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# Determination of Pesticide Residues by Chromatographic Methods for Food Safety

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**Abstract.** The paper presents a methodology for the preparation of samples of oilseeds, lettuce leaves, apple fruits for the study of xenobiotics by chromatographic control of the following chemical groups of pesticides: benzimidazole derivatives, anilinopyrimidine derivatives, bipyridyl derivatives. The following processes are considered: homogenisation of the sample, purification of the extract by solid-phase or liquid-liquid extraction methods, obtaining of plant extract, and obtaining of analytes extract. For fine-grained homogenised samples of sunflower seeds, the optimal raw material-extractant ratio is 1:20, for paste-like homogenised samples of apple fruit – 1:10, and for liquid samples of homogenised lettuce – 1:5. Analysis of the distribution of xenobiotics parameter in the octane/water system (log Pow), reference data on dielectric constant and dipole moment of solvents allowed identifying extractants capable of dissolving and removing xenobiotics from raw materials. It has been found that a mixture of acetonitrile and methanol (4:1) should be used for the extraction of benzimidazole derivatives and anilinopyrimidine derivatives, bipyridyl derivatives are best extracted with methanolic trifluoroacetic acid (9.5:0.5). The content of xenobiotics in extracts obtained from samples artificially enriched with xenobiotics was quantitatively analysed. Xenobiotics were removed from samples of crop products containing traces of fat. The most difficult process of sample preparation is the process of extracting sunflower seeds. The content of xenobiotics in extracts obtained from samples artificially enriched with analytes is affected by the temperature at which the process takes place and the duration of extraction. Based on the chemical composition of the sample matrix and the list of analytes, the conditions of the variable component of the methodology are proposed: obtaining plant extracts under the action of selective solvents, homogenised raw material-solvent with constant stirring of the extraction system at a speed of 180-200 rpm, or when exposed to ultrasonic vibrations at a frequency of 37 kHz from 4 °C to 25 °C for 5-25 minutes. Control of the qualitative and quantitative composition of the studied plant extracts and extracts of analytes was studied by methods of high-performance liquid and gas chromatography (liquid and gas) with mass-selective detectors

**Keywords**: xenobiotics, pesticides, benzimidazole derivatives, anilinopyrimidine, bipiridium, extracts, chromatography, plant extracts

## RELEVANCE

Crop production today is divided into products obtained by classical agricultural technology, which involves the use of agrochemicals and organic products. In the process of cultivation of leaf and stem vegetables, oilseeds, fruits of pome crops, and plant protection products are used, the active components of which are pesticides of different groups (Sekun M.P., 2007). At the same time, residual amounts of pesticides are xenobiotics and, together with man-made pollutants, are normalised in accordance with sanitary and hygienic rules and regulations. Their content is controlled by certain laboratories according to standardised methods (DSanPYN 8.8.1.2.3.4-000-2001). Given the approaches to analysing the content of xenobiotics, there is a need to develop a methodology for the analysis of residual amounts of pesticides of different chemical groups: mixtures of bipyridyl, benzimidazole and anilinopyrimidine derivatives in seeds, green parts and fruits of agricultural crops.

## ANALYSIS OF RECENT RESEARCH AND PUBLICATIONS

At the present stage, the methodology for measuring safety indicators in crop production is actively developing. Laboratory monitoring of such xenobiotics as a group of polycyclic aromatic hydrocarbons (PAHs) that can accumulate in oilseeds is considered in many studies (Nesterova L.O., Grybova N.Yu., Khyzhan O.I., 2018; Gribova N.Yu., 2018). The paper describes the study on xenobiotics by high-performance liquid chromatography using a fluorescence detector after the application of solid-phase extraction of analytes. Modernisation of methods for the determination of pesticide residues can include the following stages of sample preparation for research, and instrumental studies of plant extracts and analytes of plant extract purified from co-extractive chemicals. For the isolation of target analytes, specific extractants can be used, along with sorbents, and various physicochemical factors that can change the rheological properties of extraction systems and can intensify the yield of plant homogenised raw materials to the extract of the necessary components (Melo, A. *at al*., 2012; Gribova, N.Y. at al. 2008).

