https://doi.org/10.33472/AFJBS.6.Si2.2024.1892-1896

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

STUDYING THE POSSIBILITY OF SEPARATION THIACLOPRID FROM BIOLOGICAL FLUIDS BY THE A.A. VASILEVA AND V.F. KRAMARENKO METHODS

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ARTICLE INFO:

Volume 6,Issue Si2, 2024

Received:28 Mar 2024

Accepted : 29 Apr 2024

doi: 10.33472/AFJBS.6.Si2.2024.1892-1896

ANNOTATION

There were studied the possibility of isolating thiacloprid pesticide, a representative of the neonicotinoid group, which is widely used in agriculture, using traditional methods. In the practice of forensic chemistry, the methods of A.A. Vasileva and V.F. Kramarenko are widely used for the extraction of toxic substances from biological fluids in the conduct of chemical-toxicological analyzes. Thiacloprid analysis was used in practice, but no positive results were recorded. 98-27.46% was isolated. A method has been developed for the isolation of thiacloprid from biological fluids using liquid-liquid extraction and microcrystoscopy techniques.

Keywords. Thiacloprid, thiametaxam, clothianidin imidacloprid, biological object, instructions for performing actions (ABY), methods of A.A. Vasileva, V.F. Kramarenko, extraction.

Introduction. Currently, thiacloprid, thiametaxam, clothianidin, acetamiprid, and imidacloprid belonging to the neonicotinoid group, which are widely used in agriculture worldwide, are receiving a lot of attention due to their high toxicity and not being fully studied from a chemical-toxicological point of view [1]. In this regard, it is necessary to study the issues of creating optimal methods and techniques for extracting and analyzing this pesticide from biological fluids, and introducing them into the practice of forensic medicine.

When conducting tests for unknown toxic substances in chemical-toxicological research and forensic-chemical expertise, the "Practical Guidelines" (hereinafter - PG) approved by the Ministry of Health of the Republic of Uzbekistan are strictly followed. The procedure for isolation and testing of these PG toxic substances using approved methods is established, and they are performed as a rule [2]. This requires testing these methods and techniques in the investigation of substances under

investigation. It is necessary to use methods developed by A.A. Vasileva, V.F. Kramarenko to isolate PG toxic substances [3-4]. In this regard, in our further studies, the possibilities of thiacloprid separation from biological fluids were investigated using these methods.

Aim of the study. To study the possibility of isolating thiacloprid from biological fluids using traditional methods.

Materials and methods. A.A. Vasileva's method is one of the traditional methods and has been widely used in the science of toxicological chemistry for many years in the extraction of toxic substances from biological fluids [5]. To isolate the pesticide thiacloprid we studied, 10 ml of blood and 25 ml of urine samples were placed separately in a 100 ml volumetric flask. Model samples were prepared by adding 20 mg of the standard sample of thiacloprid pesticide to biological fluids and left for 2-3 hours at room temperature after thorough mixing. The mixture was adjusted to pH 2.0-2.5 with a saturated solution of oxalic acid, and the mixture was left for another 2 hours with occasional shaking. After the specified time, the sour aqueous part was extracted 2-3 times with 15-20 ml of chloroform [6]. The extracts were pooled and the acidic material was removed for analysis. The pH value of the acidic aqueous mixture remaining in the separator funnel was brought to alkaline conditions (9-10) with a 25% solution of ammonia. The liquid was extracted 2-3 times with 20 ml of chloroform. The resulting extracts were combined. The combined extract was distilled off until a small volume of organic solvent remained. The remaining liquids were transferred to porcelain plates and dried at room temperature. Dry residues were dissolved in 5 ml of 95% ethyl alcohol and analyzed [7]. The obtained results are presented in Table 1.

