U.D.C.: 613.2/3:632.95:504.064:001.8

A. A. Bedzay[1], ***O. N. Shcherbina***[2], *Candidate of Science (Pharmacy), Associate Professor,*
B. M. Mykhalitchko[2], *Doctor of Science (Chemistry), Professor,* ***I. O. Shcherbina***[3]
([1]Danylo Galytckyj National Medical University of Lviv, [2]Lviv State University of life safety,
[3]Department of the health protection, Lviv, Ukraine)

CHROMATOGRAPHIC ANALYSIS OF CHLORINATED PESTICIDES IN FOOD RAW MATERIAL

On the basis of the experimental investigation results the detection technique and quantitative determination procedure of chlorinated pesticide, namely 2,4-dichlorophenoxyacetic acid (2,4-D), in food raw material are presented in the article. The methods of thin layer and gas-liquid chromatography were proposed for use. These methods proved high resolution, fast response and separation rate. The use of thin layer chromatography for quantitative determination of 2,4-D extracted from food raw material enables the detection limit of 0.01 μg in one sample.

Keywords*:* chlorinated pesticides, food raw material, chromatography**.**

Introduction. Many chlorine-containing organic compounds (COC) are pesticides. They are broadly used in agriculture and cattle breeding. COC are toxic for people and animals. COC poisoning occurs either accidentally or purposely. Acute poisoning death amounts to20-25%.

The scale expansion and increase in assortment of the pesticides use in the agriculture as well as pollution of the environment by factory and domestic waste greatly complicates the pesticide identification in process of analyses. The reliable identification is especially important for the pesticides where their presence in foodstuffs and food raw material is debarred. Therefore, the reliable detection techniques have to be applied. The use of gas chromatograph with selective detectors (electron-capture, thermoionic or flame photometric) allows to achieve the progress. On the modern level of analytical technique development, the best results are achieved by combining the chromatograph and mass-spectrometr [1].

Nowadays, halogenated acyclic hydrocarbons (lindane, heptochlorine, chlordane, aldrin, *etc*.) and aromatic hydrocarbons (hexachlorobenzene, dichlorodiphenyltrichloroethane, methoxychlor and others) that have been broadly used against vermins of agriculture crops and for seeds treatment are prohibited.

The object of our investigations in the paper is a chemical referred to as 2,4-dichlorophenoxyacetic acid (2,4-D):

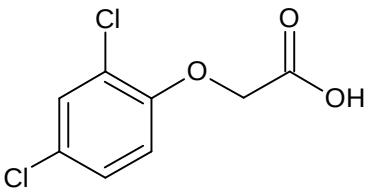

Table 1 presents the main physical and chemical properties of 2,4-D. In case of ingestion, face hyperemia and heartburn are observed after the event, and heavy breathing and cyanosis develop lately. The human fatal dose is 15 g. The maximum allowable concentration of 2,4-D in the air of the working zone is 1 mg/m^3. 2,4-D and its derivatives (salts or esters) decomposition product is 2,4-dichlorphenol, which is also a metabolite in human body [3].

In Ukraine, herbicides on basis of 2,4-D are applied for weed extirpation at grain crops sowing. 2,4-D applied in the form of salts or esters due to its low solubility in water. In soil, 2,4-D readily hydrolysis and becomes absorbed by plant, the pure (water solubility is 540 mg/l). It is known [4] that 2,4-D remains in soil for 1–6 months and is decomposed by microorganisms. More 85% brought in soil of 2,4-D is decomposed for 300 hours. However, influence of the herbicides can last from 6 to 9 months only in droughty soils. In plant, 2,4-D remains stable from 1 to 3 months [4].

In milk of cows pastured on testing ground treated by substances on basis of 2,4-D (standard of application rate 2.24 kg per hectare), insignificant amounts of herbicide were detected in four days [1]. Absorbtion of herbicide also takes place if 2,4-D is applied pastures. Today, vegetables and fruits processing by substances on basis of 2,4-D is prohibited in Ukraine.

