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Original Research

Research on the Stability of Biologically Active (E)-azastilbene Derivatives in Polish Rivers

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Abstract

The study shows potential applications of (E)-azastilbene derivatives with antimicrobial properties for surface water protection. Four derivatives of (E)-azastilbene under study interact with nine different microorganisms. The research was carried out using samples of surface waters collected from three rivers: the Bug, the Liwiec and the Muchawka. The Muchawka River flows into the Liwiec River, which, in turn, is a tributary of the Bug River. During the study, short- and long-term stability of selected stilbene derivatives in collected surface water samples was determined. The extraction process was carried out on a naphthylpropyl extraction column with a recovery rate of about 95%. The highest average reduction in the stability of the analysed derivatives after 12 months was found in water samples collected from the Muchawka River and amounted to 59%, whereas the smallest average reduction of 47% was found in water samples from the Liwiec River.

Keywords: extraction, disinfectants, surface water, stilbene derivatives

Introduction

Environmental development is defined as conscious and planned changes in the natural environment, the aim of which is the economic use of ecosystems or restoration of their ecological function. It can therefore be stated that environmental development is a deliberate and conscious impact on the natural environment, the purpose of which is to enrich it with features that are beneficial to humans and to increase the capacity of the environment. The primary objective of such measures is to create suitable conditions for environmental management that will meet the requirements of sustainable development, will ensure a higher quality of life for the residents and will increase the tourist attractiveness of a given area, while protecting the natural environment for future generations [1-5].

One of the most important elements of the natural environment is water and this is the only necessary medium for life. Any water pollution leads to various types of diseases and the death of living organisms. Contaminated water also causes diseases of aquatic plants. Harmful compounds accumulated in plants penetrate into human and animal organisms. Large amounts of industrial and municipal wastewater,

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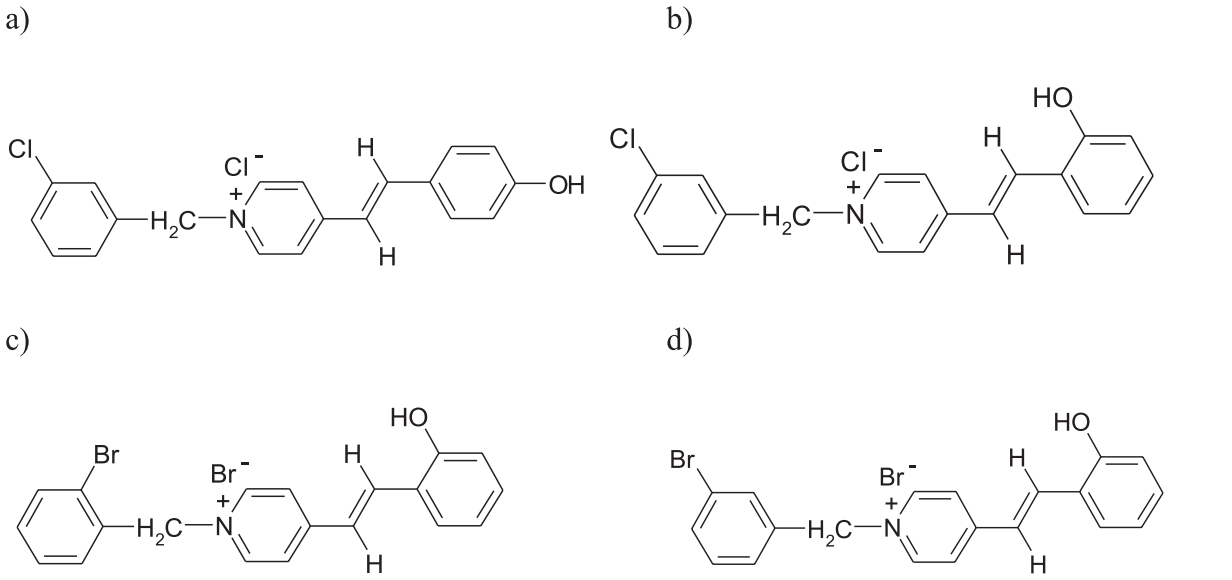

Fig. 1. Chemical structures of the analyzed derivatives: a) for chloride of (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 (A1), b) chloride of (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 (A3), c) bromide of (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 (A15), d) bromide of (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 (A17).

increasing every day, threaten the viability of lakes, rivers, seas and oceans [6-10].

