



Storage of Imidaclopride and Acetamiprid in Biological Objects and Study of Conservative Effects on This Process

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ARTICLE INFO	ABSTRACT
Published Online: 13 December 2021	In this article is studied the biological conditions of imidacloprid and acetamiprid pesticides under laboratory conditions and the storage of the objects in a state preserved in ethyl alcohol. There were determined in the composition of the biological object stored in the laboratory the imidacloprid was detected after 13.07% in 90 days and in objects preserved with ethyl alcohol - 14.05%. When the biological object containing acetamiprid was analyzed after 90 days, it was found to be 4.45%, and when the object was stored under 95% ethyl alcohol 5.72%. In the analysis of acute poisoning cases with imidacloprid and acetamiprid, it is recommended that their shelf life should be taken into consideration.
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INTRODUCTION

In forensic practice, it's important to analyze the shelf life of toxic and strong active substances. Poisoned items can be brought to forensic chemists in some cases after an indefinite period of time. If biological objects brought to forensic chemistry laboratories are stored improperly or left at room temperature, the objects will rot and decompose. As a result, the influence of external factors on the protein substances in biological objects increases, and in a short time it is observed that the toxic substance, which is chemically-toxicologically or forensically-chemical, decomposes into simple compounds. In such cases lead to the disappearance of the toxins that the forensic chemist is looking for, and as a result the analyzes may be conducted incorrectly. In most cases, biological objects are preserved using ethyl alcohol in order to prevent decay and decomposition of the objects. In order to get accurate results in the analysis process, it is important to know the shelf life of toxins in biological objects [1].

According to the literature, the shelf life of imidacloprid and acetamiprids in biological objects and the factors influencing them have not been studied.

Taking into consideration all mentioned the study of the shelf life of imidacloprid and acetamipride pesticides in biological objects, the effect of ethyl alcohol which is added to this process as a preservative is one of the important factors in resolving the questions asked by experts and giving the right conclusions to the investigating authorities.

THE AIM OF THE RESEARCH

Study of the conservation of imidacloprid and acetamiprid in the composition of biological objects under laboratory conditions and under the influence of ethyl alcohol which is added as a preservative.

THE EXPERIMENTAL PART

There were taken a piece of black beef liver in the amount of 25 g was crushed and several samples were prepared and placed in special sealed containers. 5 mg of pesticides were added to each sample separately and mixed well. The finished model objects were divided into 2 groups. The first group of jars in which the object was placed were well sealed and left at room temperature.

The samples in the second group of jars were poured into 95% ethyl alcohol to form a glass layer on the object, preserved and left at room temperature. The prepared model samples were taken and analyzed at various durations - between 5, 10, 15, 20, 30, 60, 90, 120 days using the recommended methods.

Isolation and analysis of imidacloprid and acetamiprid from biological objects. Objects stored for some time were removed and placed in a 200 ml clean dry conical flask, on top of which a 0.02 n solution of sulfuric acid was placed until a glass layer was formed. The pH condition of mixture was checked with a universal indicator added dropwise from a 10% solution of sulfuric acid until 2–2.5 and left at room temperature for 2 hours while shaking. The acidic water portion of the mixture was poured into a clean flask. To

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the remaining object was added from a 0.02 n solution of sulfuric acid to become glass layer, and the pH was checked with a universal indicator until it was 2-2.5 dropped 10% solution of sulfuric acid and soaked for another 1 hour. This process was repeated once again. The three aqueous extracts obtained were combined and centrifuged for 10 min at 3000 round speed. The clear portion of the centrifuge was transferred to a separating funnel, after the pH condition was re-checked, it was extracted using 10 ml of diethyl ether to remove foreign matter and protein, this portion was discarded.

The pH of the aqueous part was controlled using a universal indicator using a 25% solution of ammonia, when Ph became = 9.0, it extracted 3 times with 10 ml of chloroform. The chloroform extracts were combined and filtered through a filter paper containing 3-5 g of dehydrated sodium sulfate salt previously moistened with chloroform to remove moisture from the contents.

The filtrate was dried at room temperature until remained a dry residue. The dry residues were dissolved in 1 ml of ethanol, purified by the method of thin layer chromatography and analyzed.

Purification of extracts from a biological object by the method of thin layer chromatography.

