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CHROMATOGRAPHIC AND PHOTOCOLORIMETRIC DETERMINATION OF TRICHLORMETAPHOS-3 IN ENVIRONMENT

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Purpose. To study conditions of chemical separation from vegetable raw materials (sugar-beet) and to accomplish the purification of the obtained extracts as well as to carry out qualitative and quantitative analyses of trichlormetaphos-3 in the extracts. ***Methodology.*** We have used a mixture of chloroform and acetone for the separating of trichlormetaphos-3 and have performed the purification of pesticide by means of chloroform extraction. We have applied a thin layer chromatography and gas chromatographic method for the identification as well as a photocolorimetry (PhEC-56M, cuvette wide – 10 cm, red color filter) for quantitative determination of trichlormetaphos-3. ***Results.*** We have extracted trichlormetaphos-3 from crushed sugar beet using a mixture of chloroform and acetone and have used the dry residue of extract for mineralization and for the consequent quantitative detection of trichlormetaphos-3 by a thin layer chromatography (mixture solvent is chloroform – benzene (1:1), the developer – 25% aqueous solution of ammonia and 2% aqueous solution of 4-aminoantypiryn). We have performed a gas-chromatographic analysis of trichlormetaphos-3 on Chrome-5 chromatograph (duration of analysis has been 7 min) as well as have carried out the photocolorimetric analysis of pesticide by measuring the optical density of the prepared solution and using reactions of ammonium molibdate and benzidine. ***Originality.*** For the first time, we have elaborated the methods of separation, purification, qualitative and quantitative determination of trichlormetaphos-3 in food raw materials. ***Practical value.*** We have proposed the technique of fast identification and determination of extremely toxic pesticide relevant to the group of phosphorus compounds – trichlormetaphos-3 in liquids, air and food raw materials. *References 9, scheme 1, figures 1.*

Keyword: organophosphorous pesticides, separation, purification, thin layer chromatography and gel-chromatography, photocolorimetric analysis.

ХРОМАТОГРАФІЧНЕ ТА ФОТОКОЛОРИМЕТРИЧНЕ ВИЗНАЧЕННЯ ТРИХЛОРМЕТАФОСУ-3 В ДОВКІЛЛІ

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Запропоновано методики ізолювання, очистки та якісного і кількісного визначення пестициду, що належить до класу фосфорорганічних сполук – трихлорметафос-3 в рідинах, повітрі та продовольчій сировині. Обрані умови проведення аналізу є придатні для ідентифікації і визначення отриманого препарату. Ізолювання проводили за допомогою суміші хлороформу з ацетоном, очистку – екстракцією хлороформом, а ідентифікацію – методом хроматографії в тонкому шарі сорбенту та газохроматографічним методом. Чутливість методу виявлення трихлорметафосу-3 в тонкому шарі сорбенту на пластинах „Силуфол" становить 3 мкг в 0,02 $см^3$ розчину, час аналізу в системі бензен 30 хв. Тривалість газохроматографічного аналізу, виконаного на хроматографі ХРОМ-5 (фосфорний детектор, колонка з внутрішнім діаметром 3,5 мм і довжиною 100 см, заповнена хроматроном N-AW-DMCS (0,16–0,20 мм) 3 5% SE-30), склав 7 хв. Для кількісного визначення пестициду використовували фотоколориметрію, в основі якої покладена реакція з амоній молібдатом і бензидином.

Ключові слова: фосфорорганічні пестициди, трихлорметафос-3, ізолювання, очистка, хроматографія в тонкому шарі сорбенту, гель-хроматографія, фотоколориметричний аналіз.

PROBLEM STATEMENT. Organic phosphorous compounds (OPC) are widely known and used as fire retardants and catalytic agents in turbines of space vehicles as well as pesticides [1, 2]. OPC are principal class of pesticides. Particularly toxic pesticides belong to such class compounds as ethers and esters of phosphoric acids. Furthermore, compounds of this class are highly toxic and therefore they often cause professional disease, especially in countryside. Specialists of chemical industry which manufacture pesticides and workers of agriculture are mostly exposed to toxic effect of these compounds. Pesticides are one of a cause of impairment of toxicological situation in world inasmuch as they, along with direct influence on living organism, can penetrate into plant, soil, water and so forth. In this connection, the sources of poisoning of people and animals may be not only themselves pesticides, but also diverse objects of environment, for instance, plants, foodstuffs *etc*. Individual pesticides can be preserved in environment, in organisms of warm-blooded animals that eat up the plants cultivated by chemical weed-killers and, hence, these pesticides can get into people organism along with milk and meat of these animals. Pesticides in organisms of people and animals are transformed into metabolites which may be more toxic than themselves pesticides [3].