At present, water purity is a worldwide problem. In developed countries, it is not possible to drink water directly from rivers or from the tap, which raises concerns as to its purity and harmfulness. Despite various prohibitions and penalties, we still have to deal with the contamination of rivers, lakes and groundwater, from which people draw their drinking water. Water used for consumption is obviously purified, but even the most advanced technologies are insufficient to eliminate all contaminants. In addition, wastewater generated by industry, agriculture and households leads to the increasing deterioration of waters [11-15]. It is therefore necessary to protect the natural environment, and water in particular.

Stilbene derivatives with antimicrobial properties may be used for environmental protection. The interest in antiseptic (decontaminant), preserving and disinfecting substances usually results from their negative effects on human health. At the same time, these substances are essential in a number of different products, e.g. they are commonly used as additives preventing the development of mould, bacteria and fungi [15]. Stilbene derivatives under study are characterised by demonstrated fungistatic and fungitoxic properties [16]. Considering the above properties of stilbene derivatives, it has been decided to use them for environmental protection.

The objective of this study was to demonstrate the possibility of using stilbene derivatives for surface water protection by determining their short- and long-term stability in the analysed water samples. Assays were carried out with the use of high-performance liquid chromatography.

Material and Methods

Study Area

Twenty samples of surface water were collected from each of the three rivers, i.e. the Bug River in the town of Wyszków, the Liwiec River in the town of Węgrów and the Muchawka River in the city of Siedlce. The Muchawka River, with a length of 32.1 km, is a left-bank tributary of the Liwiec River. The status of water in the Muchawka River is referred to as moderate. The Liwiec River is 126.3 km long and it is the longest left-bank tributary of the Bug River; its catchment area is 2,780 km^2. The chemical status of water in the Liwca River was defined as moderate, mainly due to the exceeded annual average and maximum concentrations of benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene [17].

The Bug River, on the other hand, is a border river over a long distance and a receiver of large quantities of wastewater from Ukraine. The direct source of pollution in this river in the Mazovia province is the town of Wyszków, which discharges about 3,000 m^3 per day of

Table 1. Selected physical and chemical properties of the analyzed derivatives [16].

Compound	M.p. ºC	IR (KBr), cm^{-1} $\delta_{CH=CH}$	^{1}H-NMR δ, ppm, DMSO-d_6, -CH_2-$^+$N
(A1)	227–230	970	5.81
(A3)	209–212	965	5.82
(A15)	218–221	980	5.90
(A17)	205–208	960	5.79

Table 2. Antimicrobial properties of (E)-azastilbene derivatives included in the studies [16].

Compound	Minimal inhibitory concentration (μg · mL^{-1})								
	1	2	3	4	5	6	7	8	9
(A1)	100	500	500	500	1000	1000	>500	>500	>500
(A3)	7.5	100	100	100	1000	1000	>500	>500	>500
(A15)	5	500	500	100	1000	1000	>500	>500	>500
(A17)	5	500	100	100	1000	1000	>500	>500	>500

1–Staphylococcus aureus 209P FDA, 2–Streptococcus faecalis ATCC 8040, 3–Bacillus subtilis ATCC 1633, 4–Escherichia coli PZHO 26B6, 5–Klebsiella pneumoniae 231, 6–Pseudomonas aeruginosa 5 R1, 7–Candida albicanus PCM 1409 PZH, 8–Microsporum gypseum K_1, 9–Aspergillus fumigatus C1.

wastewater into the river, treated in a sewage treatment plant with increased nutrient removal. The Toczna and Cetynia rivers discharge significant loads of pollutants into the Bug River with wastewater from the town of Sokołów Podlaski. The characteristic contamination of the Bug River are the total suspended solids.

The reaction of water samples collected from the Muchawka River was slightly alkaline (pH = 7.23), from the Liwiec River – also slightly alkaline (pH = 7.58), and from the Bug River – alkaline (pH = 8.31).

