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**METHODOLOGICAL APPROACHES TO ENVIROMENTAL
AND ANALYTICAL DETERMINATION OF PERSISTENT ORGANIC
COMPOUNDS IN WATER BODIES***

SUMMARY. Methodological approaches to determine organic contaminants by gas liquid chromatography with electron-capture and mass-selective detection are developed. These approaches help to analyse α -, β -, γ -, δ -HCCH, DDD, DDT, DDE, aldrin, hexachlorobenzene, heptachlor, and other chlorinated pesticides in a single experiment, which significantly increases analysis accuracy. On the basis of the developed methods, the research to determine trace elements of semi-volatile organochlorine compounds in the waters of the Tyumen Region is conducted. Concentrating of organochlorine pesticides is carried out by solid-phase extraction (SPE) using the automated SPE instrument, Dionex Auto Trace 280 system. The concentrating degree is more than 1,000. The pesticide concentrations in the waters are determined in the final extracts by chromatography-mass spectrometry with mass-selective detection in selected ion monitoring (SIM) mode. The concentrations of the individual components in the waters do not exceed the maximum allowable concentration (MAC). Organochlorine pesticides, as well as dioxin-like substances, for example, polychlorinated biphenyl, are determined by the developed methods.

KEY WORDS. Organochlorine pesticides, natural water, solid phase extraction, determination, gas chromatography/ mass spectrometry (GC / MS).

Introduction. Global environmental pollution and unfavorable ecological situation in industrial areas require environmental and analytical monitoring of air pollution, drinkable and surface water quality and hazardous compound accumulation in soils and sediments.

All over the world, much attention is being paid to environmental and analytical monitoring of supertoxic pollutants, such as organochlorine pesticides (OCP), polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF). Background levels of supertoxic pollutants in air, precipitation (rain, snow), surface waters, soil, sediments, and biota have been analyzed and summarized in many regions [1-2].

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In Russia, most analytical methods for OCP determination are based on gas chromatography with electron-capture detection (GC / ECD) [3-4]. But the combination of gas chromatography and mass spectrometry (GC / MS) is now the most accurate method for trace determination analysis of OCP, PCB, PCDD, PCDF, and other persistent organochlorine compounds.

According to international agreements, about 60 chemicals were included in restricted substance lists. A group of 12 chemicals, known as «dirty dozen», are prioritized by the Stockholm Convention on Persistent Organic Pollutants (POPs); and at present, the POPs list has been significantly enlarged. The POPs list includes nine organochlorine pesticides: aldrin, endrin, dieldrin, mirex, DDT, hexachlorobenzene, heptachlor, toxaphene, chlordane; industrial chemicals: PCB, as well as unintentionally produced PCDD and PCDF. These compounds are resistant to environmental degradation through photolytic, chemical, and biological processes; and they are capable of being easily transported in the environment.

Environmental monitoring data on POPs concentrations in the waters of the Tyumen Region (from forest-steppe to tundra) are very poor. There are no analytical data obtained by high-performance and highly informative physicochemical methods, such as GC / MS.

The number of standardized environmental indicators is constantly increasing. At the same time, environmental toxicant standards are being tightened up, which in turn requires the development of new methods and measurement techniques for accurate and reliable trace determination analysis of compounds, hazardous for humans and biota. This problem calls for new highly sensitive, selective and reliable gas chromatography-mass spectrometry devices [5-7].

It is obvious that the development of an effective environmental monitoring system is becoming a more complex and multifaceted problem. Thereby, the integration of the existing methods for toxic compound determination analysis (in concentrations at ng/dm³), which will provide the most complete information on sample contaminations (in waters, sediments, biota) for making effective and valid environmental decisions is of current scientific importance.

The purpose of this research was to identify and determine background levels of OCP and PCB concentrations in the waters of the Tyumen Region by GC and GC/MS.

