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Gas and thin-layer chromatography in the control of residual levels of polychlorinated biphenyls and pesticides in foods of animal origin

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Abstract. The biological safety of food products of animal origin is closely related to the socio-economic development of society and the state of the environment. The study aimed to verify the presence of residual amounts of polychlorinated biphenyls and pesticides in food products of animal origin by gas and thin-layer chromatography methods. For the analysis of powdered milk samples, gas chromatography was used on a Bruker Scion 456-GC device equipped with an electron capture detector (ECD), an autosampler, and high-precision temperature control. Extraction of polychlorinated biphenyls was conducted with hexane followed by purification using a hexane-acetonitrile system and adsorption chromatography on silica gel. Calibration was performed with a Supelco PCB Mix standard in the concentration range of 0.001-0.250 μg/cm³ with a correlation coefficient of $R^2 = 0.9999$. For the study of fish samples, thin-layer chromatography was used with preliminary acid hydrolysis of the sample and extraction with diethyl ether.

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Visualisation of chromatographic zones was conducted by treating the plates with a silver nitrate solution followed by ammonia and UV irradiation at 254 nm. The percentage of analyte recovery was within the range of 70-120%, which confirms the reliability and accuracy of the methods used. In the samples of the studied fish, the detection limit of pesticides was 0.6 mg/kg, which ensured efficient detection of the residual amount of toxicants even at their low content. The main peaks on the milk chromatogram appeared in the interval from 9 to 14 minutes and corresponded to the standards of polychlorinated biphenyl components, and their retention time correlated with respective standard substances, which confirmed the presence of polychlorinated biphenyls in the sample and ensured quantitative determination. The significance of the study is determined by modern, reliable and effective practice-oriented information on food safety assessment to both relevant laboratories and institutions, as well as educational institutions specialising in the training of specialists in the specialty "Biotechnology and Bioengineering"

Keywords: biosafety; analyte; standards; temperature; instrument

Introduction

The issue of quality and safety of food products of animal origin becomes increasingly relevant both for consumers producers. Since proper control over the preparation, processing, storage and further sale of livestock products ensures not only monitoring of the presence of residual amounts of veterinary drugs, but also reliable control of compliance with hygiene requirements at all stages of product production. In total, compliance with each of the listed elements makes it possible to prevent the occurrence of a number of food poisonings, and deliver an exceptionally high-quality and safe product to the consumer.

The problem of food quality and safety is an issue that is actively discussed by a range of scientific communities. For instance, according to the works of Italian scientists A. Arienzo *et al.* (2022) on the monitoring the number of international publications devoted to the issue of microbiological safety of food products, an annual increase in their number (2 thousand and more) over the last decade is notable. This, in turn, confirms the significant interest in this topic from the international scientific community. Ukrainian scientists O. Mateyuk *et al*. (2025) also substantially addressed problems related to the quality and safety of food products, noting that to ensure a high level of microbiological safety of food products, it is necessary to systematically improve control and regulation systems. To minimise the risks of microbiological contamination, it is necessary to ensure systematic monitoring and compliance with control at all stages of the food chain, as well as create proper hygienic conditions at all stages of the technological process.

As T. Romanovska *et al*. (2022) highlighted, the principles of producing a safe food product ensure the quality and safety of the product at all stages of production, from the receipt of raw materials and packaging materials at the enterprise to the arrival of finished products on the market. As indicated by O. Kuzmin *et al.* (2022), increasing the efficiency of food production, while ensuring compliance with high standards of their quality and safety, are relevant issues for the competitiveness of restaurant establishments. S. Prache *et al.* (2022) emphasised that the quality of food products of animal origin is determined by the following seven main attributes: safety, commercial, sensory, nutritional, technological, convenient and image. Image encompasses ethical, cultural and environmental aspects related to the origin of food products and the method of their production and

processing. This framework highlighted the priority of different quality attributes.

According to G. Richard *et al.* (2022) the quality of food products usually depends on those properties that satisfy all the needs of the consumer. The concept of quality is becoming more complex, especially regarding products of animal origin. These changes occur in parallel and are to some extent due to concerns related to their impact on human health and the environment, agricultural methods and food processing, as well as animal welfare. Consumer habits are also changing, and this, in turn, is accompanied by a range of (sometimes contradictory) expectations. There is a demand not only for convenient and ready-to-eat meals, which are often (but not always) highly processed, but also for quality labels (in particular organic), healthy eating, etc. As the Omani scientists I. Al-Bulushi *et al*. (2026) highlighted, the quality and safety of ready-to-eat foods, which are widely consumed worldwide due to their convenience, wide availability, and relatively low prices compared to other traditionally prepared foods, must be strictly monitored. K. Hilgendorf *et al.* (2024) noted that maintenance and improvement of the quality of food products are the main objectives of research in the field of food science and technology.

Although chemical and physical approaches were always substantial in improvement of the quality of food products, while biotechnological methods have become the most innovative solutions to overcome the limitations of traditional approaches. Therefore, the primary goal of study was to verify the presence of harmful pesticides and other foreign chemicals in food products of animal origin using modern biotechnological methods.

Materials and Methods

The research was conducted from 12.05.2025 to 14.06.2025 at the Mykolaiv Regional State Laboratory of the State Service for Food Supervision and Consumer Protection of the city of Mykolaiv, Ukraine, in the relevant analytical unit, namely the gas chromatography sector, which is equipped with chromatographic equipment. Scientific processing of the results and generalisation of the obtained data were conducted at the Mykolaiv National Agrarian University.