The material of the study were also derivatives of (E)-azastilbene (Fig. 1), i.e. (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 chloride, (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 chloride, (E)-N-(o-bromobenzyl)-2'-hydroxystilbazoles-4 bromide, and (E)-N-(m-bromobenzyl)-2'-hydroxystilbazoles-4 bromide. Surface water samples were filtered through a membrane filter with a pore diameter of 3 μm, allowing bacteria to pass into the solution. The examined derivatives were obtained according to the published procedures [16]. Structures of derivatives were confirmed by nuclear magnetic resonance (Table 1) and the biological activity is presented in Table 2. The determination of the stability of derivatives was carried out between August 2018 and September 2019.

Methods

Five surface water samples collected from each site were analysed in parallel. The analysed derivatives of (E)-azastilbene are well soluble in water. In order to analyse the surface water, four azastilbene derivatives were added to each sample to obtain a 1000 μg mL^{-1} concentration each. All of the material was stirred and concentrated after 1 h, using the solid-phase extraction method. The extraction process was carried out on a naphthylpropyl extraction column (Fig. 2) [18]. The procedure of column conditioning consisted in washing the bed of 4 mL of cyclohexane (Merck, Darmstadt, Germany), 4 mL of methanol (Merck, Darmstadt, Germany) and then 4 mL of triple distilled water. Then the bed was dried in a stream of air for 15 sec. A volume of 100 mL of each solution of an analysed sample was passed through the thus prepared extraction column at a rate of 3-4 drops per second. The column was then dried in a stream of air for 10 min. The process of elution was performed using 8 cm^3 of acetonitrile. Then 2 mL of methanol was added to 8 mL of eluent and the mixture was thoroughly stirred and concentrated in a stream of air to a volume of about 1 mL. Next, 1 mL of distilled water was added. Each sample was prepared the same way. The content of each derivative was analysed by high-performance liquid chromatography with UV/Vis detection. Chromatographic conditions of the analyses were as follows: the octadecyl column, the mobile phase – methanol (100%), flow rate – 1.0 mL · min^{-1}, wavelength – 410 nm, temperature – 25ºC.

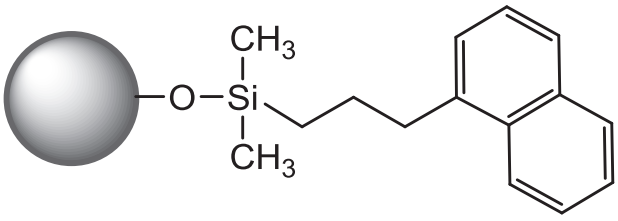

Fig. 2. Scheme of chemical structure of bonded stationary phase.

When determining the stability of the analysed derivatives, consideration was given to their possible changes over time. Assays were carried out according to the above procedure, with four time intervals: 1 h, 7 days, 28 days and 12 months. Water samples, prepared for extraction, were stored during the year in plastic bottles at a temperature range of 18-30ºC. Standard solutions with the following concentrations: 0, 50, 100, 300, 500, 750 and 1000 μg · mL^{-1} were prepared to determine recovery values of individual derivatives and to perform quantitative analysis.

The standard solutions were introduced into a chromatographic device and calibration curves were plotted based on the obtained peak areas. Using the minimum values of correlation coefficients of 0.999, the range of concentrations was determined, at which the curves were linear. The limit of detection (LOD)

and the limit of quantification (LOQ) under the chromatographic conditions and in the linearity range were expressed as the concentration of a compound for which signal/noise size ratios were 3:1 and 10:1, respectively [19-23].

All ^{1}H NMR spectra were registered on Bruker-400 in $CDCl_3$, using the internal standard HMDS, whereas the IR spectra were registered in potassium bromide using Nicollet Magna-IR 760 (Thermo Fisher Scientific, Waltham, GB). The extraction process was carried out using the naphthylpropyl extraction column at the Chair of Environmental Chemistry and Bioanalytics, Nicolaus Copernicus University in Toruń. High-performance liquid chromatography SPD-6A (Shimadzu, Kyoto, Japan) equipped with a UV detector (Shimadzu C-R6A) and an LC-6A pump with an Rheodyne dispenser (Berkeley, CA, USA), model 7125 with a volume of 20 µL, were used for qualitative and quantitative analysis.