The data and methods of the research. The research was concerned with the waters of the small lakes in the Tyumen Region (in different natural zones) located in a considerable distance from built-up areas, as well as the small lakes located in potentially contaminated areas. Lake-water sampling was carried out in August, 2011. In the summer of 2012, surface water samples were collected from the lakes located close to man-impacted areas. A total of 34 lakes were investigated, including 10 lakes located in a distance from built-up areas and 24 lakes located in potentially contaminated areas.

Methods for OCP extraction from natural waters

To obtain the concentrates of the trace elements of organic compounds from the lake-water samples, liquid-liquid extraction (LLE) and solid phase extraction (SPE) using *Diapak C16* cartridges (extraction in field conditions) and *DIONEX SolEx C18* cartridges (concentration in a laboratory using the automated SPE *AutoTrace SPE — 280* system) were applied. The extracts passed through a column

of anhydrous sodium sulfate, which was then washed out with a pure organic solvent (or a solvent mixture). The solvents were removed from the combined extracts using a rotary evaporator under vacuum at a temperature not exceeding 45°C. Re-extraction of the organic compounds, remaining after evaporation of the solvents in the form of solids or oils, was conducted with a 1:1 mixture of ethylacetate and dichloromethane, the volume of 1 ml. In some cases, the concentrates were additionally purified.

The resulting concentrated extracts were used to identify and determine the OCP by GC (ECD) and GC / MS in total ion current (TIC) and selected ion monitoring (SIM) modes.

GC and GC / MS determination of OCP in natural waters

The resulting concentrated organic extracts were investigated by GC using a gas chromatograph *Kristall 2000M* with ECD (JSC SDB *Chromatek*, Russia) and a gas chromatography-mass spectrometer *Trace GC Ultra* with a quadrupole analyzer *DSQ II* (*ThermoElectron* Corporation, USA).

Mass spectra were obtained by electron ionization at 70 eV. The chromatographic separation was accomplished on a Thermo TR-5MS capillary column (30 m x 0.25 mm i.d x 0.25 µm film thickness).

The chromatographic conditions (GC / MS) were the following: step-wise changes of the thermostat temperature were 40°C (5 min), 30°C / min to 160°C, 160°C (1 min), 10°C / min to 280°C, 280°C (15 min). The evaporator temperature was 250°C. The transfer line temperature was 250°C. The carrier gas flow rate was 1 ml · min⁻¹. An aliquot of the extract solution (1-3 µl) was injected into the evaporator of the chromatograph in *splitless* mode (1 min purge delay). To determine the chlorinated pesticides, except DDE, DDT, the fragment ions at *m/z* 237, 239, typical for all identified pesticides, were registered. In the cases, when it was impossible to separate some components by chromatographic resolution, the fragment ion analysis for each compound was reapplied.

The chromatographic analysis with electron-capture detection (GC / ECD) was carried out on a HP-FFAP capillary column (50 m x 0.32 mm i.d. x 0.5 µm film thickness). The separation conditions (GC / ECD) were the following: the changes of the temperature were 150°C (2 min. hold), heating to 240°C for 12 min., then to 290°C for 3.5 min., hold until elution complete. The carrier gas (helium) flow rate was 1 ml · min⁻¹. An aliquot of the extract solution (1.3 µl) was injected into the evaporator of the chromatograph in *splitless* mode (1 min purge delay).

To calibrate the instruments, the OCP calibration solutions were prepared from the solutions of State Standard Sample (GSO) pesticides by dilution in accordance with GOST 51209-98 (Russian State Standard). The OCP standard solution was serially diluted to make a series of the calibration solutions with the concentrations: α-, β-, γ-, δ-HCCH, 4,4'-DDD, 4,4'-DDT, 4,4'-DDE, aldrin, hexachloro-benzene — 0.025, 0.05, 0.1, 0.5, 1.0 mcg/ml; heptachlor — 0.01, 0.02, 0.1, 0.2, 0.5 mcg/ml. The massdetector calibration was carried out by the calibration solutions with the concentrations: α-, β-, γ-, δ-HCCH, 4,4'-DDD, 4,4'-DDT, 4,4'-DDE, aldrin, hexachlorobenzene — 0.02, 0.1, and 1.0 mcg/ml; heptachlor — 0.01, 0.1, and 0.5 mcg/ml. To obtain the calibration characteristics, each solution was chromatographed at least three times. Chromatogram processing was performed using the software (*Chromatec Analytic* (GC / ECD) and *Xkalibur* (GC / MS)).

