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A new method for isolating (E)-azastilbene derivatives with antimicrobial properties from aqueous samples

by

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Abstract

This paper presents the results of a study aimed at developing the extraction process and determining long-term stability of (E)-azastilbene derivatives in order to explore their possible use as preservative, antiseptic and disinfectant substances. The study was performed in three different matrices: distilled, surface and waste water. Test substances included bromide of (E)-N-(o-bromobenzyl)-4'-hydroxystilbazole-4 and chloride of (E)-N-(p-chlorobenzyl)-4'-hydroxystilbazole-4. The extraction process involved the use of three stationary phases: octyl, octadecyl and naphthylpropyl. The highest recovery values (amounting to approx. 95%) were obtained in the naphthylpropyl column for all of the above-mentioned matrices. A decline in the stability of the analysed derivatives after a 28-day period was below 14% in all matrix types.

Key words: surface water, waste water, disinfectants, extraction, (E)-azastilbenes

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Introduction

Recent years have seen a noticeable rise in demand for preservative, antiseptic and disinfectant substances. They are commonly used in, among others, pharmaceutical, cosmetic and personal hygiene products as components that suppress fungi, bacteria and mould growth (Sawa et al. 2014; Wang et al. 2001; Yahr, Greenberg 2004). The attention given to this type of compounds is due to their ever-growing use and the way they may impact the environment and living organisms.

The detection and labelling of disinfectant and preservative substances in different matrices has been the subject of an increasing number of studies around the world (Pisanello 2014; Pandey et al. 2017). The trend can be attributed mostly to legal regulations that oblige manufacturers of a wide range of products to identify, quantify and determine the stability of the components they use. This applies also to (E)-azastilbenes – compounds that have both fungistatic and fungitoxic properties, i.e. they inhibit and destroy fungal growth. Moreover, depending on the concentration used, they may also have estrogenic properties (Wyrzykiewicz, Prukała 1995; Prukała, Kędzia 1999, Prukała et al. 2008a,b).

Antiseptic(s) are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are generally distinguished from antibiotics by the latter's ability to be transported through the lymphatic system to destroy bacteria within the body, and from disinfectants that destroy microorganisms found on non-living objects. Antibacterials are antiseptics that have the proven ability to act against bacteria (Oriel, Itani 2017).

The preparation of compound samples from environmental, biological and pharmaceutical matrices is one of the most time-consuming stages of analysis, taking up to 80% of the total study duration (Castro et al. 2008; Guerrero et al. 2008; Buszewski et al. 2009; Buszewski, Szultka 2012). Sample purification usually encompasses dilution, precipitation, filtration and centrifugation. The importance of these steps is not to be underestimated: any error committed at this stage can never be rectified with even the most effective separation or detection method. The liquid-solid and liquid-liquid extraction processes are ubiquitous in standard marking due to, among others, their direct compatibility with other methods (both on- and off-line). What is more, they are characterized by high repeatability of data, accuracy, and a relatively low cost of conducting the analysis. Preliminary procedures for sample preparation should be selective, fast, inexpensive and environmentally-friendly (Buszewski, Szultka 2012; Gadzała-Kopciuch et al. 2009; Michel, Buszewski 2009b; Szumski et al. 2007). Nowadays, selectivity is more and more often achieved by employing procedures based on molecular imprinting. Time and cost of sample preparation, in turn, can be reduced by the automation of extraction procedures and the introduction of the miniaturization method. Before chromatography is applied, it is often necessary to perform extraction in order to separate analytes from interfering substances (Kataoka 2005). However, the right choice of sample preparation method is of paramount importance in the qualitative and quantitative identification of the target compounds under study. In most cases, many different compounds can be found in the matrix, from highly lipophilic to moderately polar ones, with different levels or alkalinity or acidity (Lord, Pawliszyn 2000; Buszewski et al 2009a; 2011; 2014; Mehdinia et al. 2013; Szultka-Mlyńska et al. 2016).

