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ANALYTICAL CHEMISTRY

Determination of Xenobiotic Imidacloprid Content in Surface Waters

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Abstract—Surface water is contaminated by various xenobiotics. Using standardized methods, the content of organochlorine pesticides that are environmentally stable, migrate along food chains and can accumulate in dangerous amounts in living organisms is monitored. In this case, the insecticide imidacloprid is not controlled, however, its ingress into surface and ground waters is not allowed. Seasonal detection of imidacloprid in fish tissues may indicate a violation of regulations for working with imidacloprid preparations. To ensure the necessary preventive regulation and to protect surface water from imidacloprid contamination, a methodology is needed for laboratory monitoring of the general sanitary level of imidacloprid in surface waters. In this work, imidacloprid extracts were obtained based on the liquid-liquid extraction method, the optimal conditions for the quantitative analysis of imidacloprid were determined using high-performance liquid chromatography with a spectrophotometric (diode array detector) detector (HPLC/DAD). The limit of detection of the imidacloprid, which was reached in the work, is 0.15 μg/mL, the limit of quantification is 0.7 μg/mL. Taking into account preliminary concentration (100 times), the lower limit of imidacloprid detection in water is 0.0015 mg/L, the range of determined contents is 0.007–0.028 mg/L. The developed method is simple to perform, allows us to measure the imidacloprid content in one water sample by 90 minutes. The method can be used for monitoring studies of the imidacloprid content in surface water, to collect the water pollution data and forecast its impact.

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INTRODUCTION

Water bodies of agricultural purposes are multifunctional objects in the production cycle of agricultural products. Xenobiotics of the group of pesticides may get into water bodies and pollute them while performing several agrotechnological tasks. The monitoring of water quality for their presence practically is absent except for the analysis of chlorine-containing resistant pollutants [1]. One of potentially dangerous technogenic water pollutant is 4,5-dihydro-N-nitro-1[(6-chlor-piridid)-methyl]-imidasolidin-2-ileamine known as imidacloprid acting in plant protection, sanitary, domestic and medical disinfection. However, imidacloprid is highly toxic neuro-paralitic poison, possess a destructive effect on insects (potato beetle, fleas, locusts, bugs, etc.), negatively affect aquatic organisms and birds. It is widely used in agroproduction and its content is normalized and controlled in food products, which are exported to the European Union countries [2]. Certificates of plant protection based on imidacloprid according to Order EC No. 1907/2006 indicate that when operating with the given pesticide one should avoid its getting into any surface and underground waters as well as into a complex of facilities of the system of water disposal. Despite this, imidacloprid is found in the internal organs of various fish. Detection of imidacloprid in fish tissues is seasonal, maximum levels were recorded in fish liver caught in spring and autumn [3, 4]. Identification of residual quantities of imidacloprid in fish tissues is perhaps stipulated by a violation of orders of safety work with preparations on its basis. For laboratory monitoring, methods of residual amounts of pesticides in surface waters have been established by regulatory documents [5, 6]. The method of identification of pesticides in water is based on their extraction from water by n-hexane, purification of the extract by concentrated sulfuric acid followed by quantitative determination of pesticides by the method of gas-liquid chromatography with a detector of electronic catch [5]. The analysis of residual amounts of pesticides in food products, forage, and samples from the environment is based on the removal of pesticides from the sample by an organic solvent, purification of the extract and determination the

substance content by the method of gas-liquid chromatography with the detector of the electronic catch [6]. These methods are technically obsolete, require long preparation of the samples and large costs.

One of the modern methods of analysis is high-performance liquid chromatography (HPLC) with ultraviolet detection at 270 nm with a movable phase (MP) of acetonitrile/water mixture (20 : 80). For fulfilling measurements, a substance to be analyzed is extracted by the method of solid-phase extraction (SPE) and by the method of liquid extraction [7]. The detection level (DL) constituting 0.5 mg/dm^3 was reached when SPE was used.

The quantitative analysis of the content of imidacloprid in water was conducted by the method of capillary electrophoresis [8]. In this case, satisfactory indicators were obtained characterizing the stage of sample preparation for analysis: completeness of imidacloprid extraction from river water was 81.33% and from lake water was 82.30%, whereas DL of imidacloprid was 0.005, and the range of concentrations being determined was 0.005–0.2 mg/dm^3.