Results and discussion. The procedure for the trace determination of toxic pollutants in the environment includes, as a rule, the two main stages:

1) sample pre-processing, concentrating of trace elements, sample separation of substances into groups, etc.;

2) instrumental analysis, including chromatographic separation of components, their identification and quantification [8].

In relation to the requirements for water quality monitoring results, two major approaches to environmental analysis can also be distinguished. The basic principle of the first approach is to analyze the pollutant or pollutant group for further detection and quantification, so-called «target compound analysis». It is implemented in the existing formal methods [3], [4], [9], [10].

Modern analytical instrumental base allows the second approach of a comprehensive environmental study, so-called «unknown composition sample analysis», to be implemented. It requires studying **qualitative composition of pollutants** and further assessing of the concentrations of identified toxic compounds [8].

As super toxicants (OCP, PCB) are characterized by many similar physical and chemical properties, they can be determined in the same analytical procedure. The comprehensive method of gas chromatography with mass-selective detection (GC / MS), as one of the most informative modern methods of physicochemical analysis, allows all the compounds to be determined with the required sensitivity and selectivity. Because of the differences in the molecular structures of the compounds mentioned, sample preparation, as the most time consuming and cost-based process that results in analyzable forms of extracts, is mostly difficult to be integrated.

Liquid-liquid extraction (LLE) is a classical method to extract pesticides from water samples. The extraction of water samples of 500-1000 ml is usually repeated several times in a separatory funnel with a suitable solvent [3], [4], [9], [10].

When operating with liquid samples and initial extracts, the classical methods of sample preparation can be replaced by more convenient ones, for example, solid-phase extraction (SPE). SPE allows the analytes to be isolated from a liquid sample or extracts by concentrating on a small amount of an absorbent material. The analytes are washed off with a minimal volume of a solvent. It gives an opportunity either to analyze the concentrate immediately, or to concentrate the sample additionally by the solvent evaporation with an inert gas [11-12].

Solid-phase extraction was carried out in the laboratory using concentrating *DIONEX SolEx C18* cartridges on the automated SPE *AutoTrace SPE-280* system (*Dionex* Company, USA).

Identification of pesticides by GC

GC with ECD allows volatile (isomers of HCCH) and semi-volatile pesticides (metabolites of DDT) to be identified in environmental samples.

Absolute retention time is usually used for component identification in a chromatogram. To determine the absolute retention times of individual compounds, we analyzed the calibration solutions of the pesticides ($n = 5$). Table 1 shows the absolute retention time of the pesticides on a *HP-FFAP* capillary column, the values of which were used for their identification in the real samples.

Table 1

The absolute retention time of the organochlorine pesticides

Name of OCP	Retention time, min
α -HCCH	15:50
β -HCCH	16:32
γ -HCCH	17:13
4-4'-DDT	27:39
4-4'-DDE	29:15
4-4'-DDD	31:07

Determination of herbicides by GC / ECD

The chromatography of the pesticide calibration solutions resulted in linear relationships between the peak areas of the components and their concentrations in the solutions (correlation coefficient $R^2 \geq 0.98$ for all compounds).

The chromatographic analysis of the Kuchak Lake surface waters demonstrated that the surface waters did not contain any components with the retention time presented in Table 1. Some components, such as α -HCCH, γ -HCCH, 4,4'- DDT, were added to the water samples (component concentrations were 0.35, 0.35, and 0.25 mg/L, respectively). The chromatograms of the initial water sample extracts and the water sample extracts with the pesticide additives are shown in Fig. 1. The pesticide peaks with the corresponding retention times of the additive pesticides appeared in the chromatogram of the water sample extracts with the additive components. Table 2 shows the results of the quantitative analysis for the water samples with the additive components. The calculated concentrations do not exceed 5% of the concentrations of the additives, that are in the permissible error range of the method.

