QUANTIFICATION OF PESTICIDE LEVELS IN HORSE CHESTNUT SEEDS

Tursunov Khurshid Obitovich
Yokubova Nilufar Jamshidovna
Tashkent Pharmaceutical Institute
SI "The center for pharmaceutical products safety"
*e-mail: nilufaryokubovapharmi21@gmail.com
https://doi.org/10.5281/zenodo.12684971

Abstract

In the middle of the 20th century, the use of synthetic pesticides became increasingly prevalent as a means to combat pests such as insects and weeds. Pesticides, which are substances used to eliminate or control pests, have played a pivotal role in enhancing food production to meet the rising global demand [1]. These chemicals are vital for safeguarding crops from a wide array of diseases and pests. Moreover, pesticides find extensive application in non-agricultural domains, encompassing industrial plant management, pest control in buildings, and pet care. Despite the escalating reliance on pesticides, pests have shown growing adaptability to these chemicals, prompting persistent research efforts aimed at developing effective pest control solutions [2]. Nevertheless, due to the widespread use of pesticides, complete prevention of their impact on the environment is unattainable [3]. Hence, it is imperative to regulate the quantity of pesticides present in various products. In a recent study, we analyzed pesticides, including atrazine, simazine, hydroxyatrazine, flumethrin, mepiquat, methylsulfuron, chlorimuron, and chlorsulfuron, in horse chestnut seeds using high-performance liquid chromatography. The findings from this study revealed that the levels of pesticides detected in horse chestnut seeds obtained from both Tashkent City and Qibray district adhered to the requirements stipulated in the Sanitary Rules and Regulations (SanPiN №0321-15).

Keywords: pesticide, horse chestnut, high-performance liquid chromatography, atrazine, simazine, hydroxyatrazine, flumethrin, mepiquat, methylsulfuron, chlorimuron, chlorsulfuron.

1. Introduction.

Pesticides, also known as plant protection products (PPP), are substances utilized to control pests, weeds, and diseases [4]. In the 1940s, the development of the first synthetic pesticides marked a significant milestone in agriculture, as it substantially contributed to the growth of food production. However, as early as the 1960s, increasing attention was given to the potential negative effects of

pesticides on both the environment and human health. This growing concern led to a reevaluation of the impacts of pesticide use and spurred efforts to develop more sustainable agricultural practices [5]. It is essential to note that pesticides have the potential to persist in the environment for prolonged periods, posing risks to both the environment and human health. The isolation of the Escin substance from the horse chestnut seeds necessitates adherence to established pharmacopeia standards, including the limitation of pesticide residue in plant raw materials. Even though pesticides are not typically directly applied to horse chestnuts, their presence in the environment can lead to their transfer, even in small amounts, to the horse chestnut. This research aims to analyze the pesticides commonly used in Uzbekistan in horse chestnut seeds grown in Tashkent City and Qibray district and evaluate them according to the requirements of Sanitary Rules and Regulations (SanPiN №0321-15).

2. Materials and methods.

. The study utilized acetonitrile, deionized water, sodium acetate trihydrate, Millipore filters (0.45 microns), and working standards of pesticides. Analyses were performed using the SHIMADZU Nexera 20AR chromatograph (SHIMADZU, Japan).

2.1. Conditions for determining the amount of atrazine, simazine, and hydroxyatrazine:

Chromatographic column - Zorbach Eclipse C-18, 4.6x150 mm, 5 mkm; column temperature - 40°C; detector – UF-detector, 220 nm; flow rate – 1.0 ml/min; injection volume – 10 mkl; mobile phase A – 0.01 M monopotassium phosphate (pH=7.0); mobile phase B – methanol; solvent – methanol; analysis duration - 20 minutes; concentration of substances - 0.11 mg/ml. The HPLC gradient mode is used for mobile phases A and B **(Fig.1).**

Time/min	**Mobile phase A, %**	**Mobile phase B, %**
3,00	80,0	20,0
5,00	70,0	30,0
7,00	70,0	30,0
10,00	50,0	50,0
12,00	50,0	50,0
14,00	30,0	70,0
16,00	30,0	70,0
18,00	80,0	20,0
20,00	**Finish**	

Figure 1. The HPLC gradient mode of the mobile phase.