Results and Discussion

The main objective of this study was to demonstrate the possibility of using biologically active azastilbene derivatives to protect surface waters on the basis of their short- and long-term stability in the analysed water samples. The research was conducted with the use of surface waters collected from three rivers. The following substances were included in the study: (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 chloride, (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 chloride, (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 bromide, and (E)-N-(m-bromobenzyl)-2'-hydroxystilbazoles-4 bromide. The obtained results are presented in Tables 3-5.

The process of extraction of the analysed derivatives was carried out on the naphthylpropyl extraction column (Fig. 2). The presence of an aryl group in the structure of the naphthylpropyl column attached to the alkyl chain makes it strongly involved in the process of isolating substances from different matrices using additional π–π interactions [24-28]. These interactions always occur between the isolated analyte and the terminal part of the attached ligand. Since the analysed (E)-azastilbenes contained aromatic rings in their structure, it can be concluded that they also participated in the retention mechanism.

Analyses in the liquid–solid system were conducted on surface water samples intentionally contaminated with (E)-azastilbene derivatives. The following derivatives were added to each sample: (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 chloride, (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 chloride, (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 bromide and (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 bromide, arriving at a concentration of 1000 µg · L^{-1}. Then, after mixing the solutions and concentrating them accordingly, the recovery rate of the analysed (E)-azastilbene derivatives on the naphthylpropyl column was determined for three different types of surface waters covered by this study. The obtained recovery values are presented in Table 3 and ranged from 83.5±4.9% to 96.1±3.9%. The lowest recovery value of all derivatives was determined for water collected from the Bug River. When comparing the average data of the analysed derivatives, it is easy to observe that the lowest recovery values for samples collected from the Bug River were found for derivative (A3) and the highest ones for derivative (A1). In the case of samples from the Liwiec River, the highest recovery value of 96.1±3.9% was determined for derivative (A15) and the lowest value of 91.4±3.4% was determined for derivative (A1). For samples collected from the Muchawka River, the lowest recovery value of 92.9±3.4% was obtained for derivative (A3), whereas the highest one (95.4±4.7%) for derivative (A15).

The developed method of extraction was subjected to a process of validation (Table 4), determining such basic parameters as: the linearity range, the limit of detection, the limit of quantification, the coefficient of variation and the correlation coefficient. These parameters were determined for each analysed derivative. It follows from the data presented in Table 4 that the linearity for derivative (A1) ranged from 5.1 to 310.4 µg · L^{-1}, for derivative (A3) from 4.8 to 303.6 µg · L^{-1}, for derivative (A15) from 4.9 to 321.3 µg · L^{-1} and for derivative (A17) from 4.7 to 317.2 µg · L^{-1}. Coefficients of variation ranged from 2.8-4.8 for derivative (A15) to 3.3-4.9 for derivative (A17). The lowest detection limit of

Table 3. Mean recovery values (1 hour after sample preparation) for chloride of (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 (A1), chloride of (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 (A3), bromide of (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 (A15), bromide of (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 (A17) obtained from different matrices in the naphthylpropyl column used in the study (n = 5).

Water from the river	Mean recovery values (%)				SD (%)			
	(A1)	(A3)	(A15)	(A17)	(A1)	(A3)	(A15)	(A17)
Liwiec	91.4	95.5	96.1	93.9	± 3.4	± 4.2	± 3.9	± 4.4
Muchawka	93.7	92.9	95.4	93.6	± 4.1	± 3.4	± 4.7	± 4.5
Bug	87.3	83.5	86.1	85.6	± 4.3	± 4.9	± 4.2	± 4.7

Table 4. Selected parameters of the method validation.