We can conclude that the resulting mathematical correlations can be used to determine organochlorine pesticides in surface water and drinking water samples.

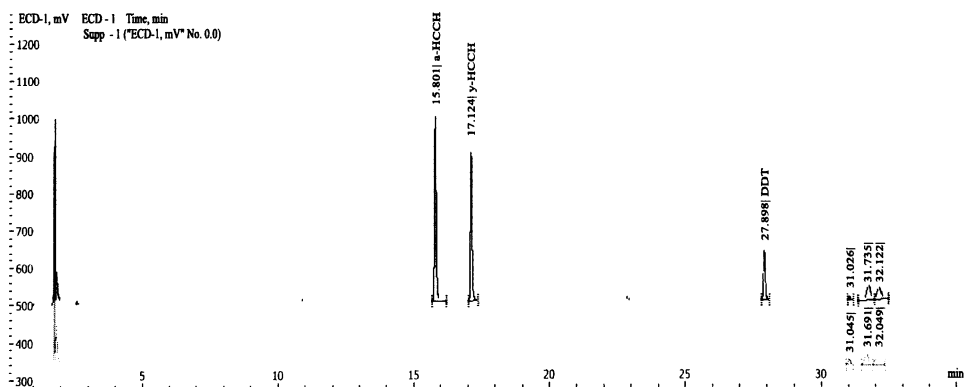


Fig. 1. The chromatograms of the initial water sample extracts (the bottom chromatogram) and the water sample extracts with the pesticide additives (the top chromatogram)

Table 2

**The results of the chromatographic analysis for the water samples
with the additive components (n=2)**

Additive component	Component concentration, mcg/l	Peak area	Peak height	Calculated concentration, µg/l
α -HCCH	0.35	2055.010	495.202	0.348
γ -HCCH	0.35	1602.164	397.543	0.335
4-4'-DDT	0.25	569.268	131.774	0.238

The main objective of the sample preparation is to achieve the most complete separation of target compounds from matrix components. The necessity to determine the trace amounts of analytes against the background of substances present in the matrix in much higher concentrations makes the POPs analysis very complicated.

The analysis of OCP extracts by GC / ECD does not often allow reliable identification of components without special procedures (addition method, determination of retention indices). The most informative method is GC / MS. Special mass spectral libraries, for example, NIST, Willey, special pesticide libraries are used for identification of unknown components.

Identification of pesticides by GC-MS

The chromatograms of the OCP calibration solutions (Fig. 2, 3) were obtained by GC / MS in total ion current (TIC) and selective ion monitoring (SIM) modes. With high probability (97-99%), the components of the pesticide solutions were identified by comparing the experimental mass spectra with the mass spectra of the NIST library.