Table 1

Results of isolation of thiacloprid from biological fluids by the method of A.A. Vasileva

Determined quantity,%				Results of metrological analysis	
blood		urine		blood	urine
mg	%	mg	%		
4,33	21,65	5,32	26,60	$\overline{X}$ =21,63; T(95%-4)=2,78	$\overline{X}$ =27,0; T(95%-4)=2,78
4,22	21,10	5,31	26,55	S^2=0,9325; S=0,3053;	S^2=0,3187; S=0,5645;
4,37	21,85	5,38	26,90	Sx=0,1365;ΔX=0,8489;	Sx=0,2524;ΔX=1,5695;
4,36	21,80	5,59	27,95	$\Delta\overline{X}$ =0,3765; Ɛ =3,92%;	$\Delta\overline{X}$ =0,7019; Ɛ= 5,81%;
4,35	21,75	5,40	27,00	$\overline{\varepsilon}$ =1,75%;	$\overline{\varepsilon}$ =2,59%;

Table 1 shows that by the method of A.A. Vasileva, thiacloprid was isolated in an average amount of 21.63% in blood, with a relative error of 1.75%, and in urine, with an average amount of 27.63%, with a relative error of 2.59%. can be determined.

The second method is V.F. Kramarenko's method, in order to isolate thiacloprid, 10 ml of blood and 25 ml of urine were placed separately in flasks with a capacity of 100 ml, and 1 ml of an alcoholic solution containing 20 mg/ml of thiacloprid was poured over them and mixed thoroughly. The mixture was left at room temperature for 2 hours. After the specified time, 0.02 M sulfuric acid solution was added to it and mixed with a glass rod. Using a universal indicator, the pH of the medium was checked and 20% sulfuric acid solution was added dropwise until the pH reached 2.0-2.5. The flask was left for 2 hours with occasional stirring. The liquid was quenched two more times with 0.02 M sulfuric acid solution for one hour. In this case, the mixture was brought to a pH of 2.0-2.5. The sulfuric acid separations were combined and centrifuged at 3000 rpm for 10 minutes, and the centrifuge was separated, the remaining sediment in the beaker was mixed with a glass rod, 20-30 ml of 0.02 M sulfuric acid solution was added to it and left for two hours, then it was centrifuged again and the centrifuge was added to the total separation. The obtained extract was saturated with ammonium sulfate salt in order to clean it from proteins and foreign substances. It was then centrifuged again after 2 hours. The separated centrifuge was checked for rN-environment, it was brought to rN=2.0-2.5 with 10% sulfuric acid solution and extracted twice with 40 ml of diethyl ether. The resulting ether extracts were discarded. Acidic aqueous layer was brought to pH 8.5-9.0 with

20% sodium alkali and extracted 3 times with chloroform. The amount of chloroform should be 3 times less than the amount of the aqueous phase. The combined chloroform extract was filtered through filter paper, flashed on a water bath until the volume of the chloroform layer was 10-15 ml, and the residue was evaporated to dryness at room temperature. The dry residue was dissolved in 10 ml of ethyl alcohol and analyzed using the methods recommended for thiacloprid. The obtained results are presented in Table 2.

Table 2

Results of isolation of thiacloprid from biological fluids by the method of V.F. Kramarenko

(added amount is 20 mg)

Determined quantity,%				Results of metrological analysis	
blood		blood		blood	пешоб
mg	%	mg	%		
5,70	28,50	4,15	20,75	$\overline{X}$ =27,46; T(95%-4)=2,78; S^2=0,6730; S=0,8203; Sx=0,3668; ΔX=2,2806; $\Delta\overline{X}$ =1,0199; Ɛ =8,30%; $\overline{\varepsilon}$ =3,71%;	$\overline{X}$ =20,69;T(95%-4)=2,78; S^2=0,2730; S=0,5224; Sx=0,2336; ΔX=1,4525; $\Delta\overline{X}$ =0,6495; Ɛ =7,02%; $\overline{\varepsilon}$ =3,13%;
5,56	27,80	4,02	20,10		
5,54	27,70	4,14	20,70		
5,28	26,40	4,08	20,40		
5,38	26,90	4,30	21,50		

As can be seen from the data in Table 2, by the method of V.F. Kramarenko, thiacloprid was isolated from biological fluids - blood in an average amount of 27.46% with a relative error of 3.71%, and from urine in an average amount of 20.69% with a relative error of 3.13% can be determined.