Compound	Linearity ($\mu g \cdot L^{-1}$)	LOD ($\mu g \cdot L^{-1}$)	LOQ ($\mu g \cdot L^{-1}$)	Coefficient of variation (%)	Correlation coefficient
(A1)	5.1 – 310.4	1.5	5.0	3.1 – 4.6	0.9995
(A3)	4.8 – 303.6	1.5	5.0	3.0 – 4.3	0.9996
(A15)	4.9 – 321.3	1.3	4.3	2.8 – 4.8	0.9991
(A17)	4.7–317.2	1.2	4.0	3.3 – 4.9	0.9994

LOQ = 3.33 x LOD

1.2 $\mu g \cdot L^{-1}$ was found for derivative (A17) and the highest one – 1.5 $\mu g \cdot L^{-1}$ for derivatives (A1 and A3). Quantification limits varied from 4.0 $\mu g \cdot L^{-1}$ for derivative (A17) to 5.0 $\mu g \cdot L^{-1}$ for derivatives (A1 and A3).

In addition to microbiological criteria, the preservation test often also consists in a test that assesses the stability of a preservative over time. Such research provides information on the breakdown and adsorption of a preservative within a specific time. Based on the loss of a preservative, the quality of preservation and its effectiveness can be assessed, as the relationship between these phenomena and the level of contamination is directly proportional [29-34]. This kind of research i.e. the stability of azastilbene derivatives over time was also used in this study.

The average content of the analysed derivatives, i.e. (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 chloride, (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 chloride, (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 bromide, and (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 bromide in the analysed surface water samples after 1 h, 7 days, 28 days and 12 months is presented in Table 5. The obtained results indicate that the content of the analysed derivatives in the studied surface waters slightly decreased over time. Since the derivatives of (E)-azastilbene used in the study are hydrolytically stable and resistant to light and oxygen, their loss is most likely associated with their effects on microorganisms. A slight decrease in the content of the analysed derivatives over time could also be partly caused by reactions with other ions or compounds present in the analysed samples, as well as adsorption on the walls of containers in which the samples were stored.

According to the literature, fungistatic and fungitoxic properties of a preparation are considered suitable if no significant decrease in antiseptic, preservative or disinfectant content occurs after 28 days at a certain temperature in a matrix

Table 5. Mean content values for chloride of (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 (A1), chloride of (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 (A3), bromide of (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 (A15), bromide of (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 (A17) in the surface waters samples after 1 hour, 7 days, 28 days and 12 months, obtained in the naphthylpropyl column.

Times	Water from the river	Average value ($\mu g \cdot mL^{-1}$)				SD (%)			
		(A1)	(A3)	(A15)	(A17)	(A1)	(A3)	(A15)	(A17)
1 hour	Liwiec	893.1	887.2	829.9	837.4	±4.5	±3.9	±4.0	±3.6
	Muchawka	867.3	882.7	858.6	823.6	±4.1	±3.5	±4.2	±4.6
	Bug	779.7	773.3	863.4	859.1	±3.4	±3.5	±4.1	±4.8
7 days	Liwiec	783.7	782.3	780.9	768.5	±3.2	±4.3	±3.5	±3.6
	Muchawka	767.5	764.7	752.1	775.1	±4.2	±4.1	±4.5	±4.0
	Bug	681.7	683.4	694.9	700.6	±3.6	±4.1	±3.7	±3.9
28 days	Liwiec	591.1	596.9	590.3	557.6	±4.4	±4.1	±4.3	±4.4
	Muchawka	566.5	591.6	595.4	583.5	±3.9	±3.4	±4.5	±4.1
	Bug	570.2	587.3	571.8	563.9	±4.9	±4.2	±4.4	±4.0
12 months	Liwiec	295.2	283.1	295.4	321.2	±3.7	±3.2	±4.4	±3.5
	Muchawka	281.6	268.9	279.7	345.5	±3.8	±3.6	±3.4	±4.7
	Bug	276.8	286.8	301.3	297.7	±3.6	±4.2	±4.6	±3.7