The gas chromatography method was used to determine the residual amounts of polychlorinated biphenyls in powdered milk samples, as well as the thin-layer chromatography (TLC) method to identify the herbicide 2,4 dichlorophenoxyacetic acid in fish (carp) samples. The research was conducted based on relevant regulatory documents and methodological recommendations, following the requirements of analytical accuracy and reproducibility of results (SANTE 11312/2021 v2, 2024). The objects of the research were received for research in the laboratory through the sample registration department. Each sample was assigned an individual registration number upon receipt, which ensured identification within the laboratory records and the anonymity of the origin of the product during analytical research. The objects of analysis were samples of powdered milk and fish, to which controlled amounts of hazardous chemical compounds were added. For each type of product, the study was conducted in triplicate.

Gas chromatographic studies were performed using a Bruker Scion 456-G gas chromatograph, serial number BR 1308M149. The device was manufactured by Bruker Chemical Analysis B.V. (Netherlands) in 2013 and is owned by the Mykolaiv Regional State Laboratory of the State Service for Food Safety and Consumer Protection. The gas chromatograph has passed state metrological certification, which is confirmed by certificate No. 130-05-FKh dated December 19, 2014, issued by the State Enterprise "Odesa Regional Centre for Standardisation, Metrology and Certification". The metrological characteristics of the gas chromatograph were defined and described in the relevant technical documentation, and the inter-verification interval is 12 months. During

the metrological certification, standard samples were used, in particular, lindane for chromatography, standard sample DSZU 042.7-96, methyl parathion and GSO 1854. The research was conducted under standard environmental conditions: air temperature 20°C, relative humidity 55%, atmospheric pressure 760 mm Hg, supply voltage 220 V, frequency 50 Hz.

Characteristics of gas chromatography. In the study of powdered milk samples, a mixture of polychlorinated biphenyls (PCBs), in particular PCB congeners No. 28, 52, 101, 138, 153, 180, was used as model contaminants. For calibration and identification, a certified analytical standard Supelco PCB Mix, manufactured by Merck KGaA (Darmstadt, Germany), was used. The standard was a solution of PCBs in isooctane with a nominal concentration of each congener of approximately 10 µg/mL. According to the certificate of analysis, the solution is a colourless liquid, the identity of the components was confirmed by gas chromatography with mass spectrometric detection, and the refractive index values corresponded to the regulatory range. The standard was stored at a temperature of 2-8°C, the shelf life is until September 2028, which guaranteed its stability throughout the entire study period.

To determine polychlorinated biphenyls in food samples, the method of extraction with an organic solvent was used with subsequent purification of the extract. PCB extraction was conducted with a non-polar solvent – hexane, which ensures the effective extraction of lipophilic compounds from the sample matrix. In the presence of a significant lipid content, the extract was purified using liquid-liquid distribution in the hexane-acetonitrile system, which reduced the content of accompanying fats and ballast substances. Further purification was conducted by adsorption column chromatography on silica gel, with elution of PCBs with a mixture of hexane and diethyl ether in a ratio typical for the determination of organochlorine compounds.

The analysis was conducted on 2 g of a sample of powdered milk. After the extraction and purification stages, the extract was concentrated to a final volume of 3 cm^3. For gas chromatographic determination of polychlorinated biphenyls, 1 µL of the prepared extract was injected into the chromatograph. According to the presented calibration protocol for the Bruker Scion 456-GC instrument, the quantitative analysis procedure was based on the following parameters:

- Concentration range. Calibration solutions were prepared by serial dilution of the stock standard with isooctane. According to the calibration table for PCB congener 28, the range of concentrations tested was from 0.001 µg/cm^3 to 0.250 µg/cm^3.
- Mathematical model. The calibration dependence was described by a linear equation of the form $y = bx + a$ (forced zero crossing: $a = 0$).
- Coefficient of approximation. The obtained value of the correlation coefficient is 0.9999, which indicates the high precision of the method and the linearity of the detector response in the specified range.

The analysis was initiated at 80°C, which was maintained for 1.00 min to stabilise the solvent front and focus the components in the initial part of the column. Then, a linear temperature increase was conducted at a rate of 20°C/min until the target value of 280°C was reached. The duration of the heating step was 10 min. After reaching 280°C, the system was maintained in isothermal mode for 5 min (for a total time of 16 min) to completely elute high-molecular-weight congeners and clean the column from matrix residues (Table 1, Fig. 1). The CompassCDS software was used for full control of the gas chromatograph operation: adjustment of temperature programmes for the column thermostat, injector, and detector, control of carrier gas flows, and configuration of sample injection modes, and support of the detector. For data collection, CompassCDS was used for stable and accurate registration

of the chromatographic signal with the ability to visualise chromatograms in real time. The programme automatically performs peak integration, determines the retention time, area, and height of peaks, and can also be used for manual adjustment of the integration to increase the accuracy of the results, which is relevant in the analysis of complex mixtures or low concentrations of analytes. Compass-CDS was also used for results documentation. The software provides data archiving, standardised report generation, export of results to common formats, and traceability of all stages of the analysis.