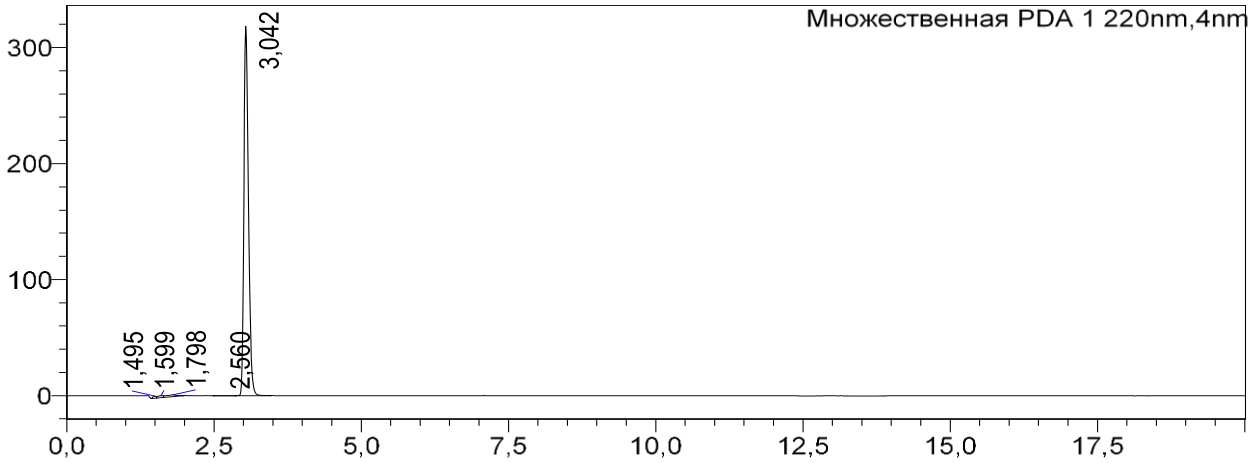


Figure 2. HPLC/DMD profile of the standard sample of simazine.

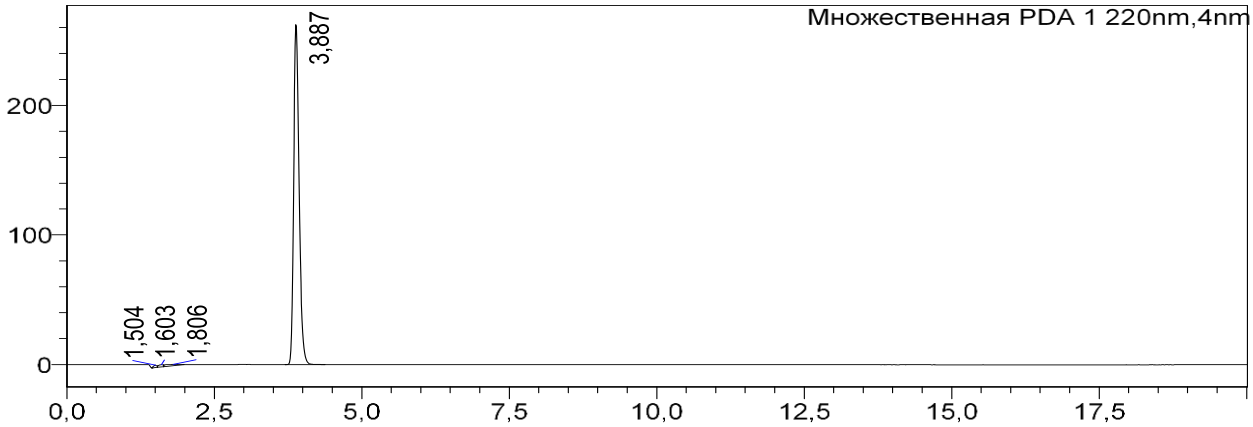
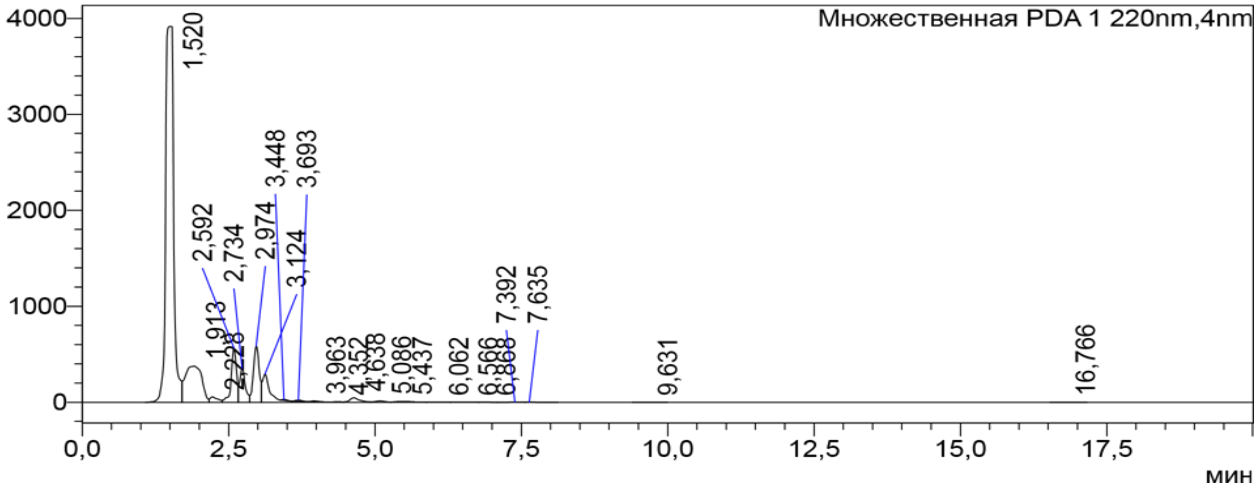