The purpose of this paper is to present an attempt at developing a procedure for analysing (E)-azastilbenes in surface and waste waters, initiated in view of their possible use as preservatives, antiseptics and disinfectants. Particular attention is given to the application of the extraction process and the determination of long-term stability of selected (E)-azastilbene derivatives. The development of such a procedure will constitute yet another step toward utilizing (E)-azastilbenes as a preservative component in a variety of products or as an antiseptic and disinfectant substance.

Materials and methods

Experimental section

The study material included derivatives of (E)-azastilbene (Fig. 1), namely bromide of (E)-N-(o-bromobenzyl)-4'-hydroxystilbazole-4 (A1) and chloride of (E)-N-(p-chlorobenzyl)-4'-hydroxystilbazole-4 (A2); a distilled water sample (with natural acidity of 6.8); a surface water sample (with natural acidity of 6.4); and a waste water sample (with natural acidity of 6.8). All tests were performed on natural samples. The pH of the analyzed samples was not modified. Waste water was filtered through a membrane filter with a 3 µm pore diameter, which allows the bacteria to pass into the solution.

The selected (E)-azastilbene derivatives were obtained based on the available literature (Wyrzykiewicz, Prukała 1995). The structures of the derived compounds were confirmed with nuclear magnetic resonance (Table 1). The biological activity

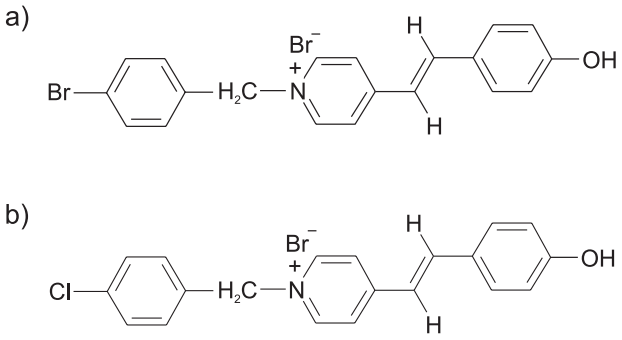

Figure 1

Structures of the analysed compounds: a) bromide of (E)-N-(o-bromobenzyl)-4'-hydroxystilbazole-4 (A1), and b) chloride of (E)-N-(p-chlorobenzyl)-4'-hydroxystilbazole-4 (A2)

Table 1

Chemical and physical data of the analysed compounds

Compound	M.p. °C	IR (KBr) (cm^{-1}) $\delta_{CH=CH}$	^{1}H-NMR δ(ppm) $-CH_2-^+N$
(A1)	217–220	981	5.91
(A2)	240–243	986	5.82

of the analysed (E)-azastilbenes is presented in literature (Wyrzykiewicz, Prukała 1995; Prukała et al. 2008a,b). Waste water samples were acquired at a sewage treatment plant, while surface water samples were collected from the Muchawka (a river on the outskirts of the city of Siedlce, Poland). The study was conducted between 2016 and 2017. Standard solutions of the derivatives under study with concentrations of 50, 100, 300, 450, 700 and 1000 μg ml^{-1} were prepared in order to determine recovery values and to perform a quantitative analysis. The studied (E)-azastilbene derivatives are highly soluble in water.

During the experiment, the derivatives under study were added to each sample (i.e. distilled water, waste water and surface water) to reach a concentration of 1000 μg ml^{-1}. The solutions were mixed and, after about 1 hour, concentrated by solid phase extraction. In order to develop optimal conditions for separation, the process was carried out simultaneously in three extraction columns packed with stationary phases of different chemical structures: octadecyl (RP Si-C_{18}) and octyl (RP Si-C_8) as well as naphthylpropyl (RP Si-NAF, Fig. 2) (Gadzała-Kopciuch et al. 2005; Kluska et al. 2008). The summary of characteristics for each stationary phase is presented in literature (Gadzała-Kopciuch et al. 2005).