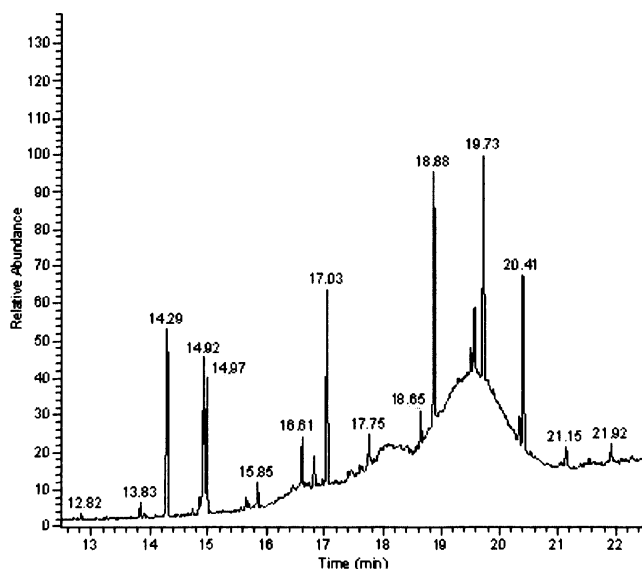


Fig. 2. The chromatogram of the calibration solution No. 2 in TIC mode

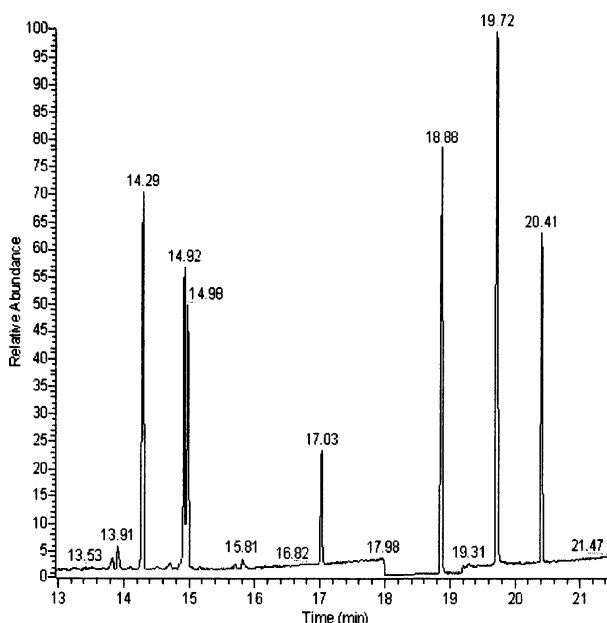


Fig. 3. The chromatogram of the calibration solution No. 2 in SIM mode

In the calibration solutions and in the extracts isolated from the real objects, we detected the interfering effect of the background compounds remaining after concentrates purification. TIC chromatograms in SIM mode were registered using the characteristic ions (Table 3), which were absent as background ions. The selection of the characteristic ions for the detection is performed in detail in [13].

Table 3

The characteristic ions for the OCP identification and determination in SIM mode

Group of ions	Pesticide	Characteristic ions at m/z		
		Group I	Group II	Group III
1	α -, β -, γ -, δ - HCCH	109,181, 219	109, 181, 183	109, 181, 183
2	aldrin	101, 263	101, 263	66, 220,263
3	hexachlorobenzene	284,286	282,284,286	214,249,282.286
4	4-4'-DDD, 4-4'-DDE	272, 387	272,387	272,287,422
5	4-4'-DDT	237	237	237

Thus, we can state that different ions in different chromatogram windows can be used for the same compound. The ions of all identified compounds are registered in each window with a narrow mass range providing maximum sensitivity and reproducibility.

Except retention time, pesticides can be identified on the bases of characteristic ions intensity ($I_{m/z 1} / I_{m/z 2} / I_{m/z 3}$). In most cases, having properly configured

devices and low background effects, pesticides can be identified by TIC in SCAN and SIM modes with a high probability using the library search.

The typical TIC chromatogram of the OCP concentrate, isolated from the lake Kuchak water samples with the additive of γ -HCCH, 4,4'-DDE and 4,4'-DDT, in SIM mode is presented in Fig. 4 (additive concentrations are 0.05, 0.025, 0.05 mcg/L, respectively).