Table 1. *Programmable temperature mode of the column thermostat*

Heating rate, °C/min	Temperature, °C	Time, min	Total time, min
Primary	80	1.00	1.00
20	280	5.00	16.00
			16.00

Source: Scion 436-GC and 456-GC gas chromatographs: Workplace requirements (2021)

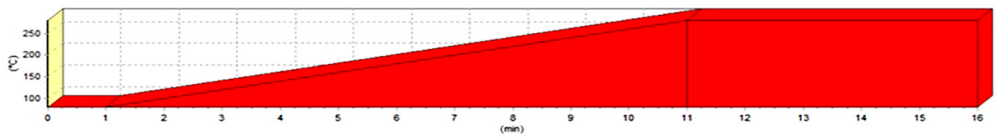

Figure 1. Programme for programming the temperature of the Bruker Scion 456-GC column thermostat

Source: Gas chromatographs Scion models 436-GC and 456-GC: Maintenance instructions (2013)

Characteristics of thin layer chromatography. For the study of fish samples, the herbicide 2,4-dichlorophenoxyacetic acid in the form of an amine salt (2,4-D) was used as a model pollutant. For quantitative determination, a standard sample of the composition of 2,4 D amine salt was used, certified following the requirements of the national metrological system. The standard was manufactured by the Special Design and Technological Bureau for Experimental Production of the O.V. Bogatsky Institute of Physics and Chemistry of the National Academy of Sciences of Ukraine (Odesa, Ukraine). The certified value of the mass concentration of 2,4-D amine salt was 0.100 mg/cm^3 with a relative error of ±1.4% at p = 0.95. Furthermore, the study used reference material (DSanPiN 8.8.1.2.3.4-000-2001, 2001).

Extraction of the analysed compounds from fish samples was conducted after acid hydrolysis of the matrix. A portion of the crushed sample weighing 30 g was treated with a 6% hydrochloric acid solution in a volume of 100 cm^3 with subsequent heating in a water bath at a temperature of 100°C for 1 hour. After cooling, 15 cm^3 of a 40% aqueous solution of phosphorotungstic acid and 100 cm^3 of distilled water were added to the mixture, after which filtration was performed. Extraction of the target components from the filtrate was conducted by liquid-liquid extraction with diethyl ether three times 50 cm^3, with a total volume of extractant of 150 cm^3. The combined ether extract was dried over anhydrous sodium sulphate and concentrated on a rotary evaporator to a final volume of 2 cm^3 for further chromatographic analysis. A standard 2,4-D solution with a concentration of 100 µg/cm^3 was used to create a model concentration of 1 mg/kg of 2,4-dichlorophenoxyacetic acid in fish samples. Incorporating the mass of the crushed sample of 30 g, the estimated amount of analyte that needed to

be added was calculated, based on the instructions registered in the quality system in the laboratory, through the proportion:

1 mg/kg – 1,000 g
X mg – 30 mg,

where 1 mg/kg – conditional calculated concentration adopted to determine the amount of analyte that must be added to a sample of a given mass; 1,000 g – mass of product corresponding to a concentration of 1 mg/kg; X – mass of 2,4-D per 30 g sample; 30 g – mass of crushed sample.

Calculation: X = (30 × 1)/1,000 = 0.03 mg. Conversion to micrograms: 0.03 mg × 1,000 = 30 mcg. Therefore, to create a concentration of 1 mg/kg in a 30 g sample, it is necessary to add 30 µg of 2,4-D. After adding the calculated volume of standard solution, the sample was thoroughly homogenised and incubated for uniform distribution of the analyte in the matrix, after which acid hydrolysis, extraction, and subsequent analysis by thin-layer chromatography were performed. The limit of quantification of the method – 0.6 mg/kg – was experimentally determined during the validation of the methods.

Visualisation of chromatographic zones was conducted following the laboratory's internal methodology for the determination of pesticides by thin-layer chromatography (Regulation (EC) No 396/2005, 2005). All computational processes met the requirements of standards and regulatory documents for laboratory quality control (Chechet *et al.*, 2023). Calculations were conducted based on the formulas and examples:

$$W = \frac{\text{QTY} \cdot \text{V}}{\text{m}}, \quad (1)$$

where QTY – concentration according to the calibration graph; V – volume of the obtained extract, cm^3; m – sample weight, g.

$$W = \frac{0{,}0994 \cdot 3}{2{,}072} = 0.144 \text{ mg/kg}.$$

Analyte recovery is the percentage of the true concentration of analyte that is detected by an analytical method. It is a key indicator of the accuracy and reliability of an analytical method, particularly during its validation. The determination of the recovery of the analyte additive to the matrix was calculated as follows (% recovery) (2):

$$\text{RP} = \frac{Xhpov}{D}, \quad (2)$$

where *Xhpov* – the amount of analyte returned; *D* – added amount of analyte, mg/kg.

$$\text{RP} = \frac{0.144\ mg/kg}{0.2\ mg/kg} \times 100\% = 72\%.$$

The range of return percentage for trials was 70-120%, hence the experiment was done correctly (Voytsitsky *et al.*, 2024). A full description of formulas, calculation algorithms, and examples of quantitative determination of pesticides based on the results of an experimental study are demonstrated below. Irregular shapes were traced with a pencil to form a square or rectangle. The area of the spot is calculated using the formula (3):

$$S = a \cdot b, \quad (3)$$

where *a* – width of the rectangle (mm); *b* – height of the rectangle (mm).

For instance, when determining pesticides at a standard concentration of 0.5 mg/kg, a spot with dimensions of 6 mm × 16 mm was obtained, which corresponds to the area:

$$S_{0.5} = 16 \cdot 6 = 96 \text{ mm}^2$$

Similarly, using a standard with a concentration of 1 mg/kg, with a spot size of 7 mm × 18 mm:

$$S_{1.0} = 18 \cdot 7 = 126 \text{ mm}^2$$

The area of the sample spot was:

$$S_{samples} = 18 \cdot 4 = 72 \text{ mm}^2$$

The quantitative determination of pesticides in the sample was conducted based on the following formula (4):

$$X = \frac{A \cdot S2}{m \cdot S1}, \quad (4)$$

where X – amount of pesticides in the test sample; A – amount of standard applied to the plate, μg; m – sample weight, g; S1 – area of the standard spot, mm^2; S2 – area of the sample spot, mm^2. Substituting the values:

$$X = \frac{50 \cdot 72}{30 \cdot 126} = 0.95 \text{ mg/kg}.$$

The processing of gas chromatographic analysis results was performed using Compass-CDS software, which was used for instrument control, chromatographic signal recording, peak integration, component identification by retention time, and quantitative determination of polychlorinated biphenyls based on calibration curves. The obtained analytical data were exported and summarised in Microsoft Excel, which was used to systematise the results, calculate descriptive statistics, and present data as mean ± standard deviation. The validation of gas and thin-layer chromatography methods was conducted by evaluating key performance characteristics, including linearity, precision within the same analysis conditions (convergence), and accuracy, which was determined by the percentage of analyte recovery. The results of experimental studies confirmed the necessary level of accuracy and reliability for food product biosafety indicator monitoring.