The conditioning of each column consisted in rinsing with 4 ml of cyclohexane (Merck, Darmstadt, Germany), 4 ml of methanol (Merck, Darmstadt, Germany) and 4 ml of triple-distilled water. Then, the columns were dried under a stream of air for 15 seconds. The next step consisted in passing 400 ml of the solution of distilled water and the analysed derivatives through the columns in vacuo at a flow rate of 3–4 drops per second. Next, each column was dried under a stream of air for 10 minutes. Once dry, adsorbed (E)-azastilbene derivatives were eluted with acetonitrile (2 × 3 ml and 1 × 2 ml); 2 ml of methanol was added to the 8 ml of the eluent. The resulting solution was concentrated under a stream of air to 0.5 ml, and 0.5 ml of triple-distilled water was added. The thus prepared samples were then analysed with high-performance liquid chromatography (HPLC) equipped with a UV-Vis absorbance detector. Chromatographic conditions: the octadecyl stationary phase, the mobile phase – methanol (100 vol. %); flow – 0.8 ml min^{-1}, wavelength – 410 nm, temperature – 26°C.

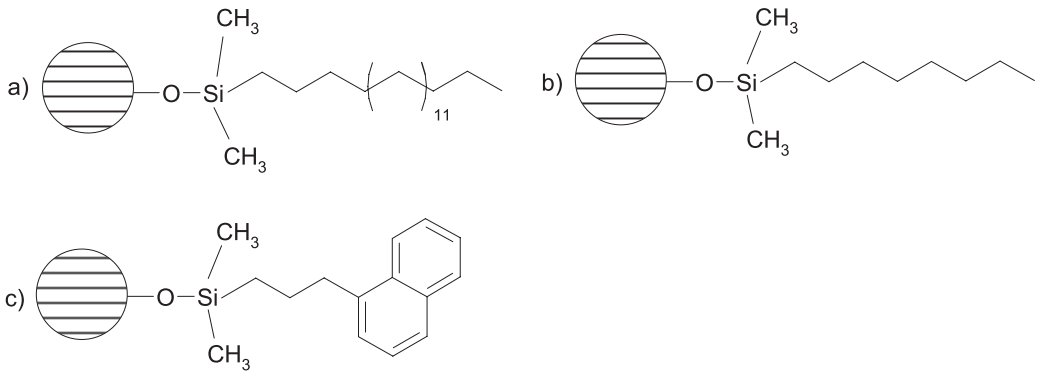

Figure 2

Chemical structures of bonded stationary phases used in the study: a) octadecyl, b) octyl, c) naphthylpropyl stationary phases

Due to the fact that the content of the analysed derivatives in distilled, surface and waste water solutions may change over time, the same procedure was repeated at four different time intervals: after 1 hour, 7 days, 28 days and 12 months. Each time, 5 samples of distilled, surface and waste water were analysed. All solutions of distilled, surface and waste water to be extracted (each 400 ml in volume) were stored in plastic bottles at temperatures between 20 and 32°C, depending on the time of the year.

The validation method of the analyzed derivatives (E)-azastilbene was then corroborated. To this end, a series of dilutions was prepared from the standard solutions to obtain concentrations of 0 to 450 µg ml^{-1}. HPLC analysis was then performed. The calibration curves were plotted on the basis of the peak area of the analyzed solution concentrations. The concentration range of the standards at which the curves were linear was based on a value of R^2 which was at least 0.999. The limit of detection (LOD) and the limit of quantification (LOQ) are expressed as the concentration of the compound for which the signal to noise ratio was 3:1 and 10:1, respectively (Konieczka, Namieśnik 2007). Linearity values, LOD, LOQ and curve equations are shown in Table 2.

Instruments and materials

The study was performed with octadecyl and octyl columns manufactured by S. Witko, Łódź, Poland. The naphthylpropyl column, in turn, was developed at the Department of Environmental Chemistry and Bioanalysis of Nicolaus Copernicus University in Toruń, Poland.

^1H NMR spectra were recorded with Bruker-200 in $CDCl_3$, with HMDS as an internal standard. The UV/Vis spectra were recorded on a spectrophotometer DU-68 (Beckman, USA). Chromatographic measurements were performed on a liquid chromatograph SPD-6A (Shimadzu, Kyoto, Japan) equipped with a pump LC-6A, a UV detector, a sampling valve Rheodyne (Berkeley, CA, USA), model 7125, with a 20 µl sample loop, and Shimadzu C-R6A. The infrared (IR) spectra were recorded with Nicollet Magna-IR 760 in potassium bromide.

