Creation and experience of using a multi-method for controlling pesticides in grain on the territory of the Russian Federation

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Abstract. This article is devoted to the development and testing of a multimethod for determining pesticides in grain, legume and oil crops using gas chromatography with mass spectrometric detection. Optimal parameters of sample preparation and chromatograph-mass spectrometric determination for the simultaneous analysis of 430 substances were established. Metrological characteristics such as uncertainty, repeatability and precision were calculated. The method was tested at the Moscow branch of the All-Russian Research Institute of Animal Husbandry on 120 samples of grain, legume and oil crops; these data are also presented in the article.

1 Introduction

Russia remains one of the main suppliers of grain on the world market, therefore, to Russia remains one of the main suppliers of grain to the world market, therefore, to ensure global food security, it is important to maintain high grain yields. In modern agriculture, high yields are ensured through the use of chemical plant protection products, starting with the processing of seed material and ending with the processing of the crop at the storage stage. To date, more than 400 medicines have been registered in the pesticide catalog, [1] this list is regularly updated.

The use of modern agricultural technologies in crop production is reduced to the use of new plant protection products during the growing season from among pesticides. In addition, in the agrotechnology of growing row crops, they resort to the desiccation process – this is the chemicalization of fields with pesticides, aimed at reducing grain moisture, thereby reducing losses during harvesting, but leading to additional chemicalization of crop products. There is also a whole arsenal of agrochemicals for harvesting, which are used in the grain storage process. These measures are aimed at combating the vital activity of pests of grain stocks [5-6].

Therefore, it can be assumed that against the background of the use of modern agrotechnologies with the use of chemicals, contamination of crop products, in particular grain, with decay products of pesticide action is possible and the development of new methods of combating pesticides in products is an urgent task. Thus, there is intense

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pollution of soil, water and air, the degree of negative impact of which has not been fully studied. This approach to farming is harmful not only to our health, but also to the general state of the environment. The influence of pesticides, which create a danger of chemical pollution of environmental objects and food products of the population, is noted. Pesticides are toxic substances of various hazard classes.

They can have a negative impact on the ecosystem and human health [7]. According to the International Health Organization, of the 320 pesticides approved for use, 66 are proven carcinogens. About 25 million people get sick due to the use of excessive amounts of pesticides, almost 500 thousand annually [16]. People with obesity and an increased content of organochlorine pesticides in the body have a higher risk of developing type 2 diabetes mellitus [8]. Pesticides acting as a fake version of natural hormones lead to metabolic syndrome and obesity [7]. More than 260 different studies have identified a link between pesticides and cancer, including lymphoma, leukemia, soft tissue sarcoma, brain, breast, prostate, bone marrow, thyroid, liver, lung and colon cancers. Pesticides also have a negative effect on the liver. Most often, it is workers of large industrial enterprises associated with chemical production who are diagnosed with toxic cirrhosis of the liver. This disease is especially common among employees of enterprises where pesticides, insecticides, phosphorus, arsenic, phenol and some other substances are involved in production processes [2].

Many effects of pesticide poisoning are difficult to diagnose directly, since as a result of their exposure, symptoms inherent in the clinical picture of completely different diseases arise [16]. Children are especially sensitive to pesticides. Pesticides have been proven to cause attention deficit hyperactivity disorder [2]. The Technical Regulation of the Customs Union "On Grain Safety" (TR CU 015/2011) establishes restrictions on the residual content of plant protection products in food grains, which ensures the free movement of grain released into circulation in the territory of the Customs Union, however, the movement of commercial grain and its processed products is prohibited. It is carried out outside the Customs Union, which makes it necessary to strengthen control over the residual content of pesticides in food grains and processed products. To do this, it is necessary to control the grain in accordance with the requirements of the importing countries. Thus, in total, in order to meet all the requirements, it is necessary to control about 1000 different pesticides in grain.

Residual amounts of pesticides are determined using chromatography-mass spectrometry [1, 4], high-performance liquid chromatography (HPLC) [8-11], polarography [12] and gas chromatography [13-14]. These papers propose methods for determining individual pesticides, but the number of pesticides used in the world has increased significantly, and their definition does not cover the entire list of pesticides.

Methods for simultaneous determination of large amounts of pesticides are gaining popularity all over the world, for example, the EN 15662 method. This method is applicable to a large list of plant products. We have developed a methodology for the determination of 430 pesticides by the GC MS method, designed for grain as the main product of Russian exports. This technique is certified and tested.

2 Materials and methods

The following measuring equipment was used in the work:

GCMS-TQ8050 gas chromatograph mass spectrometer with a SH-Rxi-5ms capillary chromatographic column, 30 meters long, 0.25 mm internal diameter, Sartorius BP 110S precision class I scales, Sartorius Biohit Proline Plus single-channel mechanical dispensers with variable capacity (20…200) µl and (100…1000) µl.