When determining the content of xenobiotics belonging to different groups of pesticides, the most commonly used method is QuEChERS – quick, easy, cheap, effective, rugged and safe (Document No SANCO/12495/2011, 2014). It should be noted that this method of laboratory monitoring does not allow for the removal of xenobiotics, such as surfactant compounds and pesticides of the bipyridyl group – chlormequat, diquat, paraquat. It is known that the spectrum of application of bipyridylium derivatives is sunflower plants, and seed crops of cabbage, table beet, carrot and radish, potato plants, sorghum, clover, fodder beans, soybeans, alfalfa.

Bipyridilium derivatives are quickly absorbed by green parts of the plant, while the contact active ingredient – diquat, quickly turns into hydrogen peroxide, which leads to the destruction of the cell membrane and drying of plants. The active substance decomposes quickly enough in the plant, so the use of diquat is considered relatively safe on seed crops, and on crops intended for food purposes.

The effect of such a drug occurs almost immediately after application, meanwhile, the visual effect of desiccation is already noticeable in 4-7 days. Based on research results (Sekun M.P., 2007) bipiridium derivatives act quickly and, depending on environmental conditions, are consumed in 50-98 hours. Therefore, laboratory monitoring of the content should be conducted with due regard to the timing of the diquat application. State sanitary rules and norms "DSanPiN 8.8.1.2.3.4-000-2001" for diquat set the maximum content of residual amounts in sunflower seeds – 0.5 mg/kg, but at the same time the research methodology is not specified. Therefore, the purpose of the study is to identify the optimal conditions of sample preparation of plant products for the extraction of xenobiotics and establish their qualitative and quantitative content by chromatographic methods of laboratory monitoring.

## MATERIALS AND METHODS

The following samples of crop production were used for the research: lettuce leaves of different varieties, oilseeds (sunflower, soybean, flax), fruits of different varieties of apples. A number of parallel laboratory samples were run, of which three samples were subject to artificial enrichment with target xenobiotics. Homogenisation of samples was performed by grinding in a glass of a laboratory homogeniser mill, at temperatures from +4°C to +25°C. To obtain the plant extract, the following chemicals were used (GR): acetone, acetonitrile, methanol, n-hexane, toluene, acids (formic, acetic, trifluoroacetic,

hydrochloric), and isopropanol. These compounds were used as individual extractants or in mixtures, including with deionised water. To buffer the solution of the layer of the homogenized sample under study and in the process of purification of the plant extract, the following chemicals of GR (Guaranteed Reagent) qualification were used: sodium chloride, magnesium sulfate, sodium citrate, aluminium oxide, calcium chloride.

The extraction was carried out in polytetrafluoroethylene tubes, dark glass flasks and polymethyl pentene flasks protected by light-tight covers. During the extraction of analytes, mass transfer was intensified by varying the ratio of raw material-extractant, subjected to stirring, temperature, ultrasonic waves with a frequency of 37 kHz (generated by Advantage Lab**)**. Phase separation of the extraction system was performed using a Thermo Scientific centrifuge, 10 min at 7000 rpm, 4°C. The obtained plant extract was subject to purification from co-extractive compounds by dispersion extraction or liquid-liquid extraction methods using organic solvents and Supelco cartridges filled with mixtures of primary and secondary amines. The concentration of the purified extract was carried out in a rotary evaporator IKA. Analysis of the number of xenobiotics in the obtained solutions was performed by high-performance liquid and gas chromatography using mass spectrometric detectors (HPLC/MS/MS and GC/MS) on Dionex and Agilent chromatographs. The results of analytical signals and spectra of analytes were processed using the calibration dependencies and databases of Cromeleon 6.0 and the installed library of mass spectra (NIST 0.5).