contaminated with microorganisms [35]. Therefore, it can be inferred from the data in Table 5 that small differences in the decline of individual derivatives were observed after 1 h, regardless of sampling sites. The analysed (E)-azastilbene derivatives showed a slight decrease in their content of about 13%, and thus good stability, also after 7 days in all the collected surface water samples. On the other hand, the average decrease in the content of individual derivatives varied significantly in the case of the 28-day time interval. The difference in the content of derivative (A1) in water from the Liwiec River compared to the content after 7 days was 192.6 $\mu g \cdot L^{-1}$, i.e. 33%, in water from the Muchawka River – 201.0 $\mu g \cdot L^{-1}$, i.e. 35%, and in the water from the Bug River – 111.5 $\mu g \cdot L^{-1}$, i.e. 20%. Similar results were also obtained for two derivatives – (A3 and A15). Derivative (A17), on the other hand, showed the highest average drop of nearly 38% in the case of water collected from the Liwiec River. After 7 and 28 days, the difference was 210.9 $\mu g \cdot L^{-1}$. A slightly smaller difference (191.6 $\mu g \cdot L^{-1}$) was recorded for samples of water collected from the Muchawka River, i.e. 33% compared to the concentration after 7 days. The smallest difference of 136.7 $\mu g \cdot L^{-1}$ was found for water samples from the Bug River, i.e. 24%.

A further decrease in the content of the analysed derivatives was observed after 12 months. In the case of derivative (A1) and water samples from the Liwiec River, the difference in the content between the last two time intervals was 295.9 $\mu g \cdot L^{-1}$, which accounts for a loss of 50%, for derivative (A3) – 47%, derivative (A15) – 50%, and derivative (A17) – 57%. Similar results were obtained for samples collected from the Muchawka River, i.e. for derivatives (A1 and A3) – a decrease by 50% each, for derivative (A15) – a decrease by 47%, derivative (A17) – a decrease by 59%. The content of derivative (A1) in water samples collected from the Bug River decreased by 48%, derivative (A3) by 49%, derivative (A15) by 52% and derivative (A17) also by 52%.

It can be concluded that the conducted research enables the use of the analysed derivatives of (E)-azastilbene for surface water treatment. Due to the reduction in the content of individual (E)-azastilbene derivatives, they can also be used with other substances in the form of mixtures that would be synergistically active when combined in solution. Literature data show that no substance has been invented so far that would fully meet all the requirements specified for antiseptics, preservatives or disinfectants. Each of the chemicals currently used has certain limitations and therefore mixtures of such substances with a synergistic effect are used in many products.

Conclusions

The research on short- and long-term stability of four biologically active derivatives of (E)-azastilbene in surface waters of three rivers was carried out. After 12 months, the highest reduction (59%) of derivative (A17) was recorded in water samples from the Muchawka River. The smallest reduction (47%) in the content of derivative (A3) was found in water samples collected from the Liwiec River. The obtained results indicate that the waters of the Muchawka River are the most susceptible to microbiological contamination. At the same time, it should be emphasised that the volume of water in this river and the flow rate are the smallest compared to the others, which undoubtedly has a negative impact on the process of dilution of pollutants and the self-purification of waters in this river. The research on short- and long-term stability indicates that the analysed derivatives of (E)-azastilbenes can be used for surface water treatment, preferably with other synergistic substances.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. SIUDEK P., FRANKOWSKI M., SIEPAK J. Atmospheric particulate mercury at the urban and forest sites in central Poland. Environ. Sci. Pollut. Res. **23**, 2341, **2016**.
2. SIUDEK P., KURZYCA I., SIEPAK J. Atmospheric deposition of mercury in central Poland: Sources and seasonal trends. Atmos. Res. **170**, 14, **2016**.
3. KLUSKA M., KRAJEWSKA E., JABŁOŃSKA J., PRUKAŁA W. New applications and analysis of (E)-azastilbenes in environmental samples. Crit. Rev. Anal. Chem. **49** (5), 395, **2019**.
4. NAWAŁA J., CZUPRYŃSKI K., POPIEL S., DZIEDZIC D., BEŁDOWSKI J. Development of the HS-SPME-GC-MS/MS method for analysis of chemical warfare agent and their degradation products in environmental samples. Anal. Chim. A, **933**, 103, **2016**.
5. Status of small-scale water supplies in the WHO European Region, Results of a survey conducted under the Protocol on Water and Health. Informal Document Working Group on Water and Health, Geneva, 26–27 November **2014**. Economic Commission for Europe and World Health Organization Regional Office for Europe.
6. KLUSKA M., KOMASIŃSKA M., JABŁOŃSKA J., PRUKAŁA W. Challenges of HPLC determination of quinoline derivatives used in the treatment of malaria. J. Liq. Chromatogr. Rel. Technol. **41** (8), 451, **2018**.
7. BINIAK S., TRYKOWSKI G., PAKUŁA M., ŚWIĄTKOWSKI A., MALINOWSKA Z., POPIEL S. Effects of ozone dissolved in water on the physicochemical properties of activated carbons applied in drinking water treatment. Adsorp. Sci. Technol. **28** (6), 521, **2010**.

