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Analysis of malathion in biological samples using thin layer chromatography

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ABSTRACT

Pesticides are major contaminating chemicals in agricultural environment and hazard to exposed population. The pesticides form a strong class of environment pollutants, as they are sometimes nonbiodegradable, damaged not only the environment and agriculture but also they have entered into the food chain thereby affecting health and development. Malathion is a broad spectrum organophosphorous insecticide generally used to control a variety of insects. The present study was planned to develop a new method for analysis of malathion in human blood samples using thin layer chromatography technique, which is simple and quick. Malathion was extracted from blood using solvent extraction methods and then identified on TLC plates.

For chromatographic separation various binary and tertiary solvent systems were used and for detection, on developed plates, palladium chlorides reagent was used which successfully increased the sensitivity without dispensing with simplicity of the method. Statistical analysis was performed on four solvent system namely benzene: methylen dichloride [80:20], hexane: ethyl acetate [50:50], hexane :ethyl acetate [20:80], and hexane: propanol [20:80] which include the calculation of mean R_f value, value of standard deviation and co-efficient of variance. It is evident from the statistical data that hexane: ethyl acetate[50:50] and hexane :ethyl acetate [20:80] are preferably good solvent systems for malathoin identification by thin layer chromatography.

KEYWORDS

Organophosphorous compound;
Malathion;
R_f;
Palladium chloride.

INTRODUCTION

Malathion is an organophosphate parasympathomim which binds irreversibly to cholinesterase. Malathion is an instecide of relatively low human toxicity, however a 2010 study has shown that children with higher levels of organophosphate pesticide metabolic in their urine are more likely to have attain deficit hyperactivity disorder[1]. Malathion is a pesticide that is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication[2]. In the US, it is the most commonly used organophosphate insecticide[3].

Malathion was used in the 1980 in California to combat the Mediterranean fruit fly. This was accomplished on a wide scale by near weekly aerial spraying of suburban communities for a period of several months. Formations of three or four agricultural helicopters would overfly suburban portions of Alameda count,

San Bernardino County, San Maleo County, San Clara County, releasing a mixture of malathion and can syrup, the corn group being a bait for the fruit flies. Malathion has also been used to combat the Mediterranean fruit fly in Australia[4].

Malathion in low dose [0.5% preparation] is used as a treatment for head lice and body lice. Malathion and lindane are the only two agents approved by the FDA for treatment of pediculosis[5]. It is claimed to effectively kill both the eggs and the adult lice, but in the fact has been shown in UK studies to be only 36% effective on head lice and less so on their eggs[6]. This low efficiency was found when malathion was applied to lice found on schoolchildren in the Bristol area in the UK and it is assumed to be caused by the lice having developed resistance against malathion.

Preparations include Derbac-M, Prioderm and Quellada-M[7] and ovide[8]. United States Environamental health effect of chronic exposure to malathion[9].

Malathion is classified by USEPA as having "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential[10].

The present paper reports the extraction of malathion from human blood samples. Various binary and tertiary solvent systems were used for purification and identification by employing thin layer chromatography method. Palladium chloride reagent was used for the detection of malathion on developed plates.

MATERIAL AND METHODS

Standard solution 1000 ppm of malathion in ethanol was prepared. Sample preparation 5 ml of blood sample was spiked with 1.5 ml of standard solution of malathion and was kept in incubator at 37°C one day. Then it was extracted by hexane thrice and all the hexane layers together were passed through anhydrous evaporation. Solvent mixture analytical grade solvents were used for preparing various solvent systems.

Equipment TLC aluminum sheet silica gel 60F 254, Merck KGa, Germany and glass chromatographic chamber were used for the experiment. A glass chamber of suitable size with an airtight lid was equilibrated with respective solvent system for 20-30 minutes prior to each experiment. For each of the solvent system separate cleaned chamber was used. Syringe is having 25 u take only 5 u were used for spotting the samples on TLC plates. Visualizing reagent 0.5 gm of palladium chloride was dissolved in 100 ml of water. Concentrated hydrochloric acid was added gradually for maintaining the P^H of the solution.

PROCEDURE

The sample which was extracted from blood along with the standard solution was spotted on the TLC plate. The spots were allowed to dry and then spotted plate was inserted in glass chamber, and sealed to maintain airtight environment. TLC plate was developed with different solvent systems, varying in composition. The approximate development time for a 20cm TLC plate was 30 min. After drying the plate visualizing agent palladium chloride was sprayed on it to get yellow colour spot surrounded by brown colour on a white background colour formation was permanent. The R_f values

	Binary solvent systems	
1.	Benzene : methylen dichloride	[80:20]
2.	Benzene : methylen dichloride	[70:30]
3.	Benzene : methylen dichloride	[60:40]
4.	Benzene : methylen dichloride	[50:50]
5.	Hexane: ethyl acetate	[10:90]
6.	Hexane: ethyl acetate	[20:80]
7.	Hexane: ethyl acetate	[50:50]
8.	Hexane: ethyl acetate	[60:40]
9.	Hexane:Propanol	[30:70]
10.	Hexane:Propanol	[40:60]
11.	Hexane:Propanol	[50:50]
12.	Hexane:Propanol	[60:40]
13.	Hexane:Propanol	[70:30]
14.	Hexane:Propanol	[80:20]
	Tertiary solvent system	
15.	Hexane : methylen	[20:40:40]
16.	Hexane : methylen	[40:30:30]
17.	Hexane : methylen	[50:25:25]
18.	Hexane : methylen	[70:15:15]
19.	Hexane : methylen	[80:10:10]
20.	Hexane :formic acid :chloroform	[70:15:15]

were calculated by ration of distance travelled by sample and distance travelled by solvents.