In the process of separation of imidacloprid and acetamipride from biological objects, co-extractive substances (foreign matters and ballast) are released with them. This interferes with the qualitative and quantitative analysis of imidacloprid and acetamiprid. According to the cases, removing pesticides from foreign substances, is used the method of thin-layer chromatography which is applying in forensic chemical treatment of toxic organic substances. Cleaning activities are carried out separately for each pesticide. Plaques CCM 20 * 20 cm Gel de silice 60 F254 from MERK, Germany, size 9.5x9.5 cm, made in Armenia, Armsorb coated with KSK silica gel, size 9.5x9.5 cm, made in Russia, “Sorbfil” coated with KSK silica gel was carried out on prefabricated plates [2]. The dry residues isolated from the biological object were dissolved in 5.0 ml of 95% ethyl alcohol. Alcoholic solutions containing acetamipride and imidacloprid were instilled in a volume of 1-5 mcl using a Fisherbant micropipette at a point marked on the starting line of the chromatographic plates, and 0.1 ml of a standard solution sample of 100 mcl / ml of pesticides was added to one side and dried at room temperature. Acetamiprid drip plates were immersed in a chromatographic camera in which

a mixture of chloroform-acetone-diethylamine was placed in a ratio of 50:30:20 and saturated with their vapor. Imidacloprid dripping plastics were dried at room temperature, immersed in a chromatographic camera prepared from a 25% solution of chloroform-acetone-ammonia in a ratio of 9: 1: 1 and saturated with their vapor. When the solvent mixture rose to a height of 10 cm and reached the finish line, the plates were removed from the camera and dried at room temperature. The following illuminating reagents were used to detect the rising spot on the chromatographic plates: UV lamp irradiated at a wavelength of 254 nm; Dragendorff reagent prepared according to Mune and 20% solution of sulfuric acid.

It is light green in UV light; Dragendorff reagent prepared according to Mune was subsequently sprayed with a 20% solution of sulfuric acid to form brown spots.

The location of the stain in the drip portion of the test substance solution was marked with a pencil and scraped into a sorbent porcelain dish. The sorbent was eluted with 5 ml of ethyl alcohol. The eluates were passed through paper filters moistened with alcohol. The filtrates were made up to a volume of 5 ml, transferred to 10 ml volumetric flasks and made up to the mark using ethyl alcohol, then mixed well. The optical densities of the solutions were measured with acetamipride at 246 nm and imidacloprid at 269 nm, and the quantities were determined using the formula below. The results of the analysis are presented in Tables 1 and 2.

Determination of imidacloprid and acetamiprid isolated from biological objects was carried out on the basis of the following formula:

$$X = \frac{D \cdot V_2 \cdot 100}{E_{1\text{CM}}^{1\% \cdot a} \cdot V_1 \cdot 100}$$

In this case

X– The amount of acetamiprid (imidacloprid) in the test object, mkg;

D – is the optical density of the solution;

$E_{1\text{CM}}^{1\%}$ – is the specific light absorption index of acetamiprid (imidacloprid);

V_1 – volume of test solution, ml;

V_2 – solution obtained for dilution, ml;

a- weight of the initial sample of acetamiprid (imidacloprid) powder.

Table 1. Study of the shelf life of imidacloprid in biological objects (amount of object - 25 g, 5 mg added, n = 5)

Days of storage of the biological object	The amount of determined imidacloprid	
	mg	%
laboratory condition		
5	3,29	65,81
10	3,08	61,76

15	2,05	41,17
20	1,79	35,95
30	1,36	27,23
60	0,92	18,51
90	0,65	13,07
120	-	-
preserved with 95% ethyl alcohol		
5	3,42	68,59
10	3,13	62,74
15	2,06	41,39
20	1,84	36,90
30	1,20	24,18
60	0,98	19,60
90	0,70	14,05
120	-	-

Table 2. Study of the shelf life of acetamiprid in biological objects (amount of the object - 25 g, 5 mg added, n = 5)

Days of storage of the biological object	The amount of determined acetamiprid	
	mg	%
laboratory condition		
5	3,77	75,42
10	3,40	68,11
15	3,20	64,14
20	3,06	61,31
30	1,83	36,61
60	0,81	16,21
90	0,22	4,45
120	-	-
preserved with 95% ethyl alcohol		
5	3,77	75,54
10	3,43	68,77
15	3,25	65,17
20	3,10	62,12
30	1,90	38,09
60	0,85	17,00
90	0,28	5,72
120	-	-

THE RESULT

There were shown the data in Tables 1 and 2 that imidacloprid was determined in 13.07% of the biological object stored in the laboratory for three months, and 14.05% in the canned biological object. It was found that the decay processes in the laboratory storage of biological objects containing imidacloprid do not significantly affect the decomposition of the substance.

The fact that biological objects stored in acetamiprid were stored under two different conditions showed that external factors have almost the same effect on the decomposition of the substance. Acetamiprid was found to contain 4.45% in the laboratory and 5.72% in the biological object preserved in ethyl alcohol.

CONCLUSION

There were studied the conditions of conservation of imidacloprid and acetamiprid pesticides with biological and ethyl alcohol in biological objects. The results show that under normal conditions, imidacloprid 13.07% and acetamiprid 4.45% could be detected in 90 days. It was found that imidacloprid 14.05% and acetamiprid 5.72% can be stored in a biological object preserved in ethyl alcohol for 90 days. In the case of acute poisoning with imidacloprid and acetamiprid, in order to obtain the correct result of forensic chemical analysis, it should be borne in mind that their storage depends on the duration.

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