Results and discussion

The results obtained are presented in Table 2 and Figures 3–5. The main objective of this study was to assess the feasibility of using two (E)-azastilbene compounds as preservatives, antiseptics or disinfectants. The analysed derivatives were bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 (A1) and chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxystilbazole-4 (A2). The secondary objective of the study, which contributed substantially to the primary one, was to develop a procedure for extraction and to determine the values of recovery from environmental samples for the analysed derivatives. The analysis was conducted on samples of distilled, surface and waste water.

In order to define optimal conditions for extraction, the study was carried out in three columns packed with stationary phases of different chemical structures (Fig. 2). The data presented in literature indicate that carbon and hydrogen loads as well as the coverage density of stationary bonded phases were high. Achieving such a high degree of coverage was possible due to the use to modify silica trifunctional silane (Gadzała-Kopciuch et al. 2005). The octadecyl phase showed the highest coverage density (4.68 µmol m^{-2}), while the lowest coverage density (4.41 µmol m^{-2}) was observed for the naphthylpropyl phase. The octyl phase had the lowest percentage of carbon (11.04%), and the chemically bonded naphthylpropyl stationary phase had the highest carbon percentage (13.60%). The presence of an aryl radical attached to the alkyl chain in the naphthylpropyl phase makes it highly selective in the isolation of compounds from different matrices through the π–π stacking interactions (Gadzała-Kopciuch et al. 2005). This was confirmed on determining breakthrough curves for each stationary phase (Fig. 3).

The breakthrough curves with shapes that are closest to the optimal one represent the octyl and octadecyl phases. They feature two types of active centres on the support surface, namely residual silanols and hydrophobic alkyl chains (Gadzała-Kopciuch et al. 2005). For the naphthylpropyl phase, the shape of the breakthrough curve slightly differs

Table 2

Concentration range of analytical compounds at which calibration curves are linear, and sensitivity of the HPLC method

Compound	Concentration range (µg ml^{-1})	Linear regression equation	R^2	LOD (µg ml^{-1})	LOQ (µg ml^{-1})
(A1)	5–300	y = 0.5060x + 0.0499	0.9999	0.152	0.457
(A2)	5–300	y = 0.4998x + 0.0421	0.9999	0.149	0.473

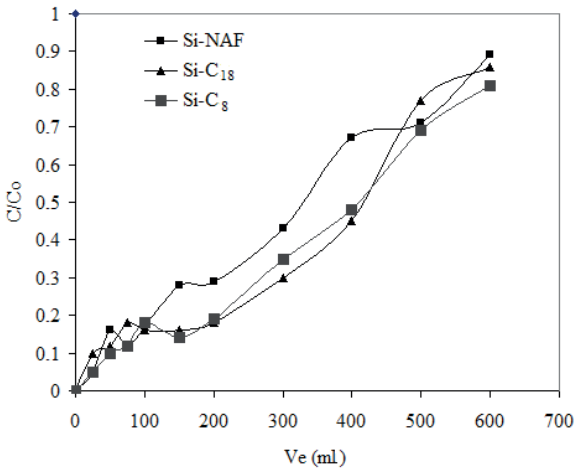

Figure 3

Breakthrough curves for bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4, obtained in the column packings used in the study

from the optimal one. This is basically due to the π–π stacking interactions between the terminal part of the bonded ligand and the isolated analyte.

In order to determine the optimal conditions for the liquid-solid extraction process, investigations were conducted on purposely tainted samples of distilled, surface and waste water. Each sample was stained with the analysed derivatives, i.e. bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxystilbazole-4 at concentrations of 1000 μg ml^{-1}. Next, after the solutions were mixed and adequately concentrated, the values of recovery for the two (E)-azastilbene compounds were determined for the three extraction columns used in the study. The obtained recovery values are presented in Figure 4. It is easily observable from the data that the highest recovery value was obtained for the (A2) derivative: it reached 95.7 ± 5.1% in the column packed with the naphthylpropyl stationary phase for distilled water matrix. The lowest recovery value was observed in the octyl column for the (A1) derivative and surface water matrix (82.7 ± 4.9%) as well as the octadecyl column and waste water matrix (83.1 ± 5.1%).