The following reagents were used: HPLC acetonitrile and HPLC water from Panreac; QuEChERS extraction kits for pesticides (LabStandard® QuE-Lab EN 15662:2018 LLe Citrate) and QuEChERS extract purification kits (LabStandard® QuE-Lab EN 15662 PSA/C18EC dSPE) from LabInstruments; LabStandard® KIT4BB3L474 pesticide standard sample solution kit from LabInstruments.

To determine the metrological characteristics of the measurement method, the measurement results obtained by measuring the mass fraction of the active substance in samples of grain and oilseed crops are provided. Samples with the introduced additive of the required analytes are used as samples for certification. In total, 4 experiments with 6 parallel determinations by different operators on different days are carried out for each sample. The experiment is carried out at 4 concentration levels: 1 μg/kg, 5 μg/kg, 10 μg/kg, 100 μg/kg.

The following metrological characteristics were calculated for the method: repeatability limit, intralaboratory precision limit, expanded uncertainty.

This method has passed state certification and is used in laboratories of the Russian Federation for grain control. The work also includes a review of data on the detection of pesticide content obtained using this method.

The methodology was tested on 120 samples, of which 50 samples were cereals (wheat, rye, barley, corn), 50 samples were legumes (peas, soybeans) and 20 samples were oilseeds (rapeseed, flax). All samples were grown in the Russian Federation in 2022, 2023 and entered the laboratory of the Moscow branch of the Federal State Budgetary Institution "ARRIAH ".

3 Results and Discussion

The following sample preparation method was established for the extraction of pesticides:

Before analysis, samples of grain and its processed products are thoroughly ground using a laboratory mill or other grinding equipment to the state of flour.

A 50 cm^3 centrifuge tube is filled with a 5.00 ± 0.05 g sample of the ground sample, 10 cm3 of deionized water is added and mixed until a homogeneous mass is obtained. Then add 10 cm^3 of acetonitrile (for matrix calibration 9 cm^3), tightly close the tube and place it in a vibration shaker for 5 minutes.

Then add the contents of the extraction kit package or a similar salt mixture to the tube. The tube is placed in a rotary shaker for 5 minutes, then centrifuged at 4 °C for 10 minutes at a rotation speed of 3500 rpm. When analyzing samples of oilseeds after centrifugation, it is recommended to place them in a freezer for 8 hours at -20 °C.

6 cm^3 of the resulting extract is transferred to a 15 cm^3 tube with a set of salts and sorbents for extract purification. The tube is placed on a rotary shaker for 5 minutes, then centrifuged at 4 °C for 10 minutes at a rotation speed of 4500 rpm.

After centrifuge, the liquid is drained into a 10 cm3 graduated tube and evaporated in a device for evaporating solvents in a nitrogen stream with a heating module at a temperature of 30 °C to a volume of 1 cm^3, the resulting extract is filtered through a syringe filter into a vial and used for chromatographic analysis. Conditions of GC MS/MS determination:

- Injector mode - without flow division.
- Injector temperature - 250 °C.
- Injected volume - 2 μl.
- Filament activation delay - 1.5 minutes.
- Column temperature mode - Heating up to 105°C for 3 minutes; Heating at a rate of 10°C/min up to 130°C; Heating at a rate of 4°C/min up to 200°C; Heating at a rate of 8°C/min up to 290°C with a delay of 6 minutes.

- The flow rate of the carrier gas (helium) through the column - 1.4 ml/min.
- - Interface temperature - 280 °C.
- Ion source temperature - 230 °C.

Peak detection is carried out using the "multiple reaction registration" (MRM) method. For each pesticide, the signal of at least three characteristic MRM transitions is measured (these data are contained in full in the published version of the measurement method FR.1.31.2022.42668 developed by us). Figure 1 shows the chromatogram of the pesticides determined according to the developed methodology.

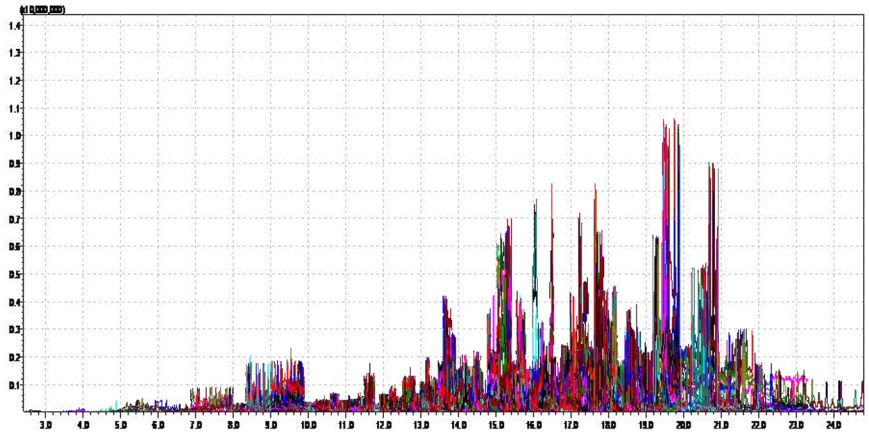

Fig. 1. Chromatogram of 430 pesticides according to the method FR.1.31.2022.42668.

