

Samples were injected using a valve with a 20-μL loop. Detection of disinfectant agents have been performed using the DAD detector with the following wavelengths: λ=227 nm for ChT, λ=245 nm for BPh, λ=265 nm for TCC, and λ=280 nm for 4C3MPh and TCS. Data acquisition was performed on the DAD ELITE HSM system manager (Merck, Germany).

Sample Preparation

Surface water samples (from rivers) and treated sewage were examined. Samples were collected (from November 2009 to March 2010) from different places in Poland, i.e. Warsaw, Gdańsk, Kielce, Zawiercie, Skoczów, Bielsko-Biała, Czerwionka, and Zawadzkie (Fig. 2). Water samples were collected according to the obligatory standard (PN-74/C-04620.00). All samples were stored in dark bottles at 4ºC until analyzed. An extraction method for determined

Fig. 2. Locations of water sample collection: 1 – Mała Panew River, Zawadzkie, 2 – Bierawka River, Czerwionka, 3 – Wisła River, Skoczów, 4 – Krzywa River, Bielsko-Biała, 5 – Warta River, Zawiercie, 6 – Silnica River, Kielce, 7 – Wisła River, Warszawa, 8 – Wisła River, Gdańsk.

compounds was established for the preparing of real water samples. Solid phase extraction procedure were used to preconcentrate the analytes that are present at trace levels in environmental samples and to remove matrix interferences.

Solid phase extraction (SPE) was done with a Bakerbond Speedisc C18 (J. T. Baker) using Bakerbond Speedisc Octadecyl (C18) (50 mm) discs (J. T. Baker). Samples of water with volume of 3 L were filtrated with dense and narrow pore filters before extraction. The discs were conditioned successively with 10 mL of methanol and 10 mL of water (acidified by sulfuric acid to pH=5) at flow rate 2 mL·min^{-1}. The flow rate of water samples by disc was 10-15 mL·min^{-1}. After the sample was passed through, the disc was dried for 2 minutes. The target compounds were eluted with 10 mL of methanol at flow rate 1 mL·min^{-1} and then the samples were ready to be investigated during chromatographic analysis.

Results and Discussion

Chromatographic System

A simple, fast, and precise chromatographic method for the determination of disinfectant agents was developed. Different chromatographic columns and mobile phase compositions were tested in the study. Columns with different packing (ranging from normal phase to reversed phase) obtained from different producers and with different dimensions (length, diameter, particle size) were used. The mobile phases consist of methanol, water, acetonitrile, water solution of trifluoroacetic acid, and water solution of formic acid. Mixtures of these solvents also were used. Except from type of column and mobile phase composition – different flow rates of mobile phase, column temperature and detection method (spectrophotometric and fluorescent) were also tested.

It was necessary to develop such a method that allows for determination of all five substances, because such a chromatographic system has not been described elsewhere. In the literature we can find GC and LC methods for simultaneous determination, for example different phenolic compounds, but none of these articles describes a chromatographic method for determining all five analytes (which are so commonly used) during one chromatographic analysis.

The use of a column with ODS packing, gradient elution of methanol, water and acetonitrile in room temperature at a flow rate 1.0 mL·min^{-1} allows for good separation for four of five chosen substances. But these chromatographic conditions turned out to be unsuitable for separation of triclosan and triclocarban; these compounds were eluted at the same time. Modification of mobile phase and its flow rate also did not give satisfactory results. It was necessary to apply a column with more non-polar packing. A Develosil RP Aqueous AR-5 RP-30 (250 × 4.6 mm, 5.0 µm) column and gradient elution of methanol and water at room temperature (20-22ºC) at a flow rate of 0.9 mL·min^{-1} turned out to be most favorable for determination of chosen disinfectants. The total time of analysis is under 10 minutes and retention times of analytes (after mobile phase gradient optimization) are presented in Table 1. A chromatogram of a standard mixture of all determined compounds is shown in Fig. 3. The application of more non-polar columns allows for very good separation of chosen substances (include triclosan and triclocarban) in a short time, using gradient elution of very popular solvents. The mobile phase consists of only methanol and water. Moreover, the chromatographic column applied gave good repeatability, resolution, and asymmetry (chromatographic parameters assigned by the system suitability test are presented in Table 2).