RESULT AND DISCUSSION

Twenty binary and ternary solvent systems were undertaken for research work. TABLE 1 and 2 envisages that the R_f values of malathion extraceted from blood is nearly equal to that of standard statistical analysis was performed on four solvent systems namely benzene:methylen dichloride [80:20], hexane: ethyl acetate [50:50], hexane:ethyl acetate [20:80], hexane :propanol [20:80] out of twenty systems [TABLE 3,4,5,6]. The mean R_f values, standard deviation, coefficient of variance were calculated. All the solvent system under study cover approximates 70% of the items which is in the close agreement with the area relationship of symmetrical distribution with mean [i.e $X \pm \sigma$ covers 67.75% items. $X \pm 2\sigma$ covers 96.48% $X \pm 3\sigma$ covers 99.80%].

It is also evident from the data a given in the table that ethyl acteate[50:50], hexane:ethyl acetate [20:80] are preferably good solvent system for malathion identification by thin layer chromatography. Quantitative analysis of sample was also performed with these solvent by estimating the area of

TABLE 1 : R_f values of malathion in different binary systems

Sr. No	Solvent System	Composition (v/v)	R_f of standard	R_f of sample
1	Benzene:methylon dichloride	80:20	85	85
2	Benzene:methylon dichloride	70:30	88	88
3	Benzene:methylon dichloride	60:40	87	85
4	Benzene:methylon dichloride	50:50	40	84
5	Hexane:ethyl acetate	10:90	39	41
6	Hexane:ethyl acetate	20:80	30	38
7	Hexane:ethyl acetate	50:50	32	30
8	Hexane:ethyl acetate	60:40	92	35
9	Hexane:Propanal	30:70	92	92
10	Hexane:Propanal	40:60	92	88
11	Hexane:Propanal	50:50	85	88
12	Hexane:Propanal	60:40	85	85
13	Hexane:Propanal	70:30	83	83
14	Hexane:Propanal	80:20	80	90

TABLE 2 : R_f values of malathion in different tertiary solvent system

Sr. No	Solvent System	Composition (v/v)	R_f of standard	R_f of sample
15	Hexane : methylen dichloride : chloroform	20 : 40 : 40	92	92
16	Hexane : methylen dichloride : chloroform	40 : 30 : 30	80	83
17	Hexane : methylen dichloride : chloroform	50 : 25 : 25	82	85
18	Hexane : methylen dichloride : chloroform	70 : 15 : 15	82	84
19	Hexane : methylen dichloride : chloroform	80 : 10 : 10	90	93
20	Hexane : formic acid : chloroform	80 : 15 : 15	94	92

TABLE 3 : Replicate Rf values of malathion in solvent system Benzene : methylene dichloride [80:20]

Sr.No	Rf (standard)	Rf (blood sample)
1	86	86
2	85	86
3	88	88
4	88	88
5	87	88
6	84	85
7	86	86
8	86	86
9	85	85
10	86	86
	Mean R_f value : 86.1	Mean R_f value 86.3
	SD-1.280	SD-1.170
	cv-1.48%	cv-1.32%

TABLE 4 : Replicate Rf values of malathion in solvent system Hexane:ethyl acetate

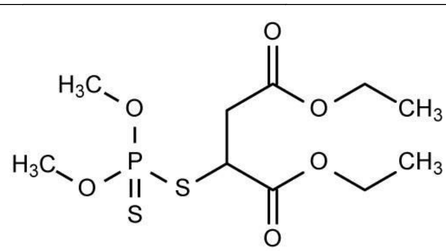
Sr.No	Rf (standard)	Rf (blood sample)
1	30	30
2	29	28
3	28	28
4	24	24
5	31	31
6	30	31
7	29	29
8	25	25
9	28	28
10	30	31
	Mean R_f value : 28.4	Mean R_f value 27.5
	SD-2.28	SD-2.45
	cv-7.8	cv-8.60%

TABLE 5 : Replicate R_f value of malathion in solvent system Hexane:ethyl acetate [20:80]

Sr.No	R_f Standard	Rf sample
1	42	43
2	44	44
3	39	39
4	39	39
5	40	41
6	43	42
7	43	42
8	39	42
9	42	39
10	40	41
	Mean value 41.1	Mean value 40.9
	SD- 1.91049	SD- 1.85
	cv- 4.63%	cv- 4.495%

TABLE 6 : Replicate R_f values of malthion in solvent system Hexane: propanol [80:20]

Sr.No	R_f Standard	Rf sample
1	86	86
2	88	88
3	85	87
4	88	87
5	88	88
6	89	89
7	88	86
8	90	89
9	87	90
10	87	90
	Mean value 87.5	Mean value 88.1
	SD- 1.4280	SD- 1.49060
	cv- 1.60%	cv- 1.70%



spot of control and sample. For quantitative analysis all samples are applied to the plates as solution and as equal volumes. Composition and thickness of layers, nature of developing solvent system, condition of environment in the nature of solvent used for dissolving the sample.

CONCLUSION

Thin layer chromatography is simple and fastest method in modern pesticide residue analysis. This method has been utilized in different areas of biological, organic, inorganic and forensic chemistry. There are many factor that may affect the R_f values such as adsorbent, solvent, temperature vapor pressure and thickness of layer.

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