However, in food and food raw material the residues of 2,4-D are still detected. This is explained by plants absorbtion of 2,4-D conjugates with amino acids, peptides, proteins, saccharides and others components of plants. The extract of vegetal raw material show that all samples contain co-extractive substances, that point out on 2,4-D.

The aim of the paper is to elaborate and improve the techniques for extraction, purification, qualitative and quantitative determination of chlorinated pesticides – 2,4-dichlorophenoxyacetic acid **(**2,4-D) extracted from food raw material. In order to obtaine the reliable results of 2,4-D content in samples of industrial raw material or foodstuff, the pesticides need to be extracted the extracts need to be purified, the analyzing techniques elaborated and the results must be adequately interpreted.

Table 1

Basic physical-chemical properties of 2,4-dichlorophenoxyacetic acid [2]

Abbreviation	2,4-D
Molecular formula	$C_8H_6Cl_2O_3$
Relative molecular mass	221.038
Color, state	White, crystalline
Melting point [°C]	140.5
Solubility	Slightly soluble in water, toluene and *n*-hexane, well soluble in ethanol, benzene, acetone and diethyl ether
Density [$g \cdot cm^{-3}$]	1.565
Vapor pressure [mm Hg]	0.4 (160°C)
pK_a at 25°C	2.64
$\underline{LD_{50}}$ [$mg \cdot kg^{-1}$]	639
Application	For weed extirpation of the cereal crop and maize
Miscellaneous	At ultraviolet radiation treatment the 2.4-D decomposes partly; on condition boiling with HCl or HBr it decomposes by forming 2,4-dichlorophenol and hydroxiacetic acid

Results and discussion. Extraction by organic solvents is the principal method that ensures high extraction level and can be applied to all pesticides. The choice of extractant and extraction conditions depend on nature of object and investigated material. The pesticides losses during extraction depend on solubility of organic solvents and pesticides in endogenous compounds (fats, lipids).

Extraction. Extraction of 2,4-D was performed by diethyl ether. Finely milled raw material (100 g of wheat grain) was added to diethyl ether (50 ml) and left for 6 hours at periodical stirring. The extract was separated, and raw material was extracted the second time by diethyl ether (by portions of 25 ml).

Purification. The extracts were acidulated with sulphuric acid to pH 1, and placed into separating funnel. Then, 25 ml of chloroform was added and shaken up of 5 min. The chloroform extract was separated, and water-based phase was processed by in 25 ml chloroform portions twice. Next, chloroform extracts were mixed and evaporated to solid residual. The solid residual was dissolved in 5 ml of diethyl ether and the flask content was hermetically sealed. The obtained solution was used for qualitative and quantitative analysis.

2,4-D identification by thin layer chromatography. The solution containing 2,4-D (2 drops) was put on “Silufol” plate on the start line. On the right side, two drops of "check" solution (solution of 2,4-D in diethyl ether) were put. The spots of solutions were dried and chromatographed in chamber saturated by dissolvent vapour (mixture of *n*-hexane and acetone 2:1). When front of the solvent lifted by 10 cm, the plate was removed dried and treated by solution of bromocresol green in aqueous-alcoholic solution of sodium hydroxide (for OH^- detection) or by solution of silver nitrate in mixture of ammonia and acetone (for Cl^- detection). Then, the dried plate was exposed to ultra-violet rays for 10 min. In presence of 2,4-D on plate, the dark spots appeared. The detection limit of 2,4-D was 2 μg in a sample.

For preparation of the solution of silver nitrate in mixture of ammonia and acetone the silver nitrate (0.5 g) was dissolved in distilled water (5 ml), then the concentrated ammonia solution (5 ml) was added and the flask was filled with acetone to 100 ml.

The reliability of 2,4-D identification by thin layer chromatography is verified by qualitative reaction with chromotropic acid. The obtained extract by means of purification (2 ml) was placed in a test-tube and evaporated to solid residual. Then, few small crystals of chromotropic acid and concentrated sulphuric acid (2 ml) were added to solid residual and heated for 2 min. After, this solution discolours to pink which shaded to dark-purple [2].