## RESULTS AND DISCUSSION

The preparation of a laboratory sample for analysis begins with the same stage for different food groups – homogenisation of the sample. In this study, the sample was homogenised in a beaker mill homogeniser at a speed of 10000 rpm. The obtained homogenised samples differed in their aggregate state due to the different chemical compositions of each matrix: pasty, fine-grained, and liquid. According to the published data on the nutritional value and chemical composition of the sample matrix (Document No SANCO/12495/2011, 2014) the mass fractions of W, % of chemical compounds ($m_{\text{-component}}$) of the matrix were calculated using the formula (1.1) and presented in Table 1:

$$W = \frac{m_{\text{component}}}{m_{\text{homogenized sample}}} \times 100\%. \qquad (1.1)$$

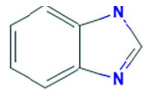
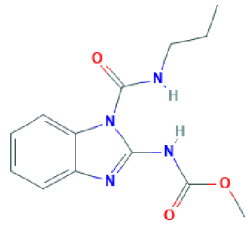
**Table 1.** Mass fraction of compounds in crop production samples

| Sunflower seed kernels | Iceberg lettuce leaves | Fruits of apples with peel |
|---|---|---|
| fats 51.46%; | fats 51.46%; | fats 51.46%; |
| proteins 20.78%; | proteins 20.78%; | proteins 20.78%; |
| hydrocarbons 10.21%; | hydrocarbons 2.97%; | hydrocarbons 2.97%; |
| water 4.73%; | water 4.73%; | water 85.56%; |
| other chemical compounds 12.82%. | other chemical compounds 12.82%. | other chemical compounds 12.82%. |

As shown in Table 1, homogenised sunflower seeds contain a significant amount of fat, and the main component of homogenised samples of lettuce leaves and apple fruits is water. Polar and non-polar, proton and antiproton solvents and their mixtures were used in the study. To create more optimal conditions for the extraction of xenobiotics from homogenised raw

materials, additives of organic and mineral acids were used to shift the equilibrium of the dissociation process of ionogenic xenobiotics towards the formation of molecules. The optimal ratio between the extractant and homogenised sample was established by visual analysis of the appearance of the extraction systems and verified by chromatographic analysis of the content of markers (Benomyl, Ciprodinil, Diquat) in plant extracts. For liquid samples of homogenised lettuce leaves, the optimal raw material-extractant ratio is 1:5, for paste-like homogenised samples of apple fruits, the ratio is 1:10, for fine-grained homogenised samples of sunflower seeds – 1:20. At the defined ratios, with constant stirring of 180-200 rpm or under the effect of ultrasonic vibrations with a frequency of 37 kHz in the extraction system, the absence of zones of compression of raw materials is established. This is a necessary condition for the realisation of the mass transfer of xenobiotics from particles of plant material into the hood due to convective diffusion. Xenobiotics belonging to the same chemical group usually differ in the presence of hydrocarbon substituents in the molecule (Table 2). These substitutes can affect the pesticide and toxic properties of the active ingredient of plant protection products, and change the physical and chemical properties (Sekun M.P., 2007). The analysis of the chemical structure of xenobiotics and the distribution parameter of xenobiotics in the octane/water system (log Pow), reference data of dielectric constant and dipole moment of solvents, made it possible to determine and analyse the effect of extractants capable of dissolving and extracting xenobiotics from raw materials, namely acetonitrile, methanol, acetone, n-hexane, toluene, isopropanol, solutions of acetic, formic, trifluoroacetic and hydrochloric acids.

**Table 2.** Hydrophobicity parameters of some xenobiotics

| Name of the chemical compound | Structural formula | log Pow |
|---|---|---|
| Benzimidazole |  | 1.3 |
| Benomil |  | 1.4 |

Analysing the data presented in Table 2, it was assumed that such xenobiotics will concentrate in the organic layer of the extraction system. Extraction of xenobiotics by various extractants and quantitative analysis of xenobiotics in extracts using chromatographic methods (Fig. 1, Table. 3), showed that for the extraction of benzimidazole derivatives and anilinopyrimidine derivatives, it is necessary to use a mixture of acetonitrile with methanol (in a ratio of 4 : 1), bipiridium derivatives are best extracted with a methanol solution of trifluoroacetic acid (at a ratio of 9.5 : 0.5).