For the gas chromatography method for the determination of PCB congeners, high linearity of the detector response was established, and the convergence and percentage recovery indicators fully met the regulatory requirements for the selected concentration range. Similarly, for the thin-layer chromatography method for the study of fish for the content of 2,4-D amine salt, the selectivity and accuracy values obtained confirmed the possibility of effective use of this technique for quantitative and qualitative analysis. All calculated validation parameters are within the permissible deviations provided for by the laboratory's internal regulations and methodological guidelines (DSTU ISO/IEC 17025:2019, 2021). To ensure the objectivity of the assessment of the biological safety of food products, the conditions and operating rules that provide stable functionality of the devices, thus accurate results, were established. These conditions were described in detail in Table 2.

Table 2. *Study parameters*

Parameter	Value
Ambient temperature	+20 °C
Atmospheric pressure	100.79 kPa (756 mm Hg)
Relative humidity (at +20 °C)	65%
Voltage in the electrical supply network	220 V

Source: Scion 436-GC and 456-GC gas chromatographs: Workplace requirements (2021)

The ambient temperature was +20°C. This temperature was most often maintained during the study, as it ensures stability of biological samples and reagents and provides comfortable working conditions for personnel. The atmospheric pressure in the conducted study was also within the normal range and was 100.79 kPa or 756 mm Hg. Constant maintenance of stable atmospheric pressure is a prerequisite for the operation of devices that use gas. An equally relevant metric that indicates the content of water vapor in the air is the humidity. In the present case, this parameter was 65%, which is a level. This level does not interfere with the operation

of the devices and, most notably, does not cause condensation on the surfaces. Continuous operation of the devices was ensured by the voltage in the electrical power supply network, which is supplied to all laboratory devices. In this experiment, it was 220 volts. The supply voltage is constantly verified and stabilisers were utilised. The advantage of the Bruker Scion 456-GC is the high-precision of the analytical instrument, which can separate complex mixtures of substances with high resolution. In addition, it operates stably during long series of analyses, has simple programming of temperature regimes and is compatible with various types of detectors. The operating mode of the device is described in Table 3.

Table 3. *Device configuration and operating mode*

Configuration	Operating mode
Gas chromatograph	Bruker Scion 456 GC
Autosampler	With automatic sample introduction
Capillary column	Rxi-5ms, Restek, 30 m × 0.25 mm × 0.25 µm
Stationary phase	5% phenyl, 95% dimethylpolysiloxane
Injector temperature	250°C
Detector	Front electronic capture detector
Detector temperature	300°C
Column temperature	80C – 1 min; 20°C – min to 200°C; 6 min – 280°C
Sample input mode	Split 1:10
Injection volume (sample introduction)	1 µl
Carrier gas	Nitrogen, 99.999% purity (1.0 mL/min)

Source: Gas chromatographs Scion models 436-GC and 456-GC: Maintenance instructions (2013)

All parameters that were set for effective chromatographic separation and subsequent quantitative determination of substances are described below. An autosampler is a device that introduces samples into the instrument in automatic mode. Therefore, operator errors were prevented, and continuous measurements without human intervention were performed. A capillary column is a thin tube located inside the chromatograph for high-quality separation of components, the diameter is 0.25 mm – a thin column, used for analytical tasks; the layer thickness of 0.25 µm determines the speed at which the substance moves through the column. The column has a polarity that is suitable for the analysis of organochlorine substances and provides high-quality peaks without overlapping them. The composition of the stationary phase was represented by 5% phenyl, 95% dimethylpolysiloxane. This is a special coating inside the column, which separates substances by their properties. Phenyl 5% ensures improved interaction with non-polar or aromatic compounds. Stable and versatile operation is provided by 95% dimethylpolysiloxane. This phase ensures effective separation of PCBs following their volatility and structure.

The injector introduces the sample. The injector temperature is 250°C, which is the temperature at which the sample evaporates after being introduced into the column, turns into a gaseous state and enters the gas stream. In front, an electron capture detector (ECD) is a type of detector, sensitive to chlorinated compounds such as PCBs. It records the change in electric current caused by the capture of electrons by analytes. In this experiment, the detector was used to detect PCBs at the milligram level. To avoid the precipitation of volatile substances and ensure signal stability of the experiment, the detector was constantly maintained at a high temperature of 300°C. At this temperature, sensitivity and reliability of measurement are ensured without loss of analytes. The column temperature was

gradually heated: the initial temperature of 80°C was held for 1 min, then at a rate of 20 degrees per minute it rose to 200°C, held for 6 min, and then increased to 280°C. This mode was used to gradually remove substances with different levels of volatility from the column and separatee with maximum efficiency.