8. KLUSKA M., PYPOWSKI K., CHRZĄŚCIK I., KOVAL T., ERCHAK N. Optimization of conditions of isotachophoretic separation and determination of new class of pentacoordinated silanes. J. Liq. Chromatogr. Rel. Technol. **32** (14), 2001, **2009**.
9. KOWALSKI A., SIEPAK M., BOSZKE L. Mercury contamination of surface and ground waters of Poznań city, Poland. Pol. J. Environ. Stud. **16** (1), 67, **2007**.
10. MICHALSKI R. Trace level determination of Cr(III)/ Cr(VI) in water samples using ion chromatography with UV detection. J. Liq. Chromatogr. Rel. Technol. **28** (18), 2849, **2005**.
11. BUSZEWSKI B., OLSZOWY P., SZULTKA M., JEŻEWSKA A. New approaches to extraction techniques in determination of 4,40-methylenebis(2-chloroaniline) in air and water solutions. Talanta **93**, 117, **2012**.
12. RAFIŃSKA K., POMOSTOWSKI P., RUDNICKA J., KRAKOWSKA A., MARUŚKA A., NARKUTE M., BUSZEWSKI B. Effect of solvent and extraction technique on composition and biological activity of Lepidium sativum extracts. Food Chem. **289**, 16, **2019**.
13. PAJEWSKA-SZMYT M., SINKIEWICZ-DAROL E., BERNATOWICZ-ŁOJKO U., KOWALKOWSKI T., GADZAŁA-KOPCIUCH R., BUSZEWSKI B. QuEChERS extraction coupled to GC-MS for a fast determination of polychlorinated biphenyls in breast milk from Polish women. Environ. Sci. Pollut. Res., **26**, 30988, **2019**.
14. PRUKAŁA W., PRUKAŁA D., PYPOWSKI K., CHRZĄŚCIK I., KLUSKA M. Chromatography of biologically active chlorides of (E)-N-o-(m- or p-) chlorobenzyl-γ-azastilbenols-2'(3' or 4'). J. Liq. Chromatogr. Rel. Technol. **31** (17), 2612, **2008**.
15. SZUMSKI M., KŁODZIŃSKA E., JARMALAVICIENE R., MARUSKA A., BUSZEWSKI B. Considerations on influence of charge distribution on determination of bio-molecules and microorganisms and tailoring the monolithic (continuous bed) materials for bioseparations. J. Biochem. Biophys. Methods **70** (1), 107, **2007**.
16. WYRZYKIEWICZ E., PRUKAŁA W. KEDZIA B. Synthesis and antimicrobial properties of N-substituted halides of (E)-azastilbenols. Il Farmaco **50** (11), 779, **1995**.
17. ANDRZEJEWSKA A., KONARZEWSKA J., PIĄTEK M., MIKULSKI M. Environmental protection program for the Liw commune for 2016-2019, Warsaw pp. 139, **2015**.
18. GADZAŁA-KOPCIUCH R., KLUSKA M., WEŁNIAK M., BUSZEWSKI B. Silicon dioxide surfaces with aryl interaction sites for chromatographic applications. Mater. Chem. Phys. **89** (2-3), 228, **2005**.
19. BOCIAN S., KRZEMIŃSKA K., BUSZEWSKI B. A study of separation selectivity using embedded ester-bonded stationary phases for liquid chromatography. Analyst, **141** (14), 4340, **2016**.
20. KLUSKA M. An application of aryl stationary phases for separation of select organogermanium compounds. J. Liq. Chromatogr. Rel. Technol. **31** (2), 210, **2007**.
21. JANAS P., BOCIAN S., JANDERA P., KOWALKOWSKI T., BUSZEWSKI B. Separation of flavonoids on different phenyl-bonded stationary phases-the influence of polar groups in stationary phase structure. J. Chromatogr. A **1429**, 198, **2016.**