Figure 3. HPLC/DMD profile of the standard sample of hydroxyatrazine.



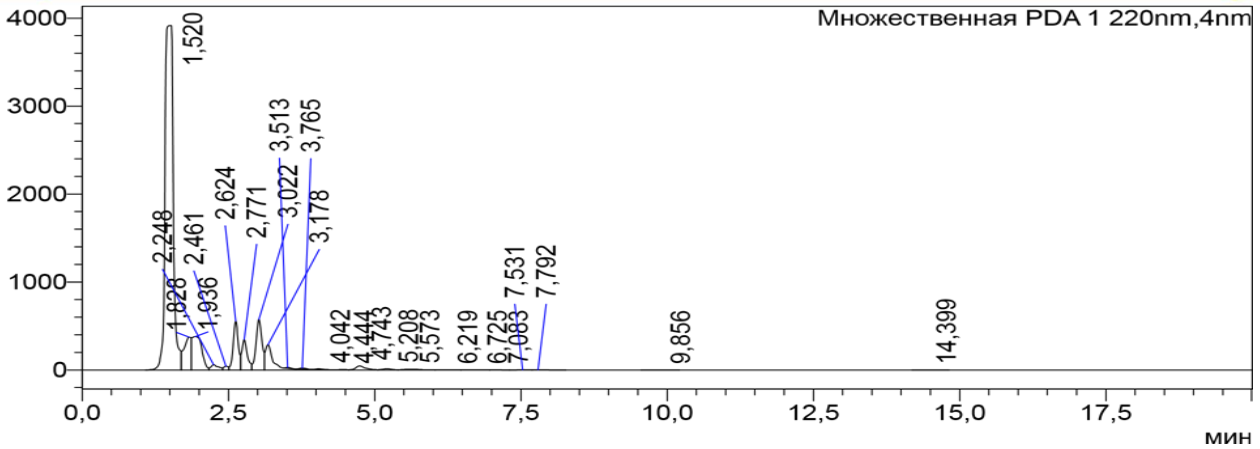


Figure 4. HPLC/DMD profile of simazine and hydroxyatrazine in horse chestnut seeds. (1-Tashkent, 2-Qibray)

2.2. Conditions for determining the amount of flumethrin:

Chromatographic column - Zorbach Eclipse C-18, 3.0x150 mm, 3.5 mkm; column temperature - 40°C; detector – UF-detector, 220 nm; flow rate – 1.0 ml/min.; injection volume – 20 mkl; mobile phase – a mixture of methanol and acetonitrile (55:45); solvent – methanol; analysis duration - 22 minutes; concentration of substances - 0.25 mg/ml.

