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Effects of Temperature & Putrefaction on the Analysis of Carbofuran & Carbaryl Insecticides

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Abstract

Pesticides are frequently encountered in forensic toxicology division of FSLs. Due to pendency of medico-legal cases; biological samples are often stored for some time from the time of collection before analyses. The role of temperature and putrefaction that may inevitably interfere with analyses results is often ignored in such cases. In our preliminary study, we have tried to produce evidence for difference in thin layer chromatography results of two insecticides- carbofuran and carbaryl as a direct consequence of changes in preservation methods of samples. This subject must further be explored taking into consideration factors like temperature, humidity, time duration, etc. so as to support research on better preservation methodology of forensic samples.

Keywords: Carbofuran; Carbaryl; Chromatography; Putrefaction; Forensic Science Laboratories (FSLs).

Introduction

The use of pesticides for criminal purposes has not only increased but their analyses complexities have also grown over decades. Insecticides have largely been abused for suicides/ homicides because of their ease of availability in regular household work like controlling mosquitoes, cockroaches, bugs, flies, etc. Deaths in suspicious circumstances are reported as medico-legal death cases and the post mortem samples are forwarded to Forensic Science Laboratories (FSLs) for analyses. Forensic Toxicology is a special area of analytical chemistry that deals with analyses of poisons in samples like blood, viscera, body fluids, etc. There are a number of factors responsible for affecting the analytical results and therefore, no standardized protocols for the identification of poisons in biological samples can be followed. In this study, we have made an effort to study the putrefaction of biological samples on the analysis of two insecticides- Carbofuran and Carbaryl.

Materials and Methods

Materials Used

HPTLC plates, Chloroform, Acetic acid, Acetonitrile were purchased from Merck Ltd. Mumbai. Methanol, n-Hexane, Acetone, Benzene were purchased from Glaxo India Ltd. Mumbai. All chemicals were of HPLC grade.

Insecticide Standards

Carbofuran and Carbaryl standards (Technical

grade) were prepared in acetone at concentrations 0.01, 0.05, 0.10, 0.50 & 1.00 mg/ml for calibration. For spiking in biological samples, standards were prepared separately in acetone at concentration 1mg/ml.

Sample Preparation

Biological Tissue samples (goat liver)- 50g each were taken in separate beakers and labeled as RT1, RT2, LT1 and LT2. Samples RT1 &LT1 were spiked with 2ml standard solution of Carbofuran at a concentration of 1mg/ml. Similarly, RT2 & LT2 were spiked with 2ml standard solution of Carbaryl at a concentration of 1mg/ml. RT1 & RT2 were kept at 37°C for 10 days without covering with lid and analyzed as putrefied samples. LT1 & LT2 were covered with aluminum foil and kept in the refrigerator in dark at 4°C for 10 days and analyzed as preserved samples.

Extraction & Purification

The samples were homogenized and refluxed with n-hexane (50ml) on a hot water bath for 90 minutes. The contents were cooled and filtered and the residue extracted twice with n-hexane (25ml) saturated with acetonitrile. The extract was dehydrated and purified by passing through sodium sulphate and silica gel-G column and evaporated to dryness on a water bath. The residue was reconstituted in 1ml n-hexane and TLC was performed with the extracted and purified sample.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC plates were activated at 110°C for 30 minutes and then cooled to room temperature before analysis. Spotting of standard solutions and extracted samples was done by HPTLC sample applicator (Desaga-AS-30, Germany). The plates were placed in developing chamber and different solvent systems of hexane and acetone were tested. After elution of spots, the plates were air dried and scanned by mutiwavelength program and λ_{max} values were noted for specific densitograms of each insectide on Desaga-Densitometer-CD20, Germany.

Results and Discussion

The extraction of insecticides from biological matrix like viscera is difficult due to interferences from fat, degraded protein, coloring matter, etc. Out of the several solvents tried, it was observed that the % recoveries of insecticides from visceral tissue were maximum for hexane as extracting solvent (85-90%) at microgram level. The recoveries may further be increased by using solid phase extraction (SPE) technique to detect the insecticides at nanogram level.

Table 1: A comparison of putrefied & preserved samples

Solvent System Used	Insecticide	Sample Name	Condition of Sample	Rf values
Hexane : Acetone (9:1)	Carbofuran	Std	Standard Solution	--
		RT1	Putrefied	--
		LT1	Preserved	--
	Carbaryl	Std	Standard Solution	Spot A= 0.42
		RT2	Putrefied	Spot A= 0.41 Spot B= 0.45
		LT2	Preserved	Spot A= 0.41 Spot B= 0.43
Hexane : Acetone (8:2)	**Carbofuran**	**Std**	**Standard Solution**	**0.53**
		RT1	Putrefied	0.61
		LT1	Preserved	0.56
	Carbaryl	Std	Standard Solution	Spot A= 0.41
		RT2	Putrefied	Spot A= 0.41 Spot B= 0.43
		LT2	Preserved	Spot A= 0.41 Spot B= 0.49

Hexane : Acetone (7:3)	Carbofuran	Std	Standard Solution	0.50
		RT1	Putrefied	0.62
		LT1	Preserved	0.55
	Carbaryl	Std	Standard Solution	Spot A= 0.41
		RT2	Putrefied	Spot A= 0.45 Spot B= 0.43
		LT2	Preserved	Spot A= 0.41 Spot B= 0.42

Various solvent systems that were used for HPTLC showed that the Rf values varied with polarity of the solvents used and thus the choice of solvent for a particular pesticide is very important. It was observed that there was a difference in Rf values of putrefied (kept at 37°C) and preserved (kept at 4°C) samples in all the three solvent systems tested (Table 1). In case of Carbofuran, the putrefied sample RT1 showed higher Rf value as compared to preserved sample LT1. In case of Carbaryl, the number of spots observed for putrefied sample RT2 was more than preserved sample LT2. The possible reason may be degradation of the insecticide due to high temperature and/or exposure to daylight. These results were also supported by HPTLC densitograms where more than one peak was observed for putrefied sample RT2. The results of HPTLC also show that below 0.01mg/ml, neither of the insecticides could be detected at their respective λ_{max}.

Conclusion

The effects of temperature and/or daylight on sample condition and analyses are very significant as the analyte of interest present in the biological matrix may be degraded by the activity of various metabolizing enzymes over a period of time. This subject needs further study for longer time durations so as to better understand the performance of putrefied and preserved samples as their analytical profiles show differences. The study needs to be replicated on other classes of pesticides as well that are commonly encountered in forensic investigations.

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