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Development of New Solvent Systems for the Analysis of Triazophos Pesticide Extracted from Blood

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Abstract

Triazophos is considered as Organophosphorous poison. Although several instrumental methods like UV-VIS, GC, and HPLC are available for separation and identification of Triazophos, but cost of analysis is very high. Therefore a simple, cheap, rapid and reliable Thin Layer Chromatography (TLC) method for separation of Triazophos has been presented. Triazophos was first extracted from blood and then identified on TLC plates by using various solvent systems. The detection of spots on the developed TLC plates was performed by using Bromophenol Blue followed by 4% Acetic acid as the spraying reagents. There were 9 different solvent systems with different volumetric ratios used in separation and identification of Triazophos.

Keywords: Triazophos; Organophosphorous; Solvent Systems; TLC; R_f; Spraying Reagents.

Introduction

Triazophos a contact and stomach poison for insects and mites. It controls a large number of insect, pest and mites that damage agricultural, horticultural and forest crops. It is mainly used in the field of cotton, sugar cane, maize, potatoes, vegetables, fruits, coffee and ornamentals [1]. Its IUPAC name is *O,O*-diethyl *O*-1-phenyl-1*H*-1,2,4-triazol-3-yl phosphorothioate. The molecular formula of the compound is $C_{12}H_{16}N_3O_3PS$ with the exact molecular mass of 313.3 g/mol [2]. The structural formula of Triazophos is depicted in Figure 1.

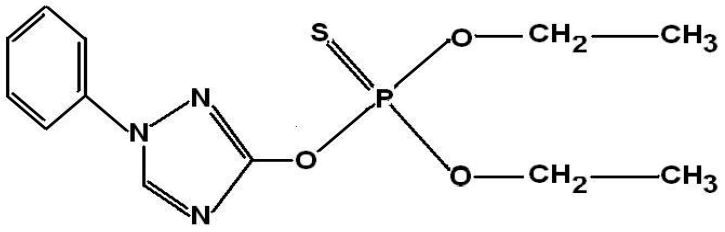


Fig. 1: Chemical structure of trizophos

It is an oily liquid having a yellowish appearance. It has a melting point of 2-5°C. The solubility of the compound is 30-40mg/kg in water, more than 500 g/L in acetone, 2-Propanol, ethyl acetate, and methanol and in Dichloromethane. Acute oral LD50 is administered to rats the dosage being 82 mg/kg and for dogs 320 mg/kg. Acute percutaneous LD_{50} is administered to rats in amount 1100mg/kg.

Reactivity Profile

Organophosphates, such as Triazophos, are

susceptible to formation of highly toxic and flammable phosphine gas in the presence of strong reducing agents such as hydrides. Partial oxidation by oxidizing agents may result in the release of toxic phosphorus oxides.

Health Hazard

Triazophos is a cholinesterase inhibitor and acts on the central nervous system. Organic phosphorus insecticides are absorbed by the skin as well as by the respiratory and gastrointestinal tracts.

Fire Hazard

Some of these materials may burn but none of them ignite readily. Container may explode in heat of fire. Fire may produce irritating or poisonous gases. Degraded by acids and alkalis.

The various techniques such as high performance liquid chromatography [HPLC][3-5], gas liquid chromatography [GLC][6], Fourier transform *infrared* spectroscopy[7], high performance thin layer chromatography [HPTLC][8,9] etc. have been used for the analysis of various organophosphorous pesticides. In present study an attempt has been made to analyse Triazophos from blood by *Thin Layer Chromatography* [TLC]. The advantages of TLC are the low cost simultaneous analysis of large number of samples and minimum sample preparation[10-11].

Experimental

Materials and Reagents

Silica gel G (Glaxo India Ltd., Mumbai), Acetone (Merck Specialties Pvt. Ltd.), Ethylacetate (Glaxo Smithkline Pharmaceutical Ltd., Mumbai), Dichloromethane, n-Hexane, Conc. Sulphuric acid, 2-Propanol, Benzene, Petroleum benzene (E. Merck India Ltd.) Chloroform, Ethanol (Merck Ltd., Mumbai).

Beaker, conical flask. TLC glass plates, glass rod, glass chromatographic chamber, separating funnel, volumetric flask and fine capillary tubes.

Preparation of Standard Solution

The 1000 ppm solution of Triazophos was prepared by dissolving 0.1 gm of Triazophos in 100 ml of n-Hexane.

Preparation of Spraying Reagent

1. 4% Acetic acid: Measure 4 ml of acetic acid and then make its volume up to 100 ml by adding 96 ml water.
2. Bromophenol blue:

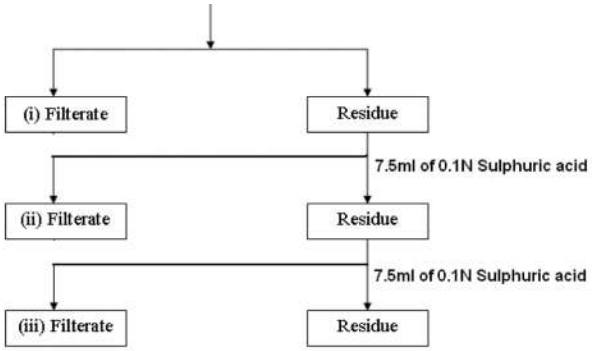
Solution 1 : 0.25gms of Bromophenol blue in 5 ml acetone.