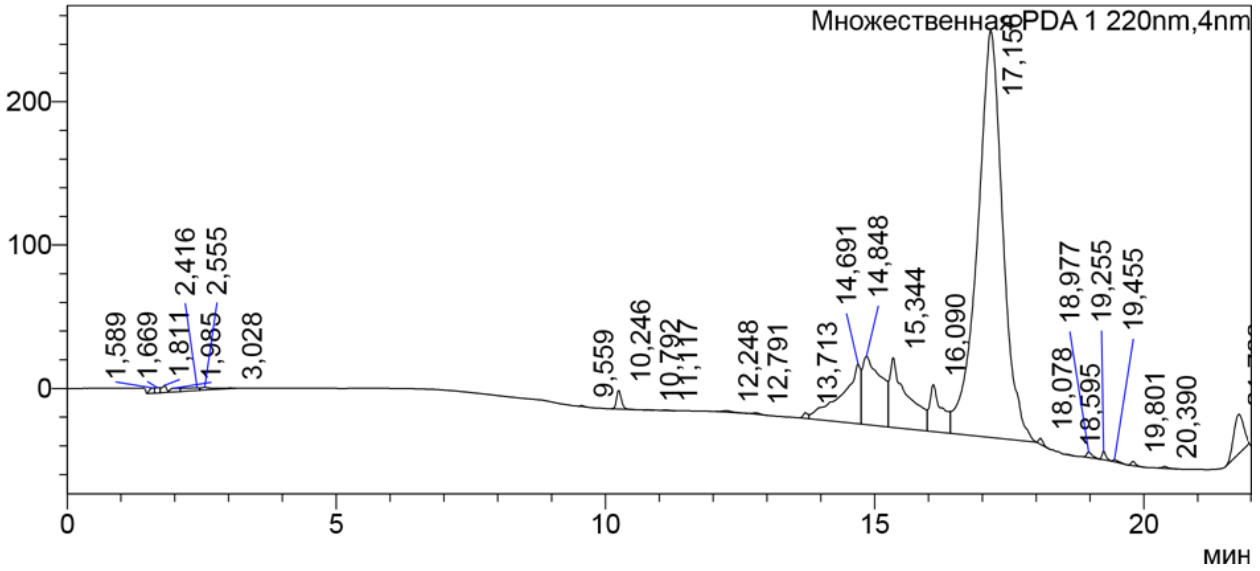


Figure 5. HPLC/DMD profile of the standard sample of flumethrin.