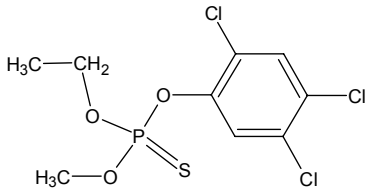
Ecologists have begun to pay attention to pesticides how to a class of toxically hazardous substances in the middle of last century when wide using of chloro-organic herbicides has led to mass diseases of people. Therefore, the studies concerning determination of toxic properties of different classes of pesticides and elaboration of methods of their analysis have started exactly from this period.

Currently, a situation with pesticides does not c hange, but, even contrariwise, it becomes more acute. There is a need every time in more effective pesticides for growth of good crop and preserving of agricultural products. It means that toxic influence of these pesticides upon people and hazard for environment will be merely stronger. Nowadays quality control of manufacturing and utilizing pesticides is put into effect by sanitary and epidemiological inspection. Owing to such activity, these supervisory organs develop steps directed to storage terms limitation of pesticides in environment. The safety code and norms of permissible concentration limits in environment, foodstuffs, grain, fruit and vegetable plants were established for the most pesticides.

All pesticides at the degree of toxicity are divided on four groups [4]. There are highly toxic pesticides (LD_{50} = 50 mg per 1 kg of animal weight), potent pesticides (LD_{50} range from 50 to 200 mg/kg), pesticides of middle toxicity (LD_{50} = 200–1000 mg/kg) and lowly toxic pesticides ($LD_{50} \geq 1000$ mg/kg). Besides, the pesticides at the chemical composition are subdivided into chlorine-containing, phosphorous-containing, amino-formic derivative organic compounds, inorganic pesticides and so forth.

Subject of our investigation were the pesticides from group of phosphorous-containing compounds. They are utilized in agriculture as insecticide, acaricide, herbicide *etc*. Their wide using are conditioned in so, that majority of them have high chemical activity and they are relatively rapidly decomposed in animal organism and environment. They are not accumulated in large quantity into animals and people tissues. But exactly these pesticides cause hard poisoning, getting into living organism.

Such pesticide as trichlormetaphos-3 ($C_9H_{10}Cl_3O_3PS$, M_r = 335.58 a.u.) [5] belongs to the organic phosphorous pesticides (OPP) and is very toxic substance. The structural formula of trichlormetaphos-3 is sketched out below.



Trichlormetaphos-3 [*o*-ethyl *o*-methyl *o*-(2,4,5-trichlorphenil)thiophosphate] is colorless or yellowish oil liquid (with boiling point of 127–133°C at 20 Pa) having hardly odor nuisance (ρ = 1.43 $g \cdot cm^{-1}$). Trichlormetaphos-3 is badly dissolved in water but good dissolved in most organic solvents. It rapidly decomposes in water solutions under the influence of chlorine and ozone. This pesticide is turned out in the form of emulsion concentrate (50 wt%) which is brown heavy oil liquid with shrilly odor. The concentrate of emulsion composed of 5 wt% of trichlormetaphos-3 and 92 wt% of mineral oil is called trichloral-5 [6].

Trichlormetaphos-3 is used as insecticide and acaricide in fight with sugar-beet vermins, viticide, chinch, and typhoid fly. The toxic effect is displayed through skin irritation and mucous membrane of eye, metabolic disorder and blood pressure reduction. The permissible concentration limits amount to 0.3 $mg \cdot m^2$ and 0.4 mg·L for working air and water, respectively [6].

Purpose of work. Taking into account toxicological importance of trichlormetaphos-3 and negative effect of this pesticide on the person's health and environment we have undertook an attempt to study possibility of application of thin layer chromatography method and gas-chromatographic analysis for trichlormetaphos-3 detection in solutions and air as well as to establish the conditions of chemicals separation from vegetable raw materials (sugar-beet), to accomplish purification of the extract and to carry out qualitative and quantitative analyses of trichlormetaphos-3 in the extracts using chromatography and photocolorimetry.