Metrological characteristics were calculated for all 430 pesticides being determined. For ease of use in the methodology, these data were summarized and limit values of these characteristics were selected. The obtained data are presented in Table 1.

Table 1. Metrological characteristics.

Measurement range	**The value of the relative expanded uncertainty, ± U, % at the coverage factor k = 2**	**Repeatability limit, r, %**	**Reproducibility limit, R, %**
from 1.00 to 100 µg/kg	14	12	21

The created technique is used in laboratories of the Russian Federation for grain control. We analyzed the data on the use of this technique in the Moscow branch of the Federal State Budgetary Institution "ARRIAH". This institution is one of the largest laboratories where food and feed products are monitored for quality and safety indicators, including grain.

The method was tested on 120 samples of grain, legumes and oilseed crops. No excess of maximum permissible concentration levels was detected, however, the range of the method allows to establish the content of trace impurities in pesticides. In the process of monitoring the safety of products, this information may be important for subsequent studies of the impact of trace amounts of these substances on human and animal health. In addition, these data will allow to identify the use of pesticides not registered for specific crops in the Russian Federation

Of the 120 samples studied, no grain was found with excess of maximum permissible levels according to TR CU 015/2011, however, pesticides were found in permissible quantities in some samples. The most frequently detected pesticides in grain are: diazinon,

premifos-methyl, malathion, fipronil. The largest amount of pesticides was contained in flax and rapeseed seeds.

Pirimiphos-methyl was contained in 5 samples of cereal crops and 3 legumes at a level of less than 10 μg/kg. According to TR CU 015/2011, the maximum permissible concentration levels of this pesticide for rice are 1.0 mg/kg (1000 μg/kg), for peas - 5.0 mg/kg (5000 μg/kg), for cereal grains - 0.1 mg/kg (100 μg/kg). According to the "State Catalog of Pesticides and Agrochemicals Permitted for Use in the Territory of the Russian Federation", perimiphos-methyl can be used both in fields to combat insect pests and for treating grain storage facilities. Based on the data obtained, no violations of the use of this pesticide during the cultivation and storage of the studied samples were revealed.

Malathion was contained in 2 samples of wheat and 5 samples of flax at a level of 10-15 μg/kg. The established maximum permissible concentration levels in the territory of the Eurasian Union: cereal grain - 3.0 mg/kg (3000 μg/kg); corn (grain), peas, soybeans (beans) - 0.3 mg/kg (300 μg/kg); peanuts - 1.0 mg/kg (1000 mg/kg); mustard - 0.1 mg/kg (100 μg/kg); sunflower (seeds) - 0.02 μg/kg (20 μg/kg). The pesticide is registered for the treatment of wheat, barley, peas, soybeans, sunflower, flax, rapeseed, some vegetable crops and fruit trees, as well as for the treatment of warehouses. It can be concluded that the found malathion content is also currently the norm.

Diazinon was found in 2 flax samples at levels of 6 μg/kg and 10 μg/kg. According to TR CU 015/2011, this pesticide is controlled in cereal grain and corn, its content should not exceed 0.1 mg/kg (100 μg/kg). These standards have not been established for flax, in addition, this pesticide is currently registered only for wheat, vegetable, berry and flower crops. Therefore, the data obtained may indicate both the receipt of experimental flax samples and violations of pesticide use. These data are analyzed by regulatory authorities and, in case of violations, sanctions are applied to producers. A higher accumulation of diazinon in oilseed crops is determined by the lyophilicity of this pesticide.

Fipronil was found in a rapeseed sample at a level of 98 μg/kg. This pesticide, according to TR CU 015/2011, has a maximum permissible concentration only for cereal grains - 0.005 mg / kg (5 μg/kg). It is registered for wheat, barley, potatoes, soybeans, corn and for pasture treatment. The content of fipronil in rapeseed does not contradict the Technical Regulations of the Eurasian Economic Union, but also provokes a check of the origin of the sample.

Thanks to the low detection limit, the new method gives an idea not only of the compliance of the sample with TR CU 015/2011 and the documents of the importing countries, but also gives a broader idea of the grain production process.

4 Conclusion

Thus, a multimethod was developed for the determination of 430 pesticides in grain using the GC MS/MS method with a detection range from 1 to 100 μg/kg. The method was validated with the determination of metrological characteristics. When testing the method on 120 samples of various grain legumes and oilseeds, the most contained pesticides were identified in quantities not exceeding the permissible values, however, the use of pesticides not registered in the Russian Federation for specific crops was detected.

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