The methods of A.A. Vasileva and V.F. Kramarenko cited by PG did not give the expected results in the isolation of thiacloprid from biological objects. When using these methods, pesticides were isolated in the amount of 21.63-27.46%. These results do not meet the requirements of chemical-toxicological studies and forensic chemistry. In this regard, it was considered desirable to develop an efficient improved method for the isolation of thiacloprid.

Improvement the method of separation of thiacloprid from biological fluids (blood, urine).

Preparation the blood sample for the analysis. For this, 5 ml of oxalic acid, 2 ml of ethyl alcohol and 5 ml of ethyl ether were added to a 10 ml blood sample in a clean dry flask with a capacity of 100 ml and left for 1 hour. It was shaken from time to time. In this case, thiacloprid forms a salt with oxalic acid and passes into the acidic water layer. With the help of alcohol, blood proteins are precipitated, and other ballast substances move to the ether layer. The acidic aqueous layer was filtered off and 10 mL of 1 M sodium hydroxide solution (pH=9) and 5 mL of chloroform were added. This mixture was placed on a mechanical shaker for 10 minutes. Then 3000 round minute centrifuged at high speed for 5 minutes. The chloroform layer was separated and filtered through anhydrous sodium sulfate. The filtrate was dried to a dry residue. The combined chloroform extracts were filtered through filter paper containing 3-5 g of anhydrous sodium sulfate to remove moisture. The filtrate was dried at room temperature to a dry residue. The dry residues were dissolved in 1 ml of ethanol, purified and analyzed by the thin layer chromatography method.

Preparation the urine sample for the analysis. To prepare a 25 ml model urine sample, 1 ml of 48% thiacloprid-containing concentrate suspension (calypso) was added to a 25 ml sample and left for 24 hours. After the specified time, extraction was carried out as follows: to extract thiacloprid from urine, 5 ml of oxalic acid, 2 ml of ethyl alcohol and 5 ml of ethyl ether were added to the sample solution and left for 1 hour. It was shaken from time to time. In this case, alkaloids form a salt with oxalic acid and pass into the acidic water layer. The acidic aqueous layer was filtered off and 10 mL of 1 M sodium hydroxide solution and 5 mL of chloroform were added. This mixture was placed on a mechanical shaker for 10 minutes. Then 3000 round minute centrifuged at high speed for 5 minutes.

The chloroform layer was separated and filtered through anhydrous sodium sulfate. The filtrate was dried to a dry residue.

The dry residues were dissolved in 1 ml of ethanol, purified and analyzed by the thin layer chromatography method. The results of the analysis are presented in

Tables 3.

Quantitative analysis results of thiocloprid extracted from a blood sample (0.5 mg added)

Allocated quantity		Statistical processing of the obtained results
µg	%	
0,260 0,264 0,264 0,256 0,256	52,00 52,80 52,80 51,20 51,20	f=4; T(95%,4)=2,78 $\overline{X}$ =52,00; S^2=0,6400; S=0,8000; S_x=0,3577; ΔX=2,2240; Δ $\overline{X}$ =0,9946; Ɛ =4,27%; $\overline{\varepsilon}$ =1,91%

As can be seen from the data in the Table 3, an average of 52.00% of thiacloprid can be isolated from blood when the improved extraction method is used.