EXPERIMENTAL PART AND RESULTS OBTAINED. *The trichlormetaphos-3 isolation and purification techniques.* Sugar beet (100 g) has been crushed. Then 70 ml of mixture of chloroform with acetone (1:1) have been added to it and this content was left onto six hours at constantly stirring. Organic layer has been separated and crushed product once more twice was extracted by means of fresh prepared mixture of chloroform with acetone (30 mL) at shaking up within 3 hours. The obtained extracts have been combined together, filtered and evaporated on sand bath to the volume of 20 mL.

The extracts have been purified in the following way: the obtained solution has been placed in separating funnel and added 40 mL of water and 20 mL of chloroform. Content of separating funnel has been shaken up within 5 minutes and, after lamination, the chloroform layer has been separated. The obtained chloroform extract was evaporated till dry. The dry residual was dissolved in 20 mL chloroform and used for detection of trichlormetaphos-3 by thin layer chromatography and gas chromatographic method.

The extracted residual was used for mineralization and subsequent determination of trichlormetaphos-3. Toward this end we used the method of chromatography in thin layer of sorbent and mixture of solvents (chloroform – benzene (1:1)), developer (25% aqueous solution of ammonia and 2% aqueous solution of 4-aminoantipyrin). There is more rational using of system that comprises solely of benzene (as solvent) and solution of bromphenyl blue (as developer).

The trichlormetaphos-3 detection by thin layer chromatography. This method is highly sensitive, high-velocity at implementation and obtainable for many chemistry laboratories. The prepared "Sylufol" plates were used for chromatographic studying of trichlormetaphos-3 [7, 8].

Two drops of trichlormetaphos-3 solution in diethyl ether (1 mg per 1 mL) were marked on starting line of chromatographic plate. Next, the plate is dried on air, placed in chamber for chromatography with mixture of chloroform – benzene (1:1) solvents. When front of solvents will transfer on 10 cm the plate is taken out of chamber and anew dried in air.

Trichlormetaphos-3 stains have been developed by 10% solution of sodium hydroxide whereupon they were stood in drying cell at 100°C during 10 min. Then the plate is sprinkled with 25% aqueous solution of ammonia and 2% aqueous solution of 4-aminoantipyrin. After exsiccation of spots at air the plate is extra sprinkled by 20% aqueous solution of ammonium peroxysulfate. At the same time the trichlormetaphos-3 spots obtain pink color on the yellow background (R_f= 0.48–0.50).

This way of trichlormetaphos-3 detection is of long duration and needs expenditures of many chemical reagents. However, there is the more reasonable way of chromatography with using of system consisting of benzene solvent and bromphenyl blue developer. For preparing a bromphenyl blue, we have mixed the equal volumes of 2% water solution of argentum nitrate and 0.4% acetone solution of a water-soluble bromphenyl blue (R_f= 0.63-0.66).

Gas-chromatographic analysis of trichlormeta-phos-3. The gas-chromatographic analysis has been carried out by means of chromatograph CHROM-5. It is equipped by phosphorus detector, column with 3.5 mm inner diameter and 100 cm length, chromatron filler N-AW-DMCS (0.16–0.20 mm) with 5% SE-30. The column and vaporizer temperature are 190°C and 220°C, respectively. The penetration rate through column with nitrogen, hydrogen and air equal 20 cm^3/min, 14 cm^3/min and 400 cm^3/min, respectively.

The solution of trichlormetaphos-3 in diethyl ether was inserted in dozer of chromatograph. The metaphos was used as standard for comparison. The chromatogram was recorded under conditions noted above. It was ascertained that retention time of trichlormetaphos-3 relatively of metaphos amount to 6 minutes. The duration of analysis is 7 min. The chromatogram of trichlormetaphos-3 solution in diethyl ether is represented in Figure.

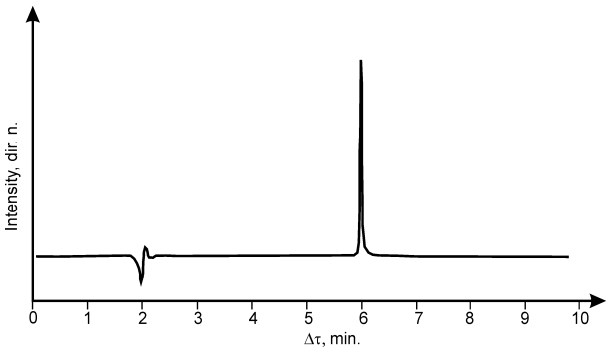


Figure – The chromatogram of trichlormetaphos-3 solution in diethyl ether.