Since maximum acceptable concentrations (MAC) of imidacloprid in water constitutes 0.007 mg/dm^3 (generally sanitary level) [1], the search for optimal conditions of performing the laboratory monitoring of its content in water samples employing modern measuring equipment remains relevant.

The objective of the present research is the development of techniques and studies of conditions for fulfilling laboratory monitoring of the accepted sanitary level of imidacloprid in surface waters by the method of high-performance liquid chromatography (HPLC) with a diode–matrix detector.

EXPERIMENTAL

Model systems based on deionized water have been used. Water samples of water bodies of agricultural purposes were enriched by solutions of the analytical standard of imidacloprid in acetonitrile manufactured by Sigma-Aldrich. Solvents and reagents for qualification were of "for chromatography" chemical grade, methanol, acetic acid, acetonitrile, deionized water, sodium chloride, sodium acetate, sodium citrate, and magnesium sulfate were of "pure for analysis" chemical grade.

Methanol, acetonitrile, deionized water and acetic acid were used in chromatography for creating MP. Acetonitrile was used for MP components and also as an agent for releasing imidacloprid from water sample that was preliminary mixed with salts of sodium acetate and sodium citrate. Sodium chloride and magnesium sulfate were put to the extraction system for enhancing the desalination of acetonitrile from the acetonitrile/water mixture. The introduction of salts results in the determination of the extraction system and allows us to separate the organic layer containing xenobiotics [9]. The extracts of imidacloprid were obtained by the method of liquid-liquid extraction with the ratio of components in the acetonitrile: aqueous solution of salts corresponded to 1 : 10. The extract (the whole organic layer of the volume 10 cm^3) was evaporated in a rotor evaporator of grade RV05 basic 2-B 1KA to 1.0–0.1cm^3, in some cases the concentration up to 1.0–0.1 cm^3 was achieved for the united extracts obtained from several prepared in parallel laboratory water samples. The prepared extract was analyzed by the method of HPLC with the spectrophotometric (diode–matrix detector (HPLC/DAD) on the HPLC UltiMate 3000 Dionex device equipped with a precolumn and column Thermo Scientific Hypersil GOLD CI8. In chromatographic control they the mode of isocratic elution by the mixture of solvents methanol–acetonitrile–deionized water–acetic acid. The control over the process of chromatographic analysis of imidacloprid extracted from samples was done with the use of the imidacloprid standard. The hold-up time, height (H) and area of signals (S) were compared between a standard solution and the extract of the water sample.

RESULTS AND DISCUSSION

At present, the monitoring of the content of residual amounts of imidacloprid in food products of the agroindustrial complex and plant is performed by using the liquid chromatography methods [7, 9, 10] with various types of detectors. Measurement is carried out by means o spectrophotometric and mass-spectrometric detectors recording at different modes of the detector operation the area or the height of the signal being analyzed obtained on the chromatogram. The most used wavelengths of the operation of the diode–matrix detector constitute 195, 230, 255, and 297 nm. The choice of the wavelength depends on the quality and quantity composition of solutions and obtained as a result of the fulfilled stage of sample preparation. Usually, these solutions are complex and contain besides imidacloprid other chemical substances that could affect the process of the chromatographic analysis, especially if they have similar to imidacloprid characteristics such as holding time and the degree of absorption in the ultraviolet spectrum.

Primary acetonitrile extracts of water xenobiotics contained only a trace amount of imidacloprid. Therefore the extracts were further subjected to 10–20-fold concentration. The quantitative analysis of the extract composition was carried out by measuring the area (S) or height (H) of the peak at different wavelengths. A comparison of chromatograms recorded at different wavelength (Fig. 1) enables reliable identification of imidacloprid. Comparing chromatograms of the extracts one may note the differences in H and S. Thus, imidalcloprid peak identified at 195 nm is characterized by the greatest in the series of $S_{peak} = 0.53 \pm 0.05$ mAU·min, $H_{peak} = 1.9 \pm 0.2$ mAU and accordingly $S_{peak} = 0.46 \pm 0.01$ mAU·min, $H_{peak} = 1.6 \pm 0.1$ mAU. The parameters of the analytical signal obtained at 225 nm ($S_{peak} = 13.7 \pm 0.5$ mAU·min, $H_{peak} = 60 \pm 3$ mAU), are much higher than such obtained at 230 and 297 nm, but lower than those obtained studied wavelengths, indicators: $S_{peak} = 227.9 \pm 0.3$ mAU·min, $H_{peak} = 141 \pm 7$ mAU, while the peak of imidacloprid identified at 230 and 297 nm, by the smallest: in the mode of operation of the detector at 195 nm. In this case, the noise value in all tested conditions had an inessential impact on the value of S and H. The minimal influence of noise was recorded for the system at 195 nm and constituted 0.15 of imidacloprid DL, the limit of quantitative determination was 0.17 mg/cm^3. With the account of the conducted preliminary concentration of imidacloprid (by 100 times) the lower boundary of determining the given xenobiotic in water constituted 0.0015 mg/dm^3, whereas the range of concentrations was 0.007–0.028 mg/dm^3. Comparing the value of established limits of