The Split 1:10 sample injection mode was the next criterion, hence only one tenth of the sample that was injected entered the column, everything else was routed to the outlet. All the above elements are performed in order not to overload the column with a large amount of substance, reduce the risk of peak deformation and ensure uniform distribution of the substance. The injection volume was 1 µL. This volume of liquid is automatically introduced into the chromatograph for further analysis. The carrier gas was nitrogen with a purity of 99.999% (1.0 mL/min). After the gas chromatograph was set up and all sample preparation steps were completed, a chromatographic analysis of the powdered milk sample was performed. For this procedure, a Bruker Scion 456-GC was used, which is highly sensitive to organochlorine compounds, including pesticide residues. The limit of qualitative determination of the method for 2,4-Dichlorophenoxyacetic acid is 0.04-0.8 mg/kg, the mass of the sample required for the work is 30 g. According to the control card of the condition of the premises during the study, the following conditions were observed throughout the experiment (Table 4).

Table 4. *Conditions for conducting the study*

Condition	Value
Ambient temperature	+22.8°C
Atmospheric pressure	101.4 kPa (761 mm Hg)
Relative humidity (at +20°C)	61%
Voltage in the electrical supply network	220 V
Frequency	50/60 Hz
Lighting	Natural, without direct sunlight on thin-layer chromatography plates
Elements required for maintaining biological safety	Availability of a hood for handling of organic solvents (acetone, ether)
Detection limit of pesticides in fish	0.6 mg/kg

Source: C. Poole (2023)

In the experiment room, the temperature was maintained at +22.8°C. This indicator is substantial for the stability of the physical and chemical properties of reagents, including standards and solvents, as well as for prevention of the influence of temperature fluctuations on the processes of extraction, sample application and migration of substances along the sorbent. The next indicator was atmospheric pressure. At the time of the study in the laboratory room, it was 101.4 kPa (which corresponds to 761 mm Hg). This pressure is standard for ensuring uniform evaporation of phases and normal volatility of organic solvents, which is a substantial prerequisite for effective chromatographic separation. For the successful completion of the study, the next condition was the relative humidity of the air. Its level was kept at 61% at a temperature of +20°C. This indicator is substantial for processing of sorbents that can absorb moisture from the air. Excessive humidity can affect the polarity of the stationary phase, reducing the clarity of the image on the chromatographic plate, causing blurring of spots and changing the nature of the distribution of substances. The electrical power supply of chromatographic equipment, auxiliary devices and lighting are also relevant factors for

the correctness of the studies. It was conducted from a standard electrical network with a voltage of 220 V and a frequency of 50/60 Hz. Throughout the entire period of analytical procedures, the parameters of the electrical network were stable, which negated possible interruptions in the operation of equipment, including drying and fume hoods, lighting devices and other equipment in the laboratory. The lighting in the laboratory was natural, but at the same time the requirements were met for the absence of direct sunlight on the surface of the chromatographic plates. This is due to the fact that direct sunlight has a negative effect on the photostability and thermal stability of the substances being analysed, especially regarding pesticides, the structure of which is photosensitive. The organisation of the workplace during the experiment was emphasised. The operating surface of the table on which the analytical equipment was placed was stable and sufficiently strong. All this was necessary to prevent even minor vibrations, which can negatively affect the reproducibility of the results and the accuracy of measurements, especially during handling of highly sensitive equipment.

Regarding compliance with biological safety requirements, all actions with reagents and samples were performed exclusively in a ShV-1 fume hood (Ukrainian company "Zapovit"). This was crucial during handling of organic solvents characterised by high toxicity and volatility (ether and acetone). The parameters and requirements of the work are presented in Table 5.

Table 5. *Parameters and operating requirements*

Parameter	Requirements
Plate	Silica gel 60 (thickness 250 microns)
Activation	110°C, 30 min
Sample application	Micropipette, distance between samples ⩾1 cm
Mobile phase	Hexane-diethyl ether-formic acid (50:50:2)
Development time	30-40 min in a closed chamber
Visualisation	Spraying $AgNO_3$ + NH_3, UV (254 nm)

Source: P. Malhotra (2023)

In the samples of the studied fish, the detection limit of pesticides was 0.6 mg/kg. This level of sensitivity of the method ensured efficient detection of the residual amount of toxicants, even incorporating their low content, which crucial for both food safety and the sanitary and epidemiology. The experiment was conducted following recommendations using Silica gel 60 (CAC/GL 40-1993, 2010). This sorbent is one of the most common in thin-layer chromatography applications and provides high resolution during the analysis of complex matrices. Uniform movement of the mobile phase and stability of the chromatographic process were guaranteed by plates with a silica gel layer 250 µm thick.

The sorbent was activated at a temperature of 110°C for 30 min before use to remove impurities and residual moisture that could affect the analysis results. Sample application was conducted manually using a micropipette. To achieve a separation of the components in the sample and avoid mixing of spots, the interval between applications of at least 1 cm was ensured. A mixture of organic solvents was used as the mobile phase: formic acid, diethyl ether and hexane in a ratio of 50:50:2. This composition provides optimal separation of the analysed substances due to compatibility with the sample matrix and effective elution ability. The development of the chromatogram was conducted in a hermetically sealed chromatographic

chamber, which ensured stable conditions for the movement of the mobile phase. The optimal development time was 30-40 min.

After the completion of chromatography, the chromatograms were visualised. For this purpose, a method was used in which the plate was sprayed with a solution of silver nitrate (AgNO3) followed by treatment with ammonia (NH3). Such treatment can detect compounds with high efficiency, which include functional groups capable of interacting with silver ions. To detect invisible spots in the study and final fixation of the results, ultraviolet radiation was used, the wavelength reached 254 nm. After preliminary sample preparation, chromatography and visualisation of pesticide spots on chromatographic plates, a quantitative assessment of the content of the analysed substances was performed. For this purpose, a planimetric method was used, based on measuring the area of the spots for both the samples under study and standard solutions.