Solution 2: 0.5gms of Silver nitrate in 50 ml of distilled water.

Took out 15 ml from solution 2 and make its volume up to 50 ml using acetone. Now add 45 ml of solution 2 to solution 1, which is called bromo-phenol blue solution.

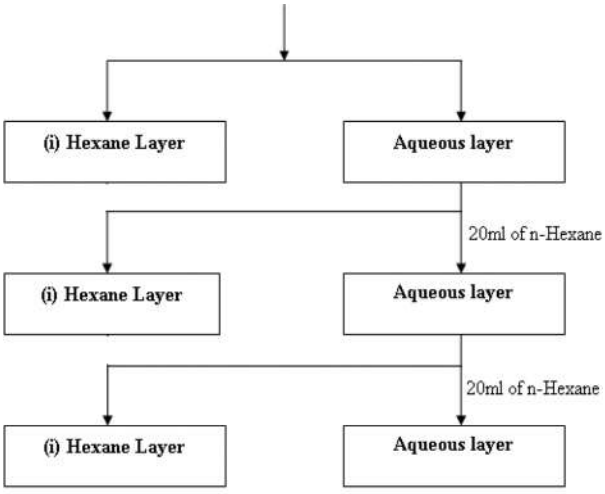
Extraction of Triazophos Pesticide from Blood[9,12-15]

4 ml blood + 6 ml of 10% Sodium tungstate solution + 15 ml of sulphuric acid, shaken for two minutes



Filterate (i), (ii) & (iii) are pooled + 15ml of n-Hexane is taken in a separating funnel, shaken for two minutes and separated by two layers.

Hexane layers (i), (ii) & (iii) are pooled and passed through the Sodium sulphate and evaporated up to 1ml.



Preparation of TLC Plates

TLC plates were prepared by dissolving 25 gm of silica gel G in 50 ml of distilled water to make slurry. This slurry was poured on the applicator and the applicator was then moved over the plate in one motion. Plates were allowed to dry at room temperature and then kept in hot air oven at 80°C for one hour.

Spotting and Development of Plates

Pesticide extracted from blood was loaded on TLC plates along with the standard with appropriate markings. Spotted plates were developed in different solvent system taken in different ratios. After developing, the TLC plates were taken out from the solvent chamber and air-dried.

Visualization of TLC Plate

The dried and developed TLC plates were sprayed with Bromophenol blue followed by 4% Acetic acid.

Results and Discussion

After the development the TLC plates are sprayed with spraying reagent such as Bromophenol blue followed by 4% Acetic acid. There were 9 different solvent systems with different volumetric ratios. The R_f value of Triazophos extracted from blood under experimental conditions was found nearly equal to standard used in some cases. The retentions factor (Rf) is defined as the ratio of the distance moved by analyte from the origin to the distance moved by solvent from the origin.The response of separation of Triazophos in all 9 solvents was analysed and presented in Tables 1,2 ,3,4,5 and 6.

Table 1: TLC parameters in solvent system Acetone : Benzene

Ratio	Time (Mins)	Distance Travelled (Centimetre)			R_f (Standard)	R_f (Sample)
		Standard	Sample	Solvent		
1:9	12	6.6	6.5	7	0.94	0.928
2:8	16	6.2	6	7.5	0.826	0.800
3:7	13	6.8	6.2	7.5	0.906	0.826
4:6	11	8.1	7.4	8.3	0.975	0.891
5:5	15	6.7	6.4	7.2	0.93	0.888
6:4	12	7.7	7	7.8	0.987	0.897
7:3	11	7.4	7.3	7.7	0.961	0.948
8:2	12	7.8	7.5	8.3	0.939	0.903
9:1	15	8.1	8	8.2	0.987	0.975
pure benzene	20	no result	-	7.7	-	-

Table 2: TLC parameters in solvent system Ethyl acetate: Hexane

Ratio	Time (Mins)	Distance Travelled (Centimetre)			R_f (Standard)	R_f (Sample)
		Standard	Sample	Solvent		
1:9	13	6.7	7.3	8.6	0.77	0.848
2:8	12	6.8	7.5	8.5	0.8	0.882
3:7	17	5	7.2	7.5	0.68	0.96
4:6	14	5.9	7.1	8	0.737	0.887
5:5	14	6.8	7	7.5	0.906	0.93
6:4	17	7.3	7.3	7.7	0.948	0.94
7:3	12	no result	-	7.8	no result	-
8:2	14	no result	-	8.5	no result	-
9:1	15	no result	-	8.4	no result	-