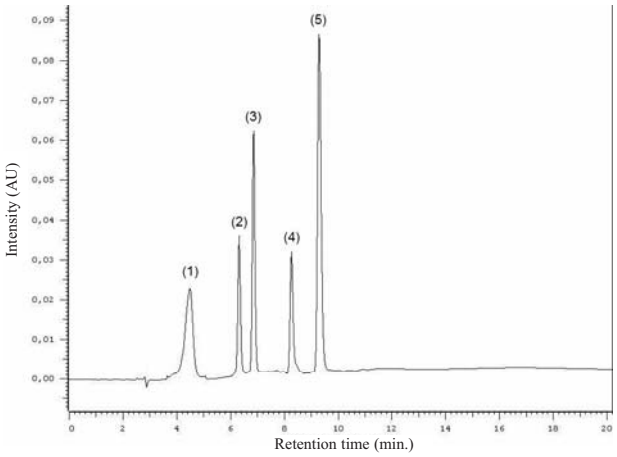

Fig. 3. Chromatogram of standard solution in methanol containing 10 µg·mL^{-1} of ChT (1), 4C3MPh (2), BPh (3), TCS (4), and TCC (5) for monitoring wavelengths λ=227 nm (0-5 minutes) and λ=265 nm (5-20 minutes).

Table 1. Analytical wavelengths, retention times, parameters of calibration curves, and LOD and LOQ values and recoveries for determined compounds.

Substance	λ [nm]	t_r [min]	Linearity range [μg·mL^{-1}]	R^2	*a* *10^5	S_a *10^3	*b* *10^4	S_b *10^4	S_{xy} *10^4	LOD* [ng·mL^{-1}]	LOQ* [ng·mL^{-1}]	R [%]	RSD [%]
ChT	227	4.46	0.10-10	0.9999	2.09	1.42	-0.98	0.87	1.12	0.6	1.8	13	1
4C3MPh	280	6.84	0.10-10	0.9992	0.55	0.91	0.19	0.56	0.71	1.4	4.2	82	2
BPh	245	7.08	0.25-10	0.9989	3.22	6.08	2.74	3.63	4.71	1.6	4.8	91	2
TCS	280	8.21	0.25-10	0.9985	0.77	1.73	1.94	1.06	1.34	1.9	5.7	87	3
TCC	265	9.39	0.10-10	0.9996	5.96	6.86	4.17	4.20	5.38	1.0	3.0	101	3

λ – wavelength [nm], t_r – retention time [minute], equation of the calibration curves: y = a x + b, y – peak area, x – concentration [μg·mL^{-1}], a – slope, S_a – standard deviation of slope, b – intercept, S_b – standard deviation of intercept, S_{xy} – residual standard deviation, R^2 – correlation coefficient, LOD* – limit of detection [ng·mL^{-1}] (calculated for initial sample), LOQ* – limit of quantification [ng·mL^{-1}] (calculated for initial sample), R – mean recovery for SPE procedure [%], RSD – relative standard deviation for recovery [%].

Table 2. Chromatographic parameters assigned by system suitability test (SST).

Substance	t_r [min]	k'	A	R	α
ChT	4.46	4.447	0.94	-	-
4C3MPh	6.84	6.833	1.03	13.19	1.72
BPh	7.08	7.080	1.16	2.82	1.05
TCS	8.21	8.204	1.09	13.74	1.24
TCC	9.39	9.380	1.06	7.87	1.14

All parameters are assigned by system suitability test (SST) for repeated injections (n=3),
t_r – retention time [min], k' – capacity factor [-], A – peak asymmetry [-], R – resolution [-], α – selectivity [-].

During the chromatographic process there were applied neutral medium of mobile phase and high non-polar stationary phase. A change (increase) of percentage amount of methanol in mobile phase causes reduction of its polarity and rise of elution strength. The order of elution of analytes is compatible to decreasing polarity of compounds. The polarity of analyzed compounds increases in the following series: triclocarban, triclosan, biphenyl-2-ol, 4-chloro-3-methylphenol, and chloramine T.

Calibration Curves, LOD, and LOQ

The limit of detection (LOD) was determined using parameters of the calibration curves (the residual standard deviation of the points and the slope of calibration curve) (LOD=3.3*S_{xy}/a). The limit of quantification (LOQ) was determined as a multiple value of LOD (LOQ=3*LOD). Additionally, LOD and LOQ values took into account degrees of sample concentrations.