2,4-D quantitative determination by gas-liquid chromatography. The vapours of etheric extracts (5 μl) were placed into *Cvet-304* chromatograph and chromatographed under operating characteristics: flame-ionization detector; chromatographic column with internal diameter of 3 mm and 180 cm in length; immobile phase composed 3 % OV-17; evaporator thermostat temperature was 210 °C; column thermostat temperature was 180 °C; azote gas-carrier (rate is 70 L/min), air rate is 300 L/min; hydrogen rate is 30 L/min. The retention time (qualitative analysis) and peaks heights relatively of 2,4-D ethylic ether (quantitative analysis) were measured by the obtained chromatograms. The detection limit of 2,4-D in food raw material using gas-liquid chromatography method was 0.01 μg in one sample.

Conclusions. Thus, the proposed methods for extraction, purification, qualitative and quantitative analysis, as well as use of all possibilities to improve the reliability of identification of investigated material ought to be elaborated for determination of 2,4-D residues in food raw material and especially in sample of unknown extraction.

References:

1. **Chmil V. D.** The state and application prospectives of modern instrumental methods for pesticides analysis. / V. D. Chmil // Sovr. Probl. Toksikol. – 2002. – Vol. 2. – P. 56 – 62 [in Russian].
2. **Mackay D.** Handbook of physical-chemical properties and environmental fate for organic chemicals / Mackay D., Shiu W.Y., Ma K.-C., Lee S.C. – London: CRC Press, 2006. Vol. 3. – P. 2761 – 2763.
3. **Kramarenko V. F.** Pesticide analisis / Kramarenko V. F., Turkevytch B. M. – M.: Khimia, 1978. – 264 p. [in Russian].
4. **Valkowsky S. H.** Handbook of aqueous solubility data / S.H. Valkowsky. – London: CRC Press, 2003. – P. 450 – 451.

А.О. Бедзай, О.М. Щербина, Б.М. Михалічко, І.О. Щербина

ХРОМАТОГРАФІЧНИЙ АНАЛІЗ ХЛОРОВМІСНИХ ПЕСТИЦИДІВ У ПРОДОВОЛЬЧІЙ СИРОВИНІ

В роботі за результатами експериментальних досліджень наведені методики виявлення і кількісного визначення хлоровмісного пестициду – 2,4-дихлорофеноксиацетатної кислоти (2,4-Д) в продовольчій сировині. Запропоновано застосовувати методи хроматографії в тонкому шарі сорбенту і газорідинну хроматографію. Ці методи мають велику роздільну здатність, високу чутливість і швидкість розділення. Використання методу газорідинної хроматографії для кількісного визначення 2,4-Д екстрагованої з продовольчої сировини дав змогу встановити поріг чутливості, який становить 0.01 мкг в пробі.

Ключові слова: хлоровмісні пестициди, продовольча сировина, хроматографія.

А.А. Бедзай, О.Н. Щербина, Б.М. Мыхаличко, И.А. Щербина

ХРОМАТОГРАФИЧЕСКИЙ АНАЛИЗ ХЛОРСОДЕРЖАЩИХ ПЕСТИЦИДОВ В ПРОДОВОЛЬСТВЕННОМ СЫРЬЕ

В работе по результатам экспериментальных исследований приведены методики качественного и количественного определения хлорсодержащего пестицида – 2,4-дихлорофеноксиуксусной кислоты (2,4-Д) в продовольственном сырье. Предложено использовать методы хроматографии в тонком слое сорбента и газожидкостную хроматографию. Эти методы имеют высокую раздельную способность, чувствительность и скорость разделения. Использование метода газожидкостной хроматографии для количественного определения 2,4-Д, экстрагированного из продовольственного сырья позволил установить порог чувствительности, который составляет 0.01 мкг в пробе.

Ключевые слова: хлорсодержащие пестициды, продовольственное сырье, хроматография.

❖ ❖ ❖