Table 3: TLC parameters in solvent system Dichloromethane: Chloroform

Ratio	Time (Mins)	Distance Travelled (Centimetre)			R_f (Standard)	R_f (Sample)
		Standard	Sample	Solvent		
9:1	17	3.8	7	7.9	0.481	0.888
8:2	18	3.2	6.5	8.7	0.367	0.747
7:3	23	3.9	7.2	8.5	0.458	0.847
6:4	16	2.8	7.3	8.1	0.345	0.901
5:5	18	2.5	7.3	8.3	0.301	0.879
4:6	18	3.3	7.5	8.7	0.379	0.862
3: 7	17	4.1	6.9	8.5	0.482	0.811
2:8	21	2.8	7	8.5	0.329	0.823
1:9	20	3.7	7.7	8.8	0.42	0.871

Table 4: TLC parameters in solvent system dichloromethane

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
100	17	3.2	7.4	7.9	0.405	0.936

Table 5: TLC parameters in solvent system Benzene: chloroform

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
8:2	17	7.6	7.2	8.5	0.894	0.847

Table 6: TLC parameters in solvent system benzene: methanol

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
8:2	18	7.7	7.3	8.2	0.939	0.890

Table 7: Solvent Systems of best Volumetric Ratios with R_f values

S. No.	Solvent system	Ratio	R_f (Standard)	R_f (Sample)
1	Acetone :Benzene	9:1	0.980	0.970
2	Ethylacetate :Hexane	6:4	0.945	0.942
3	Dichloromethane :Chloroform	3:7	0.480	0.481
4	Dichloromethane	10	0.400	0.401
5	Benzene: chloroform	8:2	0.895	0.846
6	Benzene: Methanol	8:2	0.939	0.890
7	Petroleum benzene (pure)	no result	_	_
8	Dichloromethane: Water	no result	_	_
9	Dichloromethane: Propanol	no result	_	_

Conclusion

The long time involved in screening of the Triazophos with analytical methods like GC, HPLC etc. an alternative method, TLC was searched[12-15]. This is a simple method, easy to perform, no machine is required, takes less time to analyse the sample. The solvent systems of best volume ratio with R_f values of standard samples are given in Table 7.

References

1. Pesticide Residue in food.(sponsored jointly by FAO and WHO). 1983; 1-36.
2. Tomline C, The Pesticide Manual 10th Edition, Crop Protection Publication, U.K. 1995; 104.
3. Schlett C, Multiresidue-analysis of pesticides by HPLC after solid phase extraction, Fresenius Journal of analytical chemistry. 1991; 339: 344-347.
4. Sharma V K, Jadhav R K, Rao G J, Saraf A K and Chandra H, High Performance Liquid Chromatographic Method for the analysis of organophosphorous and carbamate Pesticides, Forensic Science International. 1990; 84: 21-25.
5. Lisseter S G and Hambling S G, Chiral high-performance Liquid Chromatography of synthetic pyrethroid insecticides, Journal of Chromatography. 1991; 539: 207-210.
6. Mount D L and Churchill F C,Gas Chromatographic method for determination of p,p-DDT in DDT Technical and formulated products: Collaborative study, Journal association of analytical chemistry. 1990; 73(5): 744-748.
7. Jaiswal A K, FTIR spectrophotometric Determination of Dimethoate pesticide in formulation, Journal of the Indian of Forensic Science. 2001; 40(1&2): 17-23.
8. Jaiswal A K, Kaur A , Millo T, Kumar A and Gupta M, Determination of Malathion in blood urine using Thin Layer chromatography, Journal Indian Academy Forensic Med. 2008; 30(1): 15-21.
9. Jaiswal A K , Kashap SK, Gupta M, Mewar S K , Rana SVS . Analysis of dimethoate in viscera sample using HPTLC plates, Amity J Behavioural and Forensic Science. 2006; 2(2): 85-90.
10. Jaiswal A K, Giri N G , Gupta M & Kumar P, Development of new solvent systems for the analysis of Chlorpyrifos poison extracted from blood. Proc . Indian Natn Sci Acad. 2008; 74(4): 173-177.
11. Jaiswal A K & Lal N. Analysis of Finit in urine smple using Thin Layer Chromatography. Indian Journal of Environ. & Helth, In press (2012).
12. Chakraborty M, Archana N and Jaiswal A K . Development of New Solvent Systems for the Extraction and Identification of Fenvalerate poison from Blood . Proc . Indian Natn Sci Acad. 2010; 74(1): 5-10.
13. Jaiswal AK, Millo T and Murty O P, Extraction,

isolation and clean up methods for pesticides, insecticides and alkaloids from biological material. International Journal of Medical Toxicology and Legal Medicine. 2006; 9(1).

14. Tiwari SN Manual of Toxicology Forensic science laboratory. Agra 1st Edn. 1976; 14-15, 20-25, 56-63.
15. DFS manual of Toxicology ,1st Edition, Selective and Scientific publisher, New Deihi. 2005; 59-90.