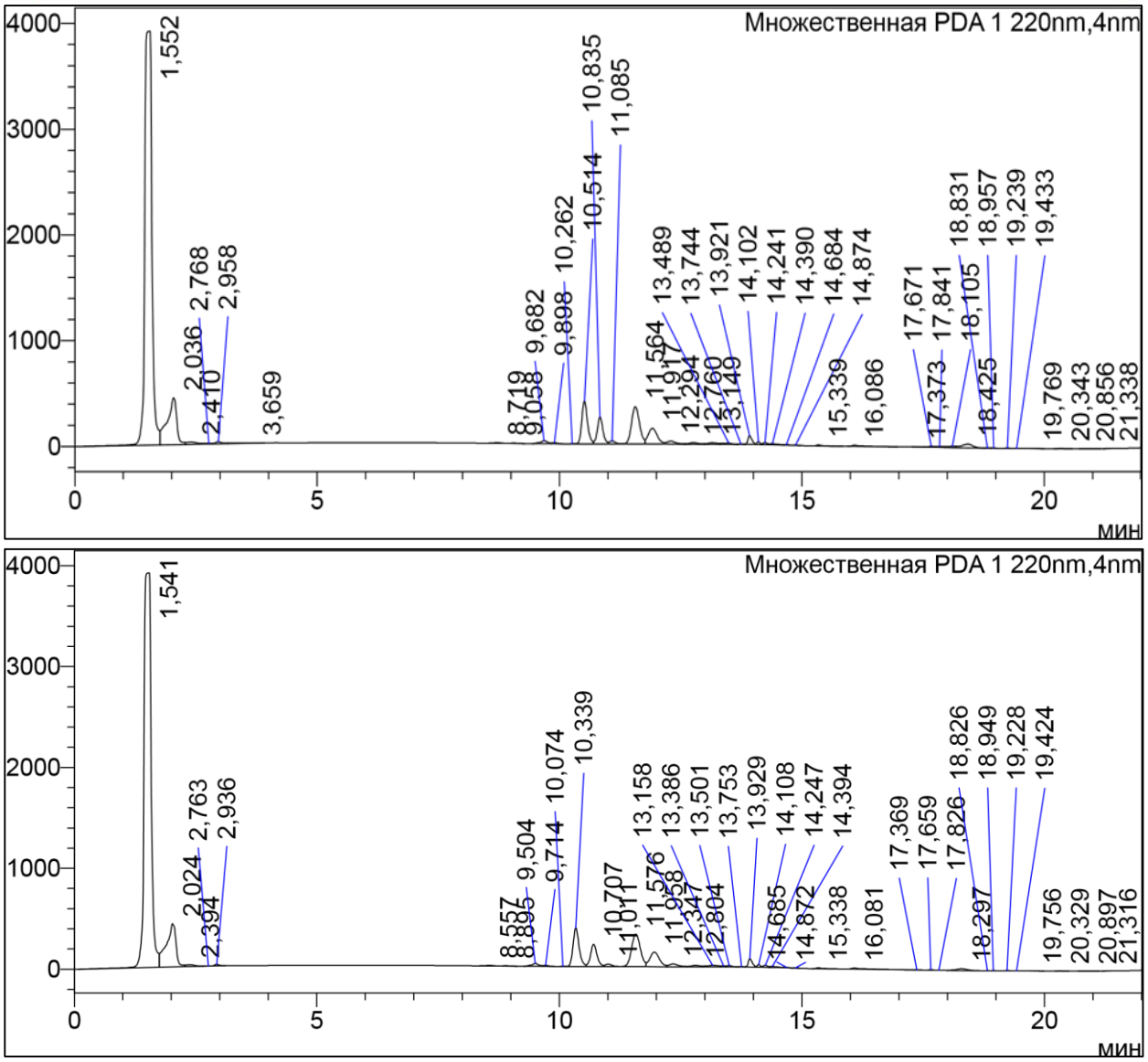


Figure 6. HPLC/DMD profile of flumethrin in horse chestnut seeds. (1-Tashkent, 2-Qibray)

2.3. Conditions for determining the amount of mepiquat:

Chromatographic column - Silasorb C-18, 4,6x150mm, 5 mkm; column temperature – 40°C; detector - UF-detector, 230 nm; flow rate-0.7 ml/min.; injection volume – 20 mkl; mobile phase – 0.05 M monopotassium phosphate (pH=2.8) and methanol mixture (95:5); solvent – water; analysis duration - 22 minutes; concentration of substances - 0.1 mg/ml.

2.4. Conditions for determining the amount of chlorsulfuron:

Chromatographic column - Zorbach Eclipse C-18, 4.6x150 mm, 5 mkm; column temperature - 40°C; detector – UF-detector, 226 nm; flow rate – 1.0 ml/min; injection volume – 20 mkl; mobile phase - a mixture of 1.4 g of Sodium 1-hexanesulfonate-containing water per liter, acetonitrile and 98.5% acetic acid

(940: 50: 10); solvent - mobile phase; analysis duration - 22 minutes; concentration of substances - 0.15 mg/ml.

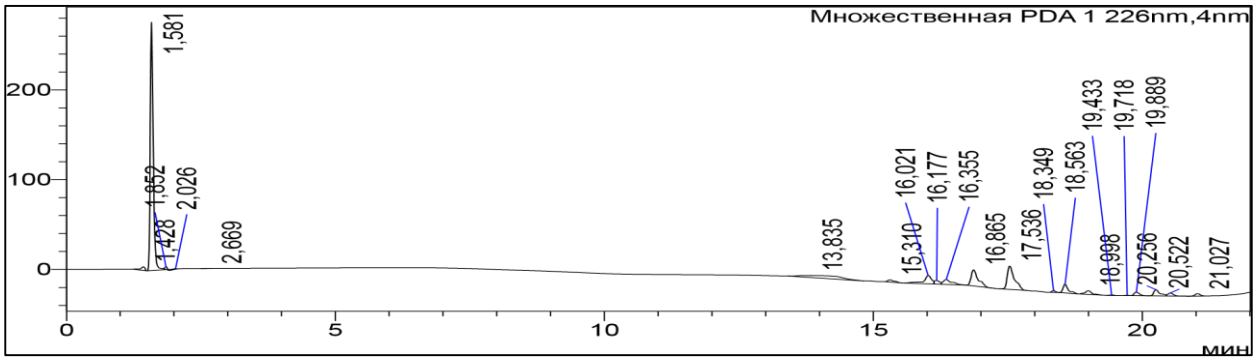


Figure 7. HPLC/DMD profile of the standard sample of chlorsulfuron.