The comparability of the recovery values may be due to the similarity in the topography as well as physical and chemical characteristics of the adsorbents under study. This is most likely the result of a specific, selective sorption with dominant π–π stacking interactions between analyte molecules and bonded aryl radicals on the silica gel surface through short carbon chains. High recovery values and low standard deviation values indicate a high reproducibility rate. They are attributable to complex molecules attached through alkyl chains to residual silanols.

As it appears from the results obtained for the effluent concentration (Fig. 3) and recovery values (Fig. 4), the column packed with the naphthyl-propyl stationary phase was effectively employed to concentrate and purify the analysed derivatives of (E)-azastilbene from the surface, distilled and waste water samples in the subsequent phases of the study. The qualitative and quantitative analyses of bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 (A1) and chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxy-stilbazole-4 (A2) were conducted with HPLC.

The stability tests for the derivatives of (E)-azastilbene were performed in three different matrices: distilled, surface and waste water. The stability of the analysed compounds was recorded after 1 hour, 7 days, 28 days and 12 months. The results are presented in Figure 5. They show a slight decrease in the content over time, which may be related mainly to the active impact on microorganisms since the analysed derivatives are hydrolytically stable and resistant to sunlight or oxygen. The gradual decrease of the (E)-azastilbene compounds under study in the above-listed matrices could also be partly explained by the derivatives reacting with other compounds in the analysed samples, adsorption taking place on container walls, etc.

This study also included the preservation test which often evaluates not only the microbiological data, but also the durability of the preservative over time, providing indirect information on its breakdown

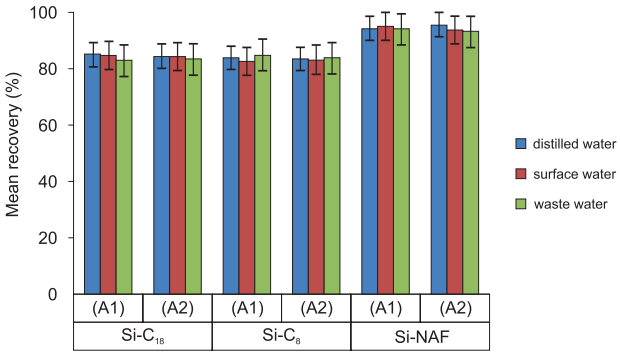

Figure 4

Mean recovery values (1 hour after sample preparation) for bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 (A1) and chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxystilbazole-4 (A2), obtained from different matrices in the columns used in the study (n = 5)

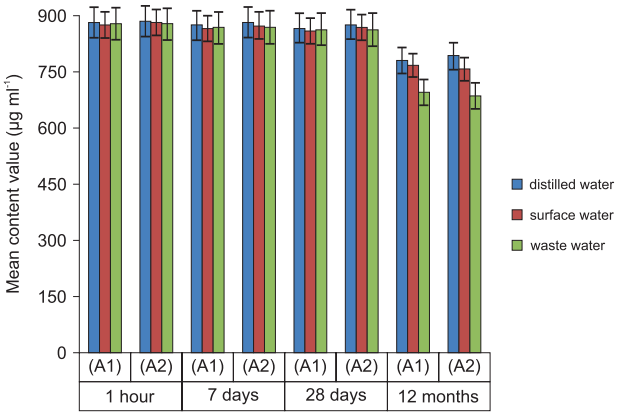

Figure 5

Mean content values for bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 (A1) and chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxystilbazole-4 (A2) in the distilled, surface and waste water samples after 1 hour, 7 days, 28 days and 12 months, obtained in the naphthylpropyl column

and adsorption. The quality and efficiency of the preservation process is assessed by estimating the loss of the preservative after the preparation is purposely tainted (Regulation 2013). This was also the method used in the present study. The results for the above criteria are directly proportional to the level of contamination. Figure 5 below presents the mean content of bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 (A1) and chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxystilbazole-4 (A2) in the analysed samples of distilled, surface and waste water after 1 hour, 7 days, 28 days and 12 months; the results were obtained in the naphthylpropyl column.