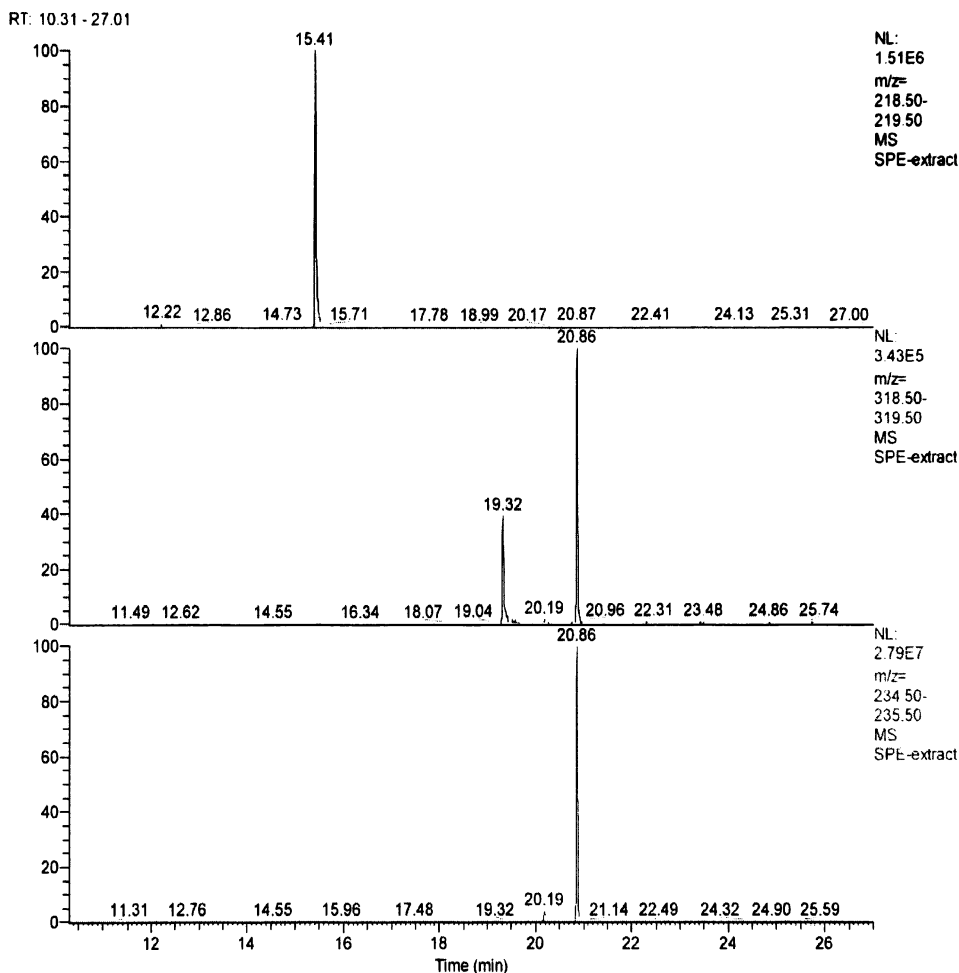


Fig. 4. The TIC chromatogram of the OCP concentrate, isolated from the Kuchak Lake water samples with the additive of γ -HCCH, 4,4'-DDE and 4,4'-DDT, in SIM mode

Calibration of gas chromatography-mass spectrometer (GC / MS)

After the analysis of the calibration solutions, relationships between peak areas and component concentrations for the herbicides were stated in the two detection modes. In Fig. 5, as an example, the calibration curves for γ -HCCH and 4,4'-DDT in TIC and SIM modes are shown.