The calibration curves were prepared in real surface water (Wapienica River, Bielsko-Biała, Poland) unpolluted by examined compounds after adding of standards of disinfectant agents in the range of 0.10 to 10.00 μg·mL^{-1} for 4C3MPh and TCC, and from 0.25 to 10.00 μg·mL^{-1} for BPh and TCS. These calibration curves take into consideration the recoveries. For ChT the calibration curve was prepared in methanol for the sake of its instability in water medium [41]. For analysis, several different wavelengths were chosen (Table 1). These wavelengths are specific for analytes and correspond to maximum of absorption. In most cases such wavelengths also eliminate water matrix interference.

Parameters of the calibration curves, values of LOD and LOQ, and analytical wavelengths are presented in Table 1.

Linearity, Accuracy, and Precision

The analytical method proposed allows us to get good linearity in the tested range, accuracy, and precision.

Correlation coefficients of the calibration curves for all compounds (Table 1) were higher than 0.9985, which confirms good linearity in the tested range.

During laboratory works intra-day and inter-day precision were assigned. For this purpose mixtures containing three different concentration levels of analytes were prepared and analyzed by HPLC-DAD method. The concentrations were selected in accordance with linearity ranges of the calibration curves of individual analytes and as values close to the limiting (0.50, 9.00 μg·mL^{-1}) and the middle (5.00 μg·mL^{-1}). For determining intra-day precision, six

Table 3. Intra-day precision, inter-day precision, and accuracy for determined compounds (n=6).

Compound	Added	Intra-day precision			Relative error	Recovery	Inter-day precision			Relative error	Recovery
		Measured	SD	CV			Measured	SD	CV		
	[μg·mL^{-1}]	[μg·mL^{-1}]	[μg·mL^{-1}]	[%]		[%]	[μg·mL^{-1}]	[μg·mL^{-1}]	[%]		[%]
ChT	0.50	0.43	0.01	2.33	-0.14	86.2	0.53	0.01	1.89	0.06	106.0
	5.00	3.69	0.05	1.36	-0.26	73.8	5.56	0.06	1.08	0.11	111.2
	9.00	7.35	0.14	1.90	-0.18	81.6	8.34	0.27	3.24	-0.07	92.7
4C3MPh	0.50	0.50	0.02	4.00	0.00	100.0	0.53	0.02	3.77	0.06	105.0
	5.00	5.16	0.17	3.29	0.03	103.2	5.13	0.16	3.12	0.03	102.7
	9.00	9.20	0.29	3.15	0.02	102.2	9.29	0.45	4.84	0.03	103.2
BPh	0.50	0.44	0.02	4.55	-0.12	88.5	0.48	0.01	2.08	-0.04	96.2
	5.00	5.14	0.13	2.53	0.03	102.7	5.14	0.23	4.47	0.03	102.7
	9.00	9.22	0.37	4.01	0.02	102.5	9.53	0.35	3.67	0.06	105.9
TCS	0.50	0.39	0.01	2.56	-0.22	78.3	0.47	0.02	4.26	-0.06	93.5
	5.00	5.69	0.23	4.04	0.14	113.8	4.33	0.19	4.39	-0.13	86.7
	9.00	9.71	0.15	1.54	0.08	107.9	7.86	0.33	4.20	-0.13	87.4
TCC	0.50	0.47	0.02	4.26	-0.06	93.8	0.51	0.02	3.92	0.02	101.5
	5.00	5.14	0.15	2.92	0.03	102.8	5.22	0.25	4.79	0.04	104.5
	9.00	9.20	0.28	3.04	0.02	102.2	9.81	0.13	1.33	0.09	109.0

injections of each prepared mixtures (on three concentration levels) were made on the same day. For determining inter-day precision, six injections of each prepared mixtures (on three concentration levels) were made over five consecutive days. The precision of the method developed was determined by calculating values of standard deviation (SD) and coefficient of variation (CV) of the concentrations for determined compounds (obtained after repeated injections (n=6) on three levels of concentrations). The SD and CV values were presented in Table 3 and its low values (CV<5% in all cases) confirm the precision of the elaborated method.

Accuracy was determined by calculating relative errors – their low values confirm accuracy of the method developed.

The recoveries are also high – in all cases exceeding 70% (Table 3).