The preservative properties of the preparation are considered adequate if, after a specified time (28 days in the case of the presented study), there is no significant decrease in the preservative content in the microbe-infected medium kept at a specified temperature under the study conditions (Kartoglu et al. 2010). As shown in Figure 5, practically no differences were observed after 1 hour as regards the content of the derivatives, irrespective of the matrix type. The actual difference of merely a few micrograms per millilitre between respective matrices was within the allowable error tolerance.

Furthermore, after 7 and 28 days, the analysed derivatives were characterized by a very high level of stability and a negligible content decrease in all matrix types. For the 28-day period, the content decrease of the (A1) compound in distilled water amounted to 131.9 µg ml^{-1}, i.e. 13.19% in proportion to the purposeful contamination of the matrix at 1000 µg ml^{-1}; 139.3 µg ml^{-1}, i.e. 13.93%, in surface water; and 135.7 µg ml^{-1}, i.e. 13.57%, in waste water. Similar results were obtained for the (A2) compound: after 28 days, the content decrease in distilled water amounted to 123.1 µg ml^{-1}, i.e. 12.31%; 129.0 µg ml^{-1}, i.e. 12.90%, in surface water; and 136.7 µg ml^{-1}, i.e. 13.67%, in waste water.

A slightly larger content decline was observed in both analysed derivatives after 12 months. For the (A1) compound and the distilled water matrix, the difference between content values after 1 hour and those obtained after 12 months amounted to 218.4 µg ml^{-1}. This is only 21.84% in proportion to the purposeful contamination of the matrix at 1000 µg ml^{-1}. Similar results were obtained for the (A2) compound: the difference of 206.6 µg ml^{-1} and a decrease of 20.66%. Slightly larger differences after the 12-month period were observed for other matrices. In surface water, this difference was 231.9 µg ml^{-1} for the (A1) compound and 241.1 µg ml^{-1} for the (A2) compound. Whereas in waste water, the decrease in proportion to the purposeful contamination of 1000 µg ml^{-1} amounted to 30.41% for the (A1) compound and 31.33% for the (A2) compound.

In conclusion, none of the chemical compounds discovered so far meets all the demands related to preservatives, antiseptics and disinfectants. Each of the chemicals currently in use has its own limitations, which is why those with a potential for synergistic action are often used in combination with each other. The two (E)-azastilbene derivatives may be safely added to the list of such chemical compounds, as demonstrated by the study presented in this paper. The analyzed derivatives can also be used as antiseptics and disinfectants.

During the analysis of the results obtained, it was noticed that the HPLC technique is characterized by high recovery, and small standard deviation analysis (Figs 4 and 5). After marking the standard deviation (for n = 5), the limit of detection was calculated, and it was equal to the value of triple deviation. Next, the limit of determination was calculated, and it was equal to the threefold value of the detection limit. The linearity did not exceed the range of 5–300 µg ml^{-1}, the detection limit reached 0.150 µg ml^{-1}, and the correlation coefficient was equal to 0.9999 (Table 2).

Conclusions

This study allowed to develop an extraction procedure for two (E)-azastilbene derivatives, namely bromide of

(E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxystilbazole-4, from different water matrices. The highest recovery values (amounting to approx. 95%) were obtained in the extraction column with the chemically bonded naphthylpropyl stationary phase. The linearity ranged from 5 to 100 μg ml^{-1}. The limits of detection of the analyzed derivatives were similar and amounted to about 0.150 μg ml^{-1}. The limits of quantification were 0.457 for bromide of (E)-N-(o-bromobenzyl)-4′-hydroxystilbazole-4 and 0.473 for chloride of (E)-N-(p-chlorobenzyl)-4′-hydroxystilbazole-4, respectively. The developed extraction procedure and the stability results will certainly allow for utilizing (E)-azastilbenes as a preservative component in a variety of aqueous solutions or as antiseptic and disinfectant substances.

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