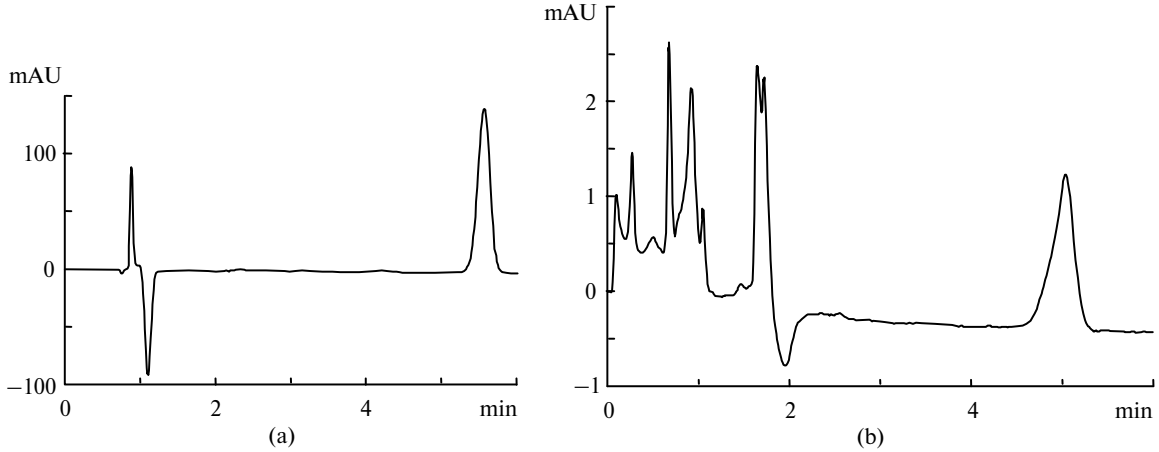

Fig. 1. Chromatogram of an extract of surface water, enriched by imidacloprid in concentration 2.8 mg/cm^3 at wave-lengths: a—195, b—297 nm.

imidacloprid determination with the value of its MAC in water (0.007 mg/dm^3) we conclude that the proposed method can be used to perform quantitative determination of imidacloprid in water at the level of its MAC and also to explore trace amounts of imidacloprid below the established value of MAC [1].

The following stage of estimating the ability of the system to fulfill the measuring of imidacloprid in water samples become the analysis of graduation dependencies of the system's response from the concentration of the substance being analyzed in the working solution (Fig. 2).

It was demonstrated that at the wavelength of 195 nm the intensity of the signal of imidacloprid is higher than at other wavelengths. The value of the respond parameter of the system when measuring the concentration of imidacloprid is described by the equation $y = 9.4x + 1.2$. The obtained two graduation dependencies have a linear nature. Analyzing and comparing the values of the coefficients of the slope of each of them one may see that at the different wavelength the detector sensitivity is not identical. The lowest sensitivity of the method was observed at wavelengths 230 and 297 nm. The dependence of the concentration of the substance being analyzed on the area of the analytical signal (S) at 230 nm is described by the equation $y = 0.20x - 0.01$ while at 297 nm respectively $y = 0.18x - 0.04$. The sensitivity achieved at 255 nm is much higher than at abovementioned wavelengths, however, it was nearly 6.5 times lower than the sensitivity achieved at 195 nm. At 255 nm the dependence of the concentration of the substance being analyzed on the area of the analytical signal is described by the equation $y = 4.3x - 0.7$. Taking into account the necessity to detect trace amounts of imidacloprid in water samples, it is recommended to use the most sensitive detection at 195 nm wavelength.