Selectivity

In order to assess the selectivity of the method developed, a blank sample (submitted SPE procedure, which ensures release and pre-concentration of analytes and also separation from matrix interferences) was analyzed to verify the absence of potential interfering substances around the retention times of determined compounds. These results demonstrate that the matrices from real samples have no effects on analysis of chosen chemicals in the studied samples.

Influence of Different Concentration of Analytes

The influence of different concentration ratios between analytes were also tested. Different concentration between substances have no influence on accuracy of analyses. There is also no interference between peak Nos. 2 and 3 because: 4-chloro-3-methylphenol (presented by peak number 2) is determined at wavelength 280 nm, and 2-phenylphenol (presented by peak number 3) at 245 nm. These different wavelengths allow for good separation of these peaks and eliminate interference between them.

SPE Procedure

Different SPE procedures were tested for the extraction of the target compounds from water samples. In this experiment different SPE columns (C18, HLB, SDB) and different solvents (for conditioning the cartridges and for eluting of analytes) were tested: methanol, water, acidified water, ethyl acetate, propanol, acetonitrile, and its combinations. It was observed that the best sorbent was C18 cartridge and using methanol and water (acidified to pH=5) for conditioning the cartridges. The first chromatographic analyses also revealed that ODS packing was a suitable phase for adsorbing chosen substances (but C18 column was not suitable for separated TCS and TCC). For extraction procedure we used C18 discs instead of C18 SPE cartridges to increase a speed of extraction (more water could be passed through the disc than by the cartridge in the same amount

Table 4. Results of analyses of environmental water samples from Poland.

Sample	Identified compounds	Concentration of identified compounds [μg·L^{-1}]
Bierawka River (Czerwionka)	TCS	7.91
Krzywa River (Bielsko-Biała)	ChT TCS	- 30.36
Warta River (Zawiercie)	TCC	5.05
Wisła River (Skoczów)	TCS	17.93
Treated sewage (southern Poland)	BPh	5.17

of time) preserving the same recoveries of analytes and with the same degree of matrix effects removing. Additionally, the application of faintly acid medium allowed to obtain better (higher) recoveries than in neutral medium. The quantitative elution of all analytes was achieved using methanol because all analyzed substances are soluble in that medium. A sample preparation method applied allows us to obtain recoveries higher than 82% for examined compounds except for Chloramine T. This should not be surprising because ChT is unstable in water medium and only residues of this compound can be determined. 13% of recovery of chloramine T – not its breakdown products and this is the value of recovery after SPE experiments (after introducing 10.00 μg of standard to 3 L of natural water). The determined compound is just chloramine T, not its breakdown products, because standard solution of chloramine T in methanol is stable and it is possible to obtain the spectrum in the range 190 to 400 nm just for chloramine T. The spectrum obtained from water solution containing chloramine T is the same as for the standard. Moreover, chloramine T is highly toxic for aquatic organisms, its primary degradation product – p-toluenesulfonamide – does not reveal toxic effects.

Recoveries (after SPE procedure) and its relative standard deviations for analytes obtained from 3 L of spiked river water samples were calculated for five levels of concentration (n=3) and results are presented in Table 1.

Application of the Method to Surface Water Samples and Treated Sewage

The HPLC-DAD method and SPE procedure described in this study were applied for analyses of real water samples (surface water and wastewater). The contents of ChT, 4C3MPh, BPh, TCS, and TCC in water and wastewater samples were determined from the calibration curves. Every eluate obtained from samples after SPE procedure was injected three times. To confirm the presence of determined compounds in real water samples a standard addition method was applied. Additionally, retention times and UV spectra of standards were compared with the retention times and UV spectra of determined substances registered for real water samples. The obtained results are shown in Table 4.

Fig. 4 presents a chromatogram of a real water sample (the Wisła River in Skoczów) after SPE procedure. This is an example of a real water sample where high a level of disinfectant agents (triclosan, 17.93 μg·L^{-1}) was determined. It can be caused by the specific location of this river. There are mainly food industries in Skoczów, so there are places where a lot of disinfectants are used. Apart from that, a close neighborhood of health resort (for example Ustroń) could also have an influence on the level of disinfectant residues in water.