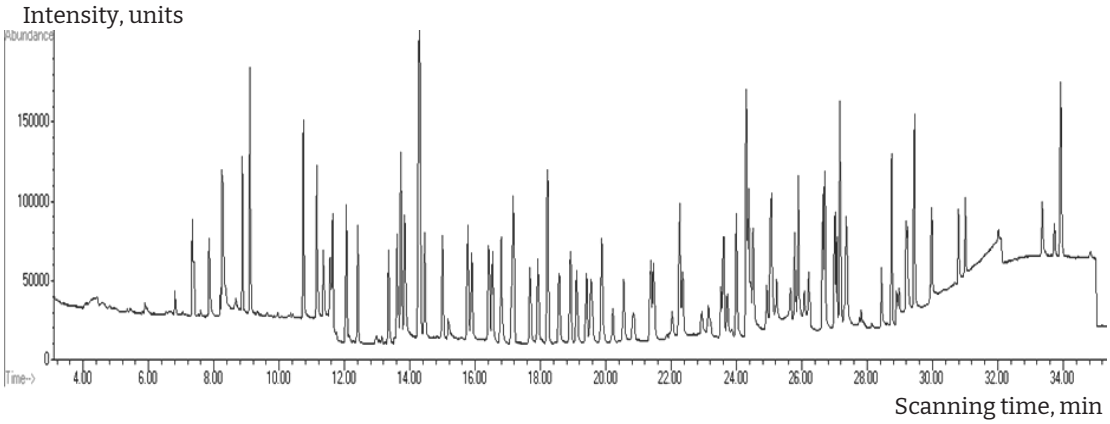



**Figure 1.** . Chromatogram of plant extract derived by exposure to acetonitrile : methanol (4 : 1) on homogenised apple fruits artificially enriched with xenobiotics (Benomyl, Ciprodinil, Diquat)

**Table 3.** Quantitative analysis of xenobiotic content in extracts obtained from samples artificially enriched with xenobiotics

| Marker name | Extractant | Introduced, µg/kg | Determined, µg/kg | Removals, % |
|---|---|---|---|---|
| Sunflower seeds | | | | |
| Benomil | mixture of acetonitrile and methanol (4:1) | 0.50 ± 0.01 | 0.41 ± 0.03 | 82.0 ± 6 |
| Ciprodinil | | 0.50 ± 0.01 | 0.40 ± 0.03 | 80.0 ± 6 |
| Diquat | methanol solution of trifluoroacetic acid | 0.50 ± 0.01 | 0.43 ± 0.03 | 86.0 ± 6 |
| Lettuce leaves | | | | |
| Benomil | mixture of acetonitrile and methanol | 0.50 ± 0.01 | 0.49 ± 0.01 | 98.0 ± 2 |
| Ciprodinil | | 0.50 ± 0.01 | 0.49 ± 0.02 | 98.0 ± 4 |
| Diquat | methanol solution of trifluoroacetic acid | 0.50 ± 0.01 | 0.51 ± 0.03 | 102.0 ± 6 |
| Apple fruits | | | | |
| Benomil | mixture of acetonitrile and methanol | 0.50±0.01 | 0.48±0.01 | 96.0±2 |
| Ciprodinil | | 0.50±0.01 | 0.49±0.02 | 99.0±4 |
| Diquat | methanol solution of trifluoroacetic acid | 0.50±0.01 | 0.47±0.02 | 94.0±4 |

Peaks were identified from the library of mass spectra by comparing two parameters: the retention time of the xenobiotic peak and the retention time of the analytical standard peak, the values of characteristic ions of xenobiotics and the corresponding values of analytical standards. Quantitative analysis was carried out by calibration dependences, the mean value and error of he value obtained during the measurement were calculated using the Exel programme.