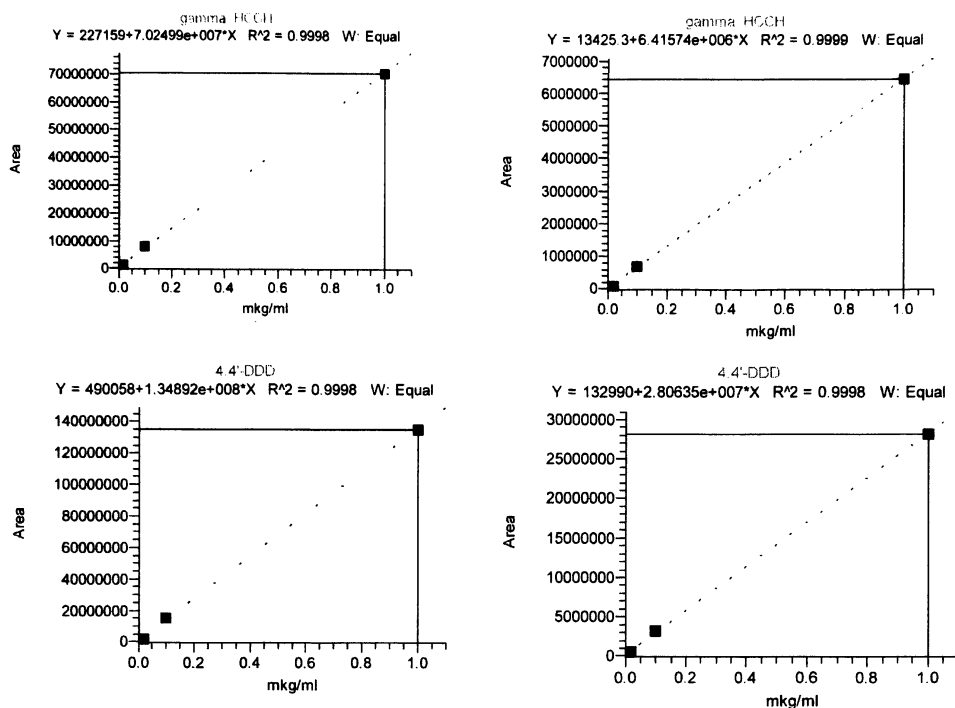


Fig. 5. The calibration curves in TIC and SIM modes for γ -HCHH (the top graphs) and for 4,4'-DDD (the bottom graphs)

In all calibration curves graphs, the standard deviation is close to 1, which allows us to use the obtained graphs for the further quantitative determination of the organochlorine pesticides in the surface water samples by GC / MS.

Verification of pesticides in SIM mode

To verify the accuracy of selected ion monitoring (SIM), a certified hexachlorobenzene (HCB) sample* was analyzed in hexane with the certified concentration value of 0.1 $\mu\text{g}/\text{ml}$. The chromatograms of the certified HCB sample in TIC and SIM detection modes are shown in Fig. 6 and 7, respectively.

In TIC detection mode, the HCB signal with the retention time of 11.49 min. is weak against the background of other components (Fig. 5). In SIM detection mode, the HCB signal significantly increases (Fig. 6). Therefore, it is possible to calculate the concentration of HCB in peak height or peak area. Three combining determination analyses of the certified solution concentrations revealed an average HCB concentration, which proved to be 0.10 mg/ml. The resulting value coincided with the certified concentration one. It leads to the conclusion that SIM detection mode is reliable.

* Our special gratitude to A.P. Bagdasarova, the head of the laboratory in the Centre of Hygiene and Epidemiology in the Yamal-Nenets Autonomous Okrug in Noviy Urengoy, for the certified HCB sample.