High levels of triclosan (30.36 μg·L^{-1}) and the presence of chloramine T also were observed in the Krzywa River (Bielsko-Biała, Poland) where the most common industries are food, textile, and automotive. It was not possible to determine amounts of ChT for the sake of its instability and low recovery, and only qualitative analysis is possible in that case. The chromatogram obtained for the Krzywa River is presented in Fig. 5. This case confirms the necessity of such analyses in surface water. In spite of low stability (13% recovery after SPE experiments and 90% degradation due to

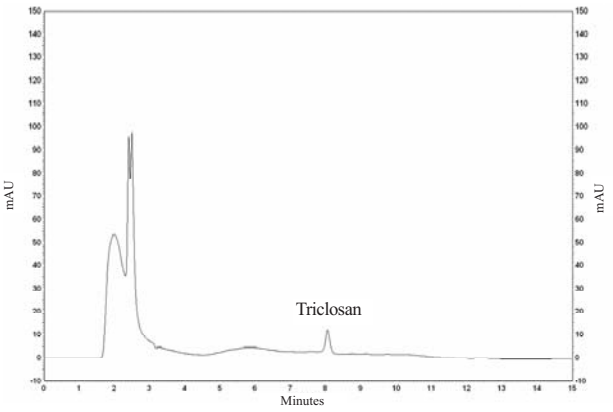

Fig. 4. Chromatogram of real water samples from Wisła River (Skoczów, Poland) submitted to SPE procedure for monitoring wavelength λ=280 nm.

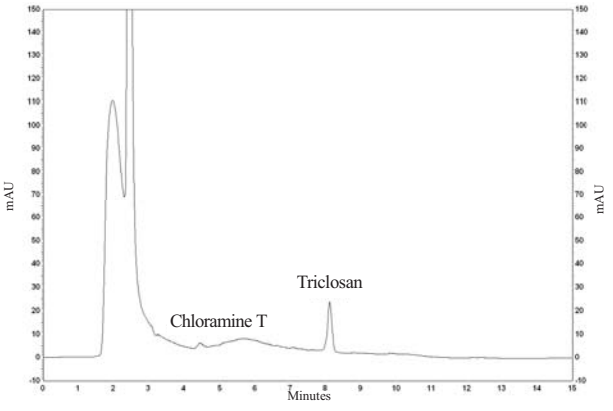

Fig. 5. Chromatogram of real water samples from Krzywa River (Bielsko-Biała, Poland) submitted to SPE procedure for monitoring wavelength λ=227 nm (0-5 minutes) and λ=280 nm (5-15 minutes).

data sheet) the presence of this substance could be identify in real water samples. It is worth mentioning that aqueous chloramine T solutions are stable in alkaline medium as opposed to acid medium [41], especially there is a big loss in concentration with increase of chloride content.

Water samples from Mała Panew (Zawadzkie, Poland), Silnica (Kielce, Poland), and Wisła (Gdańsk and Warszawa, Poland) rivers were also analyzed, but no analytes were identified in spite of the amount of samples (3 L) submitted to SPE procedure.

Conclusions

The chromatographic method validated and described in this paper has been successfully applied for the separation and determination of selected disinfectant agents in water samples. The SPE procedure described in this article allows the pre-concentration of chosen compounds from real water samples. The chromatographic method developed is simple, rapid, and sensitive. The extraction procedure worked out in this study is characterized by high values of recoveries.

Such a chromatographic method and SPE pre-concentration procedure have not been described before for ChT, BPh, 4C3MPh, TCS, and TCC. This method could be very useful for environmental research because it enables simultaneous determination of selected disinfectant agents and excludes the necessity of using different methods for individual substances.

The analysis of water from several rivers from different locations in Poland showed that the problem of residues of disinfectant agents in surface water is significant. The determined concentrations were high – several to tens $\mu g \cdot L^{-1}$ levels.

The method developed (both HPLC-DAD and SPE) can be used for environmental monitoring and evaluating the degree of water contamination by residues of disinfectant agents. Such methods could be especially useful for water monitoring in the industrial, medical and breeding areas. It is forbidden to release manufacturing and hospital sewage directly to surface water, but a lot of factories do not observe these regulations. In some branches of industry a lot of disinfectant products are used, for example: medical branches (medicine, veterinary, stomatology), pharmaceutical and cosmetic industries, food industry, and different breeding (i. e. stock-farming, poultry farming, fish farming). The method developed allows for monitoring rivers and lakes in the neighborhood of such places.

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