To ensure the reliability of the results obtained and to control the accuracy of the analysis, the recovery coefficient of the analytical standard added to the sample was additionally calculated. This indicator was used to assess the accuracy of quantitative determination by the selected method and the efficiency of extraction. When validating the method for determining pesticides in food samples, one of the most relevant stages is the assessment of the efficiency and accuracy of the extraction, purification, and subsequent quantitative analysis process. For this purpose, the percentage of recovery of the analytical standard was calculated, which was used to determine what part of the introduced pesticide was detected after passing through the entire analytical process. This indicator is relevant for assessment of the reliability of the results obtained, since in the case of low analyte recovery, it is possible to state that certain substances were lost during analytical procedures (chromatography, concentration, filtration) or the extraction was ineffective. On the contrary, a high percentage of recovery indicates the correctness of the method and its suitability for further use in routine analysis. The application of samples was conducted manually, using a micropipette in the form of narrow or dotted lines, while maintaining a distance between them to effectively prevent mixing or overflow of components. After the chromatogram development process was completed, the plate was treated with a special reagent – a silver nitrate solution, followed by treatment with ammonia, which revealed traces of the substances under investigation.

Results and Discussion

Result of the gas chromatography method

One of the main food products studied in the experiment was powdered milk. For this purpose, a matrix of powdered milk with an additive was prepared (the additive was a standard of polychlorinated biphenyls). The results obtained in the process of scientific research are presented in the form of a chromatogram, which records signals from the target analytes and auxiliary components that were part of the sample (Fig. 2).

The chromatogram from the analysis of a powdered milk sample, to which a polychlorinated biphenyl standard was previously added to control the accuracy of the research results, is demonstrated above. This additive was used to verify the effectiveness of the method and calculate the percentage of analyte recovery during the analysis process. The study by B. Güzel & O. Canli (2022) confirmed that the gas chromatography method is sufficient for the rapid, selective and correct determination of indicator PCBs not only in food products, but also in solid samples of waste oils. Figure 2 demonstrates that the retention time (set in minutes) is plotted on the X axis, which characterises the moment the substance leaves the column, and the detector signal (expressed in microvolts) is plotted on the Y axis, which indicates the concentration of the corresponding compound.

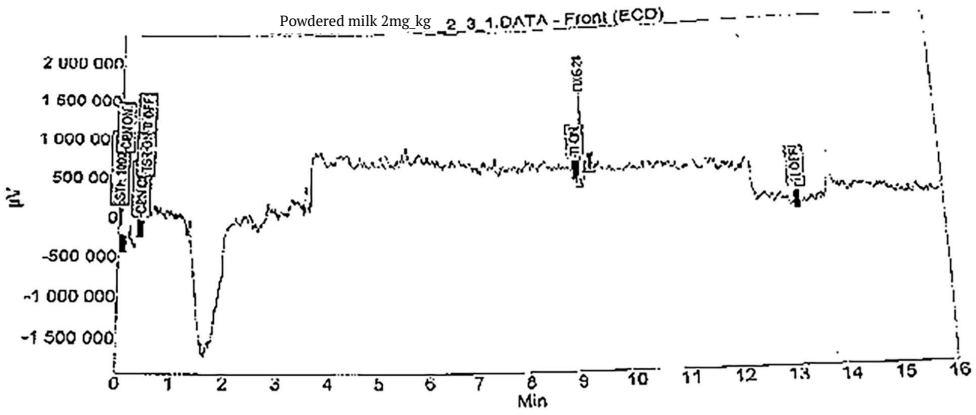

Figure 2. Results of the experiment on powdered milk

Source: compiled by the authors using CompassCDS software

At the beginning of the chromatographic analysis, at approximately 1.5 min, a pronounced hexane peak is notable. This is the solvent that was used during the extraction of the analysed sample. Despite the fact that hexane is not the target analyte, presence is normal, since it is part of the sample that was introduced into the chromatograph. Its presence is also due to the fact that hexane was used not only as an extractant during sample preparation, but also for washing the chromatograph liner before each sample injection. As an analytical substance, hexane does not interact with the electron-capture detector, therefore its presence is manifested in the form of a negative (downward) signal. This is a normal phenomenon for the used detectors, which does not affect the analysis results. This peak then reduces, hence, has a negative signal value recorded by the detector. This direction of the peak is determined by the specifics of the detector operation and the physical properties of hexane.

The main peaks, which appeared in the interval from approximately 9 to 14 min, correspond to the standards of the PCB component. Their retention time coincided with the corresponding standard substances, which made it possible to confirm the presence of PCBs in the sample and perform quantitative determination. The peak is symmetrical, well distributed and has a clearly defined shape, which indicates optimally selected chromatography conditions and correct operation of the column. The intensity of the peak was used for quantitative determination of the analyte content by comparison with calibration standards. The performed chromatographic analysis confirms the effectiveness of the sample preparation method and ensures confident conclusions on the quality and safety of the milk powder samples that were examined. The obtained level of chromatographic separation could only be achieved if the controlled analysis conditions were carefully observed. F. Wang *et al.* (2022), in their studies of dairy products, also confirmed the satisfactory linearity, sensitivity, accuracy, and precision of the gas chromatography method.

The stable temperature of the chromatograph oven ensured linear evaporation of the sample components, which in turn prevented the displacement and superposition of the analytes on each other. In this experiment, a temperature programme was used that consolidated the initial isothermal heating with a gradual increase in temperature in the future, which ensured efficient separation of both heavy and lighter chlorinated components. In addition to temperature control, a substantial factor that

ensured the clarity of the signal was the stability of the carrier gas (flow) – nitrogen. Even a slight fluctuation in the flow rate or pressure could cause their deformation or shift the time of peak emergence. However, in the chromatogram image presented above, exceptional symmetry is observed, which in turn indicates the absence of turbulence in the column and the accuracy of the system calibration.