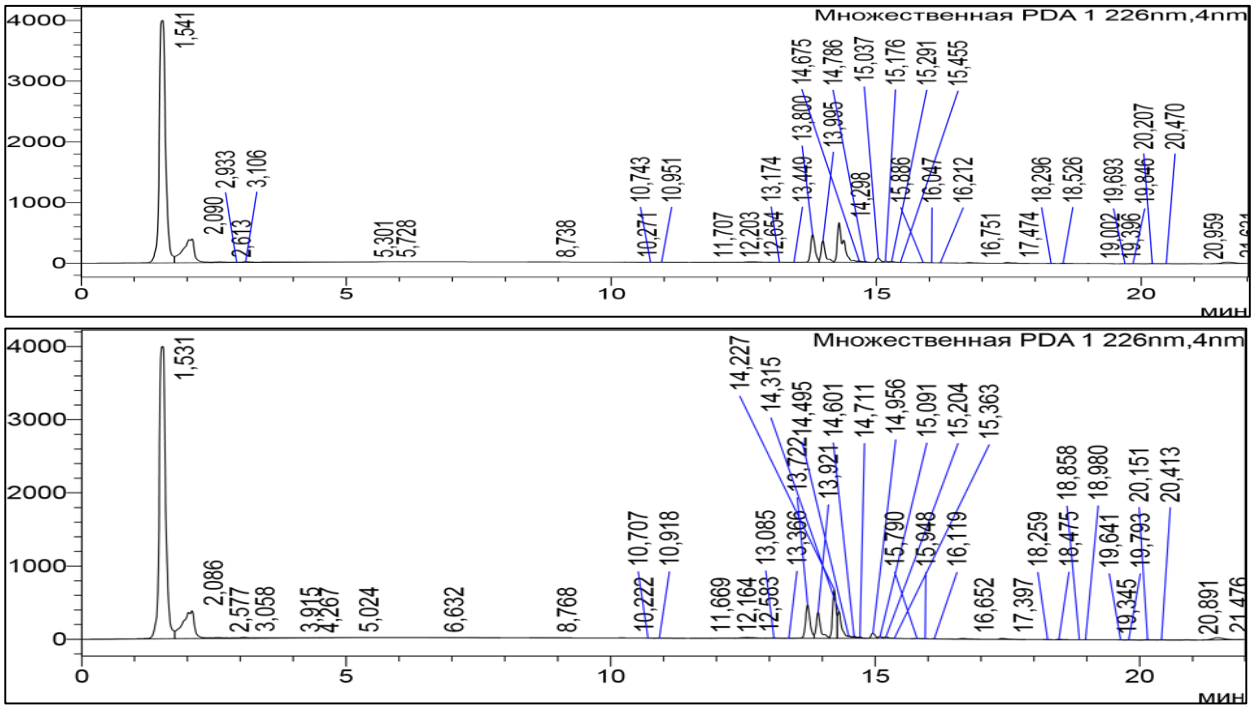


Figure 8. HPLC/DMD profile of chlorsulfuron in horse chestnut seeds. (1-Tashkent, 2-Qibray)

2.5. Conditions for determining the amount of methylsulfuron and chlorimuron:

Chromatographic column - Zorbach Eclipse C-18, 4.6x150 mm, 5 mkm; column temperature - 40°C; detector – UF-detector, 234 nm; flow rate – 1.0 ml/min; injection volume – 20 mkl; mobile phase A – 0.05 M sodium acetate (pH=2.5); mobile phase B – a mixture of methanol and acetonitrile (3:2); analysis duration - 28 minutes; concentration of substances - 0.1 mg/ml. The HPLC gradient mode is used for mobile phases A and B **(Fig.9).**

Time/min	**Mobile phase A, %**	**Mobile phase B, %**
0	0%	100%
1,5	1%	99%

17,0	57%	43%
18,0	98%	2%
23,0	98%	2%
26,0	1%	99%
28,0	**Finish**	

Figure 9. The HPLC gradient mode of the mobile phase.

Working standard preparation:

0.050 grams of methylsulfuron working standard sample and 0.0500 grams of chlorimuron working standard sample is placed into a 100 ml volumetric flask, dissolved in a small amount of purified water, diluted to volume with water, and mixed thoroughly. 10 µL of the resulting solution, 100 µL of the saturated sodium tetraborate solution, and 290 µL of the reagent A are placed in a microtube and shaken for 3 minutes.