Table 3 shows that xenobiotics were completely removed from samples of crop products

containing trace amounts of fat (Table 2). The most difficult from the point of view of conducting the process of sample preparation for research and according to the percentage of extraction of xenobiotics (Table 3), is the process of obtaining a plant extract from sunflower seeds. The percentage of isolated xenobiotics from sunflower seeds is less than the percentage of the same markers extracted from lettuce leaves and apple fruits. However, the process of performing sample preparation of sunflower seeds is satisfactory, since the established average percentage of extraction of an artificially introduced xenobiotic is more than 80%. The content of xenobiotics in extracts obtained from samples artificially enriched with analytes (Table 3) is affected by the temperature at which the process takes place and the duration of the extraction. Optimal conditions are the temperature from 4°C to 25°C and exposure to the extractant for 5-25 minutes.

## CONCLUSIONS

Analysis of the quantitative and qualitative content of xenobiotics in plant extracts and extracts purified from coextractive substances made it possible to establish the optimal conditions for the variable component of the methodology for preparing samples of crop production to the stage of determining the content of xenobiotics by chromatographic methods of laboratory control. Optimal extractants are mixtures of acetonitrile with methanol (4:1) and a mixture of methanol with trifluoroacetic acid (9.5:0.5). The ratio of components of the extraction system varies depending on the aggregate state of the homogenised sample. It was found that the optimal ratio of raw materials and extractant for sunflower seeds is 1:20, for apples – 1:10, for lettuce leaves – 1:5. Extraction is carried out under conditions of constant stirring of the extraction system at a speed of 180-200 rpm, or by exposure to ultrasonic vibrations at a frequency of 37 kHz at a temperature from 4°C to 25°C for 5-25 min.

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## Визначення залишкових кількостей пестицидів хроматографічними методами для безпечності харчових продуктів

**Анотація.** У статті представлено методику підготовки зразків насіння олійних культур, листя салату, плодів яблуні для дослідження ксенобіотиків методом хроматографічного контролю наступних хімічних груп пестицидів: похідні бензімідазолу, похідні анілінопіримідину, похідні біпіридилу. Розглядаються наступні процеси: гомогенізація зразка, очищення

екстракту методами твердофазної або рідинно-рідинної екстракції, отримання рослинного екстракту, отримання екстракту аналітів. Для дрібнозернистих гомогенізованих зразків насіння соняшнику оптимальне співвідношення сировина-екстрагент становить 1:20, для пастоподібних гомогенізованих зразків плодів яблук - 1:10, а для рідких зразків гомогенізованого салату - 1:5. Аналіз розподілу параметра ксенобіотиків у системі октан/вода (log Pow), довідкових даних щодо діелектричної проникності та дипольного моменту розчинників дозволив визначити екстрагенти, здатні розчиняти та вилучати ксенобіотики із сировини. Встановлено, що для екстракції похідних бензімідазолу та анілінопіримідину доцільно використовувати суміш ацетонітрилу та метанолу (4:1), біпіридильні похідні найкраще екстрагуються метанол-трифтороцтовою кислотою (9,5:0,5). Вміст ксенобіотиків в екстрактах, отриманих із зразків, штучно збагачених ксенобіотиками, було проаналізовано кількісно. Ксенобіотики вилучали із зразків рослинницької продукції, що містять сліди жиру. Найскладнішим процесом пробопідготовки є процес екстрагування насіння соняшнику. На вміст ксенобіотиків в екстрактах, отриманих із зразків, штучно збагачених аналітами, впливає температура, при якій відбувається процес, і тривалість екстракції. Виходячи з хімічного складу матриці зразків та переліку аналітів, запропоновано умови проведення варіативної складової методики: отримання рослинних екстрактів під дією селективних розчинників, гомогенізованої системи сировина-розчинник при постійному перемішуванні екстракційної системи зі швидкістю 180-200 об/хв, або при дії ультразвукових коливань з частотою 37 кГц від 4 °С до 25 °С протягом 5-25 хвилин. Контроль якісного та кількісного складу досліджуваних рослинних екстрактів та екстрактів аналітів вивчали методами високоефективної рідинної та газової хроматографії (рідинної та газової) з мас-селективними детекторами

**Ключові слова:** ксенобіотики, пестициди, похідні бензімідазолу, анілінопіримідин, біпіридій, екстракти, хроматографія, рослинні екстракти