The low level of the background line shown in the graph, which is almost noise-free, is notable. This is evidence that the sample introduction liners were thoroughly cleaned, the column phase was maintained in proper condition, and the system was free of contaminants. Background noise is often a positive indicator of the quality of the analytical process: under conditions of temperature fluctuations or uncontrolled humidity, it increases, which significantly complicates the detection of small concentrations. In this case, there is no such effect in the image, so the analysis was conducted under conditions that are maximally conducive to analytical reliability and accuracy. After the procedure for preparing a powdered milk sample with the addition of a standard was performed and gas chromatographic analysis was performed using an electron capture detector, one main peak was recorded in the processed chromatogram file, which corresponds to the substance PCB-28 (Table 6).

Table 6. *Peak study results*

Index	Name	Time, min	Quantity, mkg/mL	Area, pV min	Height, uV	Signal/noise	Result, mg/kg
1	PCB 28	9.12	0.0994	43,838.7	990,555.0	1.25	0.144
Total			0.0994	43,838.7	990,555.0		

Source: compiled by the authors

Table 6 demonstrates that the retention time of the substance on the column was 9.12 min, which confirms its identification based on comparison with the standard sample. According to the software CompassCDS used for analysis, the following peak parameters were determined:

➢ Analyte concentration Quantity (μg/mL) – this is the calculated concentration of the analyte in the extract, which was 0.0994 μg/mL.

➢ Area (peak area) – 43,838.7 pKV min, which is proportional to the amount of substance in the sample. The larger the peak area – the higher the concentration.

➢ Height (peak height) – 990,555.0 μV, which indicates the intensity of the signal recorded by the detector.

➢ Signal/noise – 1.25, i.e. the signal is more than 1 time higher than the noise level. This is an acceptable ratio for low concentrations, although ideally it should be higher.

➢ Result, mg/kg – recalculation of the amount of analyte considering the volume of extract and the mass of the sample. In this case, the result was 0.144 mg/kg.

The peak characteristics of PCB-28 fully corresponded to the expected parameters of the standard. The accuracy and reliability of the analyte detection were clearly indicated by the presence of a clearly pronounced peak, the correspondence of the retention time and the stability of the signal.

The results of the work by thin layer chromatography method

The establishment of biological safety and norms compliance of the thin-layer chromatography is also a priority of the experiment. In the

specified subsection of the experiment, the main sample (matrix) was a food product in the form of fish (carp), and the main additive was 2,4-D amine salt in the amount of 1 mg/kg. The results obtained indicate that the method is highly efficient and sufficiently accurate, since the level of permissible return in the experiment is from 70 to 120%, depending on the type of research being conducted and the sample matrix.

Figure 3 shows a chromatographic plate after the completion of the analysis of a fish sample by thin-layer chromatography. This image demonstrates the practical result of the study performed and confirms the efficiency of the applied method for detection of residual amounts of the pesticide 2,4-D amine salt. Traces of chromatographic separation are evident on the surface of the plate, namely grey or dark spots that were formed as a result of the movement of substances along the sorbent layer under the influence of the mobile phase. Thanks to these manipulations, characteristic spots appeared on the plate, which differed in size and colour depending on the amount of analyte in the sample. The figure demonstrates the difference between the spots of the test and standard samples, which highlights the presence of traces of PCBs in the sample, and can be used for quantitative determination based on the ratio of areas. The shape of the spots is clear, mostly oval or elliptical, with even contours, which indicates the quality of the sorbent, the correctness of the plate preparation and stable conditions for conducting the analysis. The spot of the test sample is located at the same level with the spot of the standard, with an identical Rf value, i.e. the ratio of the migration distance of the substance to the front of the mobile phase. This, in turn, confirms the identity of the substance detected in the sample under study with the specified standard 2,4-D.

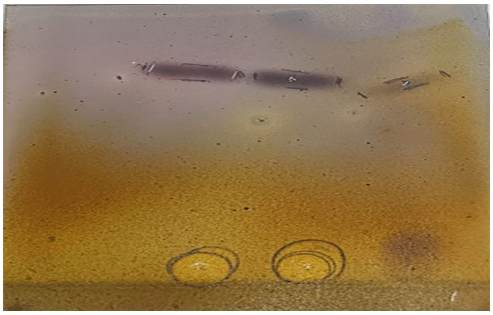

Figure 3. Result of the thin layer chromatography method
Source: compiled by the authors

The stability of the conditions of the experiment was confirmed by the visual appearance of the chromatographic plate. Thus, the clarity and shape of the spots indicated that the temperature and humidity remained within the permissible values, and the absence of smearing or blurring indicated the effective avoidance of excess moisture. The spots did not lose their colour intensity due to the absence of direct sunlight, and this moment is relevant when visualising with silver nitrate. Summarising the above, it is possible to state that the resulting image is not only the result of the reaction but also an indicator of the correctness of the entire process. In general, Figure 3 illustrates the final stage of the experiment, which shows not only the detected target analyte in the food sample, but also the quality of compliance with the methodological instructions. The resulting visual result provides not

only analytical, but also visual confirmation of the reliability of the research conducted. Therefore, it is possible to conclude that the applied methodology was sensitive, reliable, and effective for controlling pesticide residues in fish products. Similar results were obtained by A. Tan *et al*. (2025) in an analysis of the detection of residues of banned substances in shrimp based on the combined method of thin-layer chromatography and surface-enhanced Raman spectroscopy. The results demonstrated the effectiveness of the TLC-SERS method (combines thin-layer chromatography with surface-enhanced Raman spectroscopy) for the rapid, sensitive and accurate detection of residues of banned substances in seafood, which has significant implications for monitoring the quality and safety of food products.