22. KLUSKA M., PYPOWSKI K., Separation of tribenzylhydrogermanium nitrile derivatives by means of HPLC with participation of π-π interactions. J. Liq. Chromatogr. Rel. Technol. **30** (14), 2059, **2007**.
23. STUDZIŃSKA S., BOCIAN S., SIECIŃSKA L., BUSZEWSKI B. Application of phenyl-based stationary phases for the study of retention and separation of oligonucleotides. J. Chromatogr. B **1060**, 36, **2017**.
24. BOCIAN S., SKOCZYLAS M., GORYŃSKA I., MATYSKA M., PESEK J., BUSZEWSKI B. Solvation processes on phenyl-bonded stationary phases - The influence of polar functional groups. J. Sep. Sci. **39** (22), 4369, **2016**.
25. PRUKAŁA W., PYPOWSKI K., CHRZĄŚCIK I., KLUSKA M. Separation of biologically active isomers of (E)-N-meta- and para-nitroazastilbenes by the HPLC technique. J. Liq. Chromatogr. Rel. Technol. **31** (4), 578, **2008**.
26. KLUSKA M., PYPOWSKI K., ERCHAK N. Separation of hexabenzyldigermoxane and hexabenzyldigermanium by HPLC. J. Liq. Chromatogr. Rel. Technol. **30** (12), 1777, 2007.
27. PYPOWSKI K., USZYŃSKA I., KLUSKA M. Chromatographic separation of isomers of tribenzylgermanium nitrile derivatives using chemically bonded aryl stationary phases. J. Liq. Chromatogr. Rel. Technol. **29** (20), 2989, **2006**.
28. MAŁKIEWICZ K., TURŁO J., MARCINIUK-KLUSKA A., GRZECH-LEŚNIAK K., GĄSIOR M., KLUSKA M. Release of bisphenol A and its derivatives from orthodontic adhesive systems available on the European market as a potential health risk factor. Ann. Agric. Environ. Med. **22** (1), 172, **2015**.
29. BAJKACZ S., BARANOWSKA I., BUSZEWSKI B., KOWALSKI B., LIGOR M. Determination of flavonoids and phenolic acids in plant materials using SLE-SPE-UHPLC-MS/MS method. Food Anal. Methods **11**, 3563, **2018**.
30. POPIEL S., NAWAŁA J. Detoxification of sulfur mustard by enzyme-catalyzed oxidation using chloroperoxidase. Enzyme Microb. Technol. **53** (5), 295, **2013**.
31. POPIEL S., WITKIEWICZ Z., NALEPA T. The reactions of sulfur mustard with the active components of organic decontaminants. J. Hazard. Mater. **123** (1-3), 269, 2005.
32. WITKOWSKA-KRAJEWSKA E., KLUSKA M., PRUKAŁA W., MIKULEWICZ M., CHOJNACKA K., MAŁKIEWICZ K. Study on stability of (E)-azastilbenes as disinfectants and preservatives as well as their recovery from aqueous solutions. Przem. Chem. **97** (8), 1320, **2018**.
33. KLUSKA M., WITKOWSKA-KRAJEWSKA E., PRUKAŁA W. A new method for isolating (E)-azastilbene derivatives with antimicrobial properties from aqueous samples. Oceanol. Hydrobiol. Stud. **47** (1), 19, **2018**.
34. PRUKAŁA D., PRUKAŁA W., MAŁKIEWICZ K., SZYMALSKA M., WITKOWSKA-KRAJEWSKA E., KLUSKA M. New methodology of separation and determination of biologically active isomers of nitrobenzyl azastilbene derivatives, J. Liq. Chromatogr. Rel. Technol. **33** (6), 761, **2010**.
35. KIEŁBASA A., KRAKOWSKA A., RAFIŃSKA K., BUSZEWSKI B. Isolation and determination of saponin hydrolysis products from Medicago sativa using supercritical fluid extraction, solid-phase extraction and liquid chromatography with evaporative light scattering detection. J. Sep. Sci. **42** (2), 465, **2019**.