The trichlormetaphos-3 detection in air. The sample drawing of air was fulfilled by means of the appliance which is made up of aspirator connected with pipe filled by absorbent cotton (0.5 g). The 20 L of air polluted by trichlormetaphos-3 is run through pipe with rate in 1 L per min. Next, absorbent cotton was withdrawn out of pipe, put into beaker and washed out thrice by diethyl ether at 5 cm^3. Ether extracts have been combined together and evaporated on the water-bath to the volume of 0.5 cm^3.

The obtained solution was used for trichlormetaphos-3 detection by method of chromatography in thin layer of sorbent and Gas-chromatographic analysis at conditions mentioned above.

The determination by photocolorimetry. The quantitative determination of trichlormetaphos-3 has been performed by photocolorimetry. At first we carry out mineralization using mixture of sulfate acid and nitrate acid as is described in literature [9]. The role of these acids reduces to oxidation of the organic substances that are a part of investigation object. The sulfate acid has a low oxidation potential but as dehydrating agent, this acid assists the increasing of oxidation effect of nitrate acid.

Then we have prepared phosphomolybdic acid that have transformed in molybdenic blue by means of reduction by benzidine. Further, the 10 mL of mineralizing have been placed into test tube, added solution of ammonium molybdate and acidated by 10% solution of nitrate acid. So, the yellow of solution was appeared. After addition of saturated aqueous solution of benzidine hydrochloride and 10 % solution of ammonia to reaching alkaline reaction the given solution has become blue. The optical density of this solution was measured using photocolorimeter FEC-56M, 10 mm cuvette and red light-filter.

CONCLUSIONS. Thus, in this article the methods of isolation, purification, qualitative and quantitative determination of pesticide – trichlormetaphos-3 that belongs to the organic phosphorous compounds in liquids, air and food raw materials were proposed. The

results of investigations have revealed that the chosen conditions of undergoing tests are suitable for the identification and quantification of trichlormetaphos-3 isolated from food raw materials. The isolation of pesticide was performed using a mixture of chloroform with acetone (1:1). The purification was carried out by extraction from chloroform. The identification was accomplished by a thin layer chromatography and gas-chromatography. The qualitative analysis has lasted seven minutes. Trichlormetaphos-3 quantitative determination has been fulfilled by means of photocolorimetric method using reaction of ammonium molybdate with benzidine.

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ХРОМАТОГРАФІЧНЕ ТА ФОТОКОЛОРИМЕТРИЧНЕ ВИЗНАЧЕННЯ ТРИХЛОРМЕТАФОСУ-3 В ДОВКІЛЛІ

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Предложены методики изолирования, очистки, качественного и количественного определения пестицида, принадлежащего к классу фосфорорганических соединений – трихлорметафос-3 в жидкостях, воздухе и продовольственном сырье. Подобранные условия проведения анализа пригодны для выявления и определения упомянутого препарата. Изолирование проводили с помощью хлороформа с ацетоном, очистку – экстракцией хлороформом, а выявление – методом хроматографии в тонком слое сорбента и газохроматографическим методом. Чувствительность метода выявления трихлорметафоса-3 в тонком слое сорбента на пластинах „Силуфол" составляет 3 мкг в 0,02 см^3 раствора, время анализа в системе бензол – 30 мин. Продолжительность газохроматографического анализа, выполнено на хроматографе ХРОМ-5 (фосфорный детектор, колонка с внутреннем диаметром 3,5 мм и длиной 100 см, заполнена хроматроном N-AW-DMCS (0,16–0,20 мм) 3 5% SE-30), составила 7 мин. Для количественного определения пестицида использовали фотоколориметрию, в основе которой лежит реакция с аммоний молибдатом и бензидином.

Ключевые слова: фосфорорганические пестициды, трихлорметафос-3, изолирование, очистка, хроматография в тонком слое сорбента, гель-хроматография, фотоколориметрический анализ.