Relevant areas such comparative analysis of the two applied research methods – thin-layer chromatography and gas chromatography, is notable. Both methods are quite effective, but at the same time they differ in sensitivity, accuracy and principle of operation, which makes it possible to assess compliance with the standards of biological safety of food products more fundamentally. Similar findings were reported in other studies. For instance, Y. Li *et al.* (2023) confirmed that liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-MS/MS (GC-MS/MS) are the main methods with excellent selectivity, sensitivity and specificity used for target analysis. LC-MS/MS and GC-MS/MS can also be used simultaneously to determine several HCPs with instrumental detection limits down to 0.010 ng mL −1, such as PBDEs, polychlorinated biphenyls and organochlorine pesticides.

Gas chromatography was used to analyse milk powder samples, which is a method used to detect even microscopic levels of harmful substances with high reproducibility of results with high accuracy. This subsequently prevented the presence in products even in minimal concentrations. Y. Shao *et al.* (2023) also confirmed that approaches such as thin-layer chromatography, high-performance liquid chromatography, gas chromatography, and high-performance liquid chromatography/gas chromatography-mass spectrometry are successfully used for pesticide detection. This method was described as highly efficient in the analysis of chlorine-containing organic compounds due to the high sensitivity of the detector, modern equipment and automated sample introduction. Thin-layer chromatography, which was used at that time to examine fish samples, is an accessible method that can quickly assess the presence of pesticides and does not require complex laboratory equipment. However, this method had a lower sensitivity and depended largely on visual assessment of the result. Therefore, the use of both methods in one scientific study ensure an integrated approach to assessing the safety and quality of food products.

Conclusions

Based on the conducted scientific research, using gas and thin-layer chromatography methods, residual amounts of polychlorinated biphenyls and pesticides were detected in food products of animal origin, namely powdered milk and fish. For the analysis of powdered milk samples, gas chromatography was used – a method that can detect even microscopic levels of harmful substances with high reproducibility of results and high accuracy. This subsequently prevented their presence in products even in minimal concentrations. This method was highlighted as a significant level of efficiency for the analysis of chlorine-containing organic compounds due to the high sensitivity of the detector, modern equipment and automated sample introduction. To verify the accuracy and reliability of the method, the percentage of analyte recovery was calculated, which was within the range of 70-120%, which, in turn, confirms the reliability and accuracy of the method. Thin-layer chromatography, which was used at that time to study fish

samples, is an accessible method that can quickly assess the presence of pesticides and does not require complex laboratory equipment. In the samples of fish studied, the detection limit of pesticides was 0.6 mg/kg. The main peaks that appeared in the time interval from approximately 9 to 14 min correspond to the standards of the PCB component. Their retention times coincided with the corresponding standard substances, which confirmed the presence of PCBs in the sample and was used for a quantitative determination. Further research on this topic should continue the study and use chromatographic methods to determine the presence of any harmful substances not only in animal products, but also in other food products.

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Conflict of Interest

None.

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Газо- та тонкошарова хроматографія в контролі залишкових кількостей поліхлорованих біфенілів і пестицидів у харчових продуктах тваринного походження

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Анотація. Біологічна безпека харчових продуктів тваринного походження тісно пов'язана з соціально-економічним розвитком суспільства та станом навколишнього середовища. Метою проведеного дослідження було здійснити перевірку наявності залишкової кількості поліхлорованих біфенілів і пестицидів в харчових продуктах тваринного походження методами газової та тонкошарової хроматографії. Для аналізу зразків сухого молока використовували газову хроматографію на приладі Bruker Scion 456-GC з електронно-захоплюючим детектором (ECD), автосемплером і високоточним температурним контролем. Екстракцію поліхлорованих біфенілів проводили гексаном з наступним очищенням системою hexane-acetonitrile та адсорбційною хроматографією на силікагелі. Калібрування виконували стандартом Supelco PCB Mix у діапазоні концентрацій 0,001-0,250 мкг/см^3 з коефіцієнтом кореляції R^2 = 0,9999. Для дослідження зразків риби застосовували тонкошарову хроматографію з попереднім кислотним гідролізом проби та екстракцією діетиловим ефіром. Візуалізацію хроматографічних зон проводили обробкою пластин розчином нітрату срібла з наступною обробкою аміаком та УФ-опроміненням при 254 нм. Відсоток повернення аналіту перебував у межах 70-120 %, що підтверджує надійність і точність проведених методів. У зразках досліджуваної риби межа виявлення пестицидів становила 0,6 мг/кг, що дозволило ефективно знаходити залишкову кількість токсикантів навіть при їх низькому вмісті. Основні піки на хроматограмі молока з'являлися в інтервалі від 9 до 14 хвилин і відповідали стандартам компонентів поліхлорованих біфенілів, а час їх утримання співпадав із відповідними стандартними речовинами, що дало можливість підтвердити наявність поліхлорованих біфенілів у зразку та здійснити кількісне визначення. Цінність виконаної роботи полягає в забезпеченні сучасною, достовірною та ефективною практико-орієнтованою інформацією з оцінки безпечності харчової продукції як відповідних лабораторій і установ, так і освітніх закладів, що спеціалізуються на підготовці фахівців за спеціальністю «Біотехнологія та біоінженерія»

Ключові слова: біобезпека; аналіт; стандарти; температура; прилад