The linearity of the signal was set within the range from 0.5 to 4 MAC, the error of determination was estimated via the relative standard deviation (S) (table).

The values given in the Table characterize the completeness of imidacloprid extraction. The extraction of imidacloprid is most fully achieved from the model systems based on deionized water and constitutes 91.4–100%. The decline in the imidacloprid extraction rate (up to 80.0–96.4%) occurs while using the experimental water samples. Such drop in the extraction efficiency could be explained, most likely, by the presence in the experimental water samples of components capable of binding to imidacloprid and preventing its extraction into the organic layer. According to Directive 2002/657/EC concerning expected content of pesticide in the object being analyzed the degree of its removal within the range from 50 to 100% may be considered satisfac-

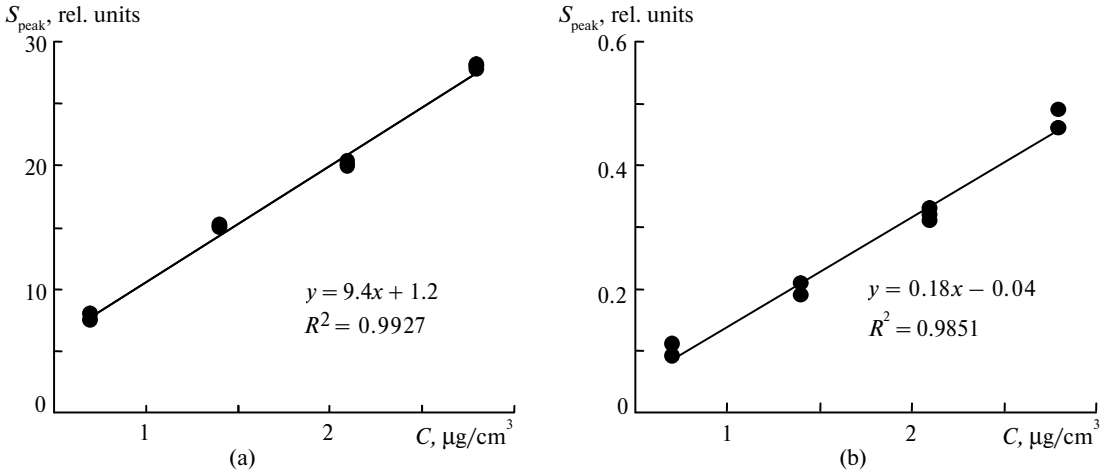

Fig. 2. Graduation dependence of the system's response at wavelengths: 185 (a) and 297 nm (b) of the concentration of imidacloprid in the standard working solution.

tory. For residual quantities of pesticides, larger or equal to 10 mg/kg, the average value of the removal percentage measured in three parallel determinations should be in the range 80–110%. Since in our study the extraction efficiency of imidacloprid from the samples of surface water constituted 80 to 97%, the developed method of sample preparation by the mean of the liquid-liquid extraction is considered as satisfactory.

Determination of imidacloprid and characteristic of measuring error ($n = 3$; $P = 0.95$)

Water	Introduced	Determined	Removed	S_r
	mg/dm^3		%	
Deionized	0.0035	0.0032	91.4	4.7
	0.007	0.0068	98.6	2.5
	0.009	0.009	100	2.5
	0.014	0.0139	99.3	2.1
	0.028	0.028	100	2.0
Surface	0.0035	0.0028	80.0	9.0
	0.007	0.0068	97.1	7.2
	0.009	0.0084	93.3	5.9
	0.014	0.0135	96.4	5.8
	0.028	0.027	97.0	5.4

CONCLUSIONS

Thus, residual amounts of imidacloprid in water bodies of agricultural design can be controlled by the methods of high- performance liquid chromatography with the diode-matrix detector. The extraction of this xenobiotic should be carried out by acetonitrile using the water sample after it had been mixed with sodium and magnesium salts at the ratio of the volumes of the sample and acetonitrile 10 : 1. For analysis of trace amounts of imidacloprid such acetonitrile extracts should be further concentrated. The time of the analysis of one water sample constitutes 90 min.

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