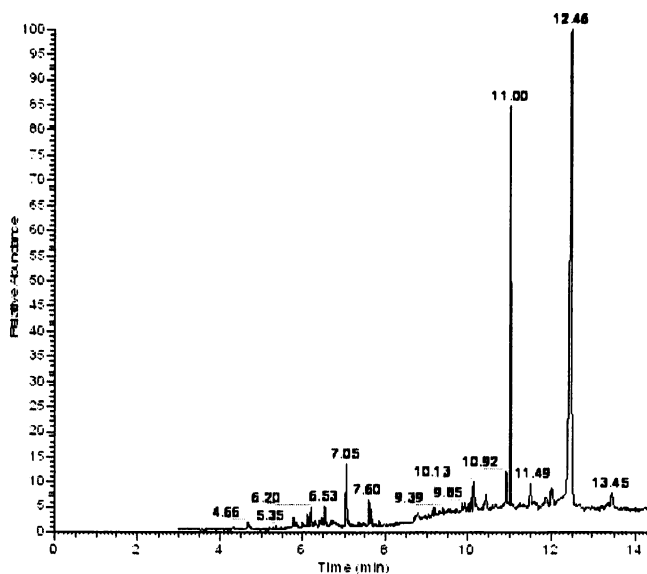


Fig. 6. The chromatogram of the certified HCB sample in TIC mode

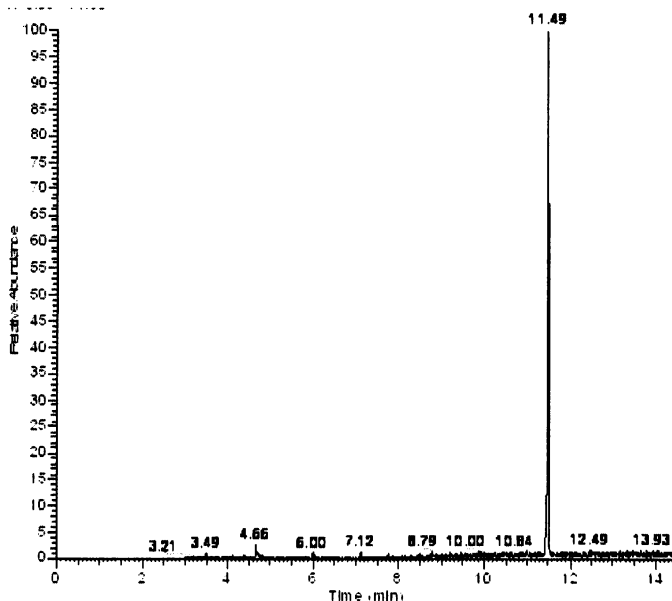


Fig. 7. The chromatogram of the certified HCB sample in SIM mode

Analysis of water samples by GC-MS

The pesticide concentrations were determined by the calibration curves (functions), distinguished on the basis of the characteristic ions with maximum intensities of the ion current at m/z : 181 (α -, β -, γ -, δ -HCCH), 246 (4.4'- DDE) 235 (4.4'- DDD, 4.4'- DDT), 284 (HCB), 263 (aldrin).

Taking into account linearity of calibration curves, the concentration of a certain pesticide in a water sample can be calculated by the formula:

$$C_{xi} = S_{xi} \cdot (C_{oi} / S_{oi}) \cdot (1 / k),$$

where: S_{oi} and S_{xi} are peak areas of the individual pesticides in a chromatogram in a calibration mixture and a mixture isolated from a real object, respectively; C_{oi} is a pesticide concentration in a calibration mixture; C_{xi} is a pesticide concentration in a mixture isolated from a real object; $k = V_{ws} / V_{concent}$ is the concentration coefficient that is equal to the ratio of the water sample volume to the concentrate volume.

The results of the OCP determination in the final extract obtained from the surface waters, showed that all water bodies were not polluted by the following substances: α -, β -, γ -, δ -HCCH, 4,4'-DDD, 4,4'-DDT, 4,4'-DDE, aldrin, heptachlor, hexachlorobenzene.

Resume. The research on the concentrating of the organochlorine pesticides from the surface waters by solid-phase extraction was conducted. The mass-selective detection of OCP with their gas chromatography-mass spectrometric determination by TIC in SIM mode to eliminate the interfering effects of the matrix components was studied. We can state that in the period of the analysis, the small lakes of the Tyumen region, located in a distance from built-up areas, did not contain the organochlorine pesticides: α -, β -, γ -, δ -HCCH, 4,4'-DDD, 4,4'-DDT, 4,4'-DDE, aldrin, hexachlorobenzene, heptachlor.

Conclusion. Despite the fact that mass spectrometry is a more time consuming and expensive method of analysis, its benefits are justified not only for the determination of the trace concentrations of organochlorine pesticides but other dioxin-like compounds (PCB). It will significantly improve the reliability of the analysis for the water bodies located in a large distance from built-up areas, which is particularly important in the study of transboundary persistent organic pollutants (OCPs, PCBs, PCDDs, PCDFs) [7], [14], [15]. The research showed that the levels of the organochlorine pesticides in the bodies of water were below the detection limit.

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