Drug solution preparation:

1.0 grams of accurately weighed sample is placed into a 25 ml volumetric flask. Then, 15 ml of water is added and treated with ultrasound for 15 minutes (can be slightly heated). The resulting solution is then cooled, diluted to volume with water, mixed, and filtered through a membrane filter with a hole size of 0.45 µm.

Test solution preparation:

10 µL of the drug solution, 100 µL of saturated sodium tetraborate solution, and 290 µL of the reagent A are placed in a microtube and shaken for 3 minutes.

Reagent A preparation.

The first solution:

0.05 g of phthalaldehyde is placed into a 10 ml volumetric flask, dissolved in a small amount of methanol, and diluted to volume with the same solvent.

The second solution:

50 ml of 2-mercaptoethanol is diluted to 100 ml with methanol. 1.25 ml of the first solution and 25 ml of the second solution are mixed.

3. Results and discussion.

The study involved analyzing the levels of various pesticides, including atrazine, simazine, hydroxyatrazine, flumethrin, mepiquat, methylsulfuron, chlorimuron, and chlorsulfuron, in horse chestnut seeds harvested from Tashkent City and Qibray district using high-performance liquid chromatography **(Fig.10).**

№	Name of PESTICIDES	Sanitary Rules and Regulations (SanPiN №0321-15).	Amount of pesticides in seeds collected from Tashkent, mg/kg	Amount of pesticides in seeds collected from Qibray, mg/kg
1	Atrazine	Less than 30 mg/kg	-	-
2	Simazine	Less than 30 mg/kg	0,15	0,28
3	Hydroxyatrazine	Less than 30 mg/kg	-	0,012
4	Flumethrin	Less than 30 mg/kg	0,245	0,29
5	Mepiquat	Less than 30 mg/kg	-	-
6	Methylsulfuron	Less than 30 mg/kg	-	-
7	Chlorimuron	Less than 30 mg/kg	-	-
8	Chlorsulfuron	Less than 30 mg/kg	0,084	0,0024

Figure 10. Results of the analysis of pesticides in horse chestnut seeds.

The findings revealed that horse chestnut seeds from Tashkent contained 0.15 mg/kg of simazine, 0.245 mg/kg of flumethrin, and 0.084 mg/kg of chlorsulfuron, with no trace of atrazine, hydroxyatrazine, mepiquat, methylsulfuron, and chlorimuron. In contrast, horse chestnut seeds from Qibray contained 0.28 mg/kg of simazine, 0.012 mg/kg of hydroxyatrazine, 0.29 mg/kg of flumethrin, and 0.0024 mg/kg of chlorsulfuron, with the same absence of atrazine, hydroxyatrazine, mepiquat, methylsulfuron, and chlorimuron.

4. Conclusion.

The horse chestnut seeds collected from Tashkent City and Qibray district were subjected to a comprehensive analysis to identify the presence of pesticides. Specifically, the pesticides atrazine, simazine, hydroxyatrazine, flumethrin, mepiquat, methylsulfuron, chlorimuron, and chlorsulfuron were meticulously examined. Their concentrations were accurately determined using high-performance liquid chromatography, ensuring precise results. The findings of the study revealed that the levels of pesticides in the horse chestnut seeds from

both Tashkent City and Qibray district were found to be within the permissible limits outlined by the Sanitary Rules and Regulations (SanPiN №0321-15).

References:

1. Yusà, V., Coscollà, C., & Millet, M. (2014). New screening approach for risk assessment of pesticides in ambient air. Atmospheric Environment, 96, 322-330.
2. Tankiewicz, M., Fenik, J., & Biziuk, M. (2011). Solventless and solvent-minimized sample preparation techniques for determining currently used pesticides in water samples: a review. Talanta, 86, 8-22.
3. Gupta, D. K., Chatterjee, S., Datta, S., Voronina, A. V., & Walther, C. (2017). Radionuclides: accumulation and transport in plants. Reviews of Environmental Contamination and Toxicology Volume 241, 139-160.
4. Cilia, N., & Kandris, I. (2023). Training in the evaluation of pesticides (plant protection products and active substances) according to Regulation (EC) No 1107/2009. EFSA Journal, 21, e211007.
5. Van Der Werf, H. M. (1996). Assessing the impact of pesticides on the environment. Agriculture, Ecosystems & Environment, 60(2-3), 81-96.