Table 4

Thiacloprid quantification results from urine sample (added amount 0.5 mg)

The amount of thiacloprid detected		Statistical processing of the obtained results
µg	%	
0,316 0,314 0,316 0,307 0,313	63,20 62,80 63,20 61,40 62,60	f=4; T (95%,4)=2,78; $\overline{X}$ =62,64; S^2=0,5480; S=0,7402; S_x=0,3310; ΔX=2,0579; Δ $\overline{X}$ =0,9203; Ɛ =3,28 %; $\overline{\varepsilon}$ =1,46%;

From the data in Table 4, it can be seen that when the improved extraction method is used, thiacloprid can be detected in the biological object in an average amount of 62.64% with a relative error of 1.46%.

A moderate method was developed for the extraction of thiacloprid from biological objects using the liquid-liquid extraction method. The improved method is effective, fast and convenient, proteins are purified using an organic solvent (diethyl ether) and only in certain cases, that is, if the object is rotten or improperly stored, the separation is centrifuged. Using the proposed method, thiacloprid was isolated in relatively large quantities from the composition of model objects. This method is recommended for the practice of forensic chemistry.

Conclusions.

1. Thiacloprid is widely used in forensic chemistry practice by A.A. Vasileva and A.F.Thiacloprid in biological objects was metrologically analyzed using Kramarenko methods.

2. By the method of A.A. Vasileva, thiacloprid was isolated from a biological object in an average amount of 21.63%, with a relative error of 1.75%, and by the method of A.F. Kramarenko, an average amount of 27.46% was isolated, and a relative error of 3.71% was determined. .

3. Using the methods of A.A. Vasileva and V.F. Kramarenko given in the instruction manual did not give the expected results in the isolation of thiacloprid from biological fluids. When using

these methods, pesticides were isolated in the amount of 21.63-27.46%. These results do not meet the requirements of chemical-toxicological studies and forensic chemistry. In this regard, an effective improved method for the isolation of thiacloprid was developed and recommended for the practice of forensic chemistry.

References.

1. Нурматова М.И., Юлдашев З.А. Обнаружение ацетамиприда в биологических объектах // IV международная научно-практическая конференция «Лекарства-человеку. Современные проблемы фармакотерапии и назначения лекарственных средств» Харьков, 2020. – С. 413-414.

2. Юлдашев З.А., Нурматова М.И. Annals of Development Analysis Methods of Pesticides Acetomipride Isolated from a Biological Object by UV-Spectrophotometry III Халқаро илмий амалий анжуман материаллари. – Тошкент, 2021. – Б.154- 155.

3. Нурматова М.И., Юлдашев З.А.»Изучение оптимальных условий экстракции имидоклоприда из водных растворов.» IV международная научно-практическая конференция «Лекарства-человеку. Современные проблемы фармакотерапии и назначения лекарственных средств» Харьков, 2021. – С.619-620.

4. Zulfikarieva D.A., Urinboeva I.R.Development of analysis conditions for clothianidin pesticide by HPLC method // III Международная научно-практическая конференция «Современная фармация: новые подходы в образовании и актуальные исследования. Астана, 2023. 27 октября. – С. 246-247

5. Usmanaliyeva Z.U., Zulfikarieva D.A. Express Analysis in Acute Poisoning with Some Antihelminth Drugs //Journal of Pharmaceutical Negative Results: Volume 13, Special Issue 8, 202

6. Ganieva K., Yunuskhodjaev A. Development and validation of uv-spectrophotometric method for quantitative assessment of metronidazole in the “metromed® neo” solution for infusion. Indian Journal of Forensic Medicine and Toxicology, 2020, 14, 4, pp. 7312 – 7318

7. Usmanaliyeva Z.U., Zulfikarieva D.A. Determination of accumulation and distribution of effective antigelmint drugs in biological objects // Turkish journal of Physiotherapy and Rehabilitator 32(3). 2021. -P. 31995-32003.

8. Нурматова М.И., Юлдашев З.А. “Development of techniques of the analysis of Imidokloprid by the method of HPLC” Journal of Critical Reviews (JCR). Malaysia .Vol. 7, Issue 5, 2020, Pages. 1657-1663. Received 22.01.2020; Accepted 12 February 2020. jcr 07.05.283. http://www.jcreview.com/