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Research article

ANALYSIS OF METHYL PARATHION IN BIOLOGICAL SAMPLES USING THIN LAYER CHROMATOGRAPHY

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ABSTRACT: Pesticides are major contaminating chemicals in agriculture environment and a 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 have entered into the food chain thereby affecting health and development. Methyl parathion is a broad-spectrum organophosphorus insecticide generally used to control a variety of insects. The present study was planned to develop a new method for analysis of Methyl parathion in human blood samples using thin layer chromatography technique, which is simple and quick. Methyl Parathion was extracted from blood using solvent extraction methods and then identified on the TLC plates. For chromatographic separation, various binary and tertiary solvent systems were used and for detection on developed plates, palladium chloride reagent was used which successfully increased the sensitivity without dispensing with the simplicity of the method. Statistical analysis was performed on four solvent systems namely benzene: chloroform (80:20), hexane: carbontetrachloride (50:50), hexane: carbontetrachloride (20:80), hexane: propanol (20:80) which included the calculation of mean R_f value, value of standard deviation and coefficient of variance. It is evident from the statistical data that hexane: carbontetrachloride (50:50), hexane: carbon tetrachloride (20:80) is preferably good solvent systems for parathion identification by thin layer chromatography.

Keywords: Organophosphorous compound, Methyl parathion, Rf, Palladium chloride.

INTRODUCTION

Our environment has always been under natural stress but it degradation was not severe as it is today. The indiscriminate use of pesticide in agriculture as a plant protection agent for boosting production, apart from being an occupational hazard in the developing world, has been posing a serious threat to human and animal life. (Posecion, et.al., 2006) (Damgaard, et. al., 2006). Initially the use of pesticides reduced pest attack and paved the way for increasing the crop yield as expected. Simultaneously, increased use of chemical pesticides has resulted in contaminating the environment and the long-term implications on the society are found multidimensional.(Rathore,et.al.,2002), (John, et al.,2001). Several hundred compounds are available for use as pesticides and the widespread applications of these agents in contemporary agriculture have resulted in considerable environmental contamination and over 20,000 deaths per year as WHO estimated. (Ebadi, et.al. 2005) Synthetic pesticides can be grouped by the presence of their active ingredient. Major chemical groups of pesticides are: organochlorine, organophasphates, carbamates, and pyrethriods. (Gupta and Salunkhe ,1985). Organochlorine pesticides, being lipophilic and persistent, tend to accumulate in lipid rich tissues of organism. Thereafter, they come into a steady state, bioconcentrate in the lipid part of organism according to the equilibrium pattern. (Kumar, et. al., 2006) (Bhatnagar, et. al., 2004).

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One of the principle advantages claimed for organophosphate pesticide as opposed to hard-chlorinated pesticides such as aldrin, dieldrin, DDT is the ability to breakdown or be altered chemically only a few hours or day after their application. (Mathur, et.al., 2005). Methyl parathion is an organophosphorous pesticide generally applied as a spray, mainly as an emulsifiable concentrate formulation. It is used to control chewing and sucking insects in a wide range of crops. The World Health Organization classifies methyl parathion as a class I A 'extremely hazardous' pesticide. It is highly toxic by inhalation and ingestion, and moderately toxic by dermal adsorption. Like other organophosphate insecticide, methyl parathion is a cholinesterase inhibitor. When inhaled, the first adverse effects are bloody or runny nose, coughing, chest discomfort and difficulty breathing. Skin contact may cause localised sweating and involuntary muscle contractions. Following exposure by any route, other systemic effects may begin within a few minutes, or be delayed for up to 12 hours. (WHO report ,1993). Pure methyl parathion is a white crystalline solid m.p. 35-36°C, b.p. 143°C, specific gravity is 1.20 - 1.36 at 20°C,It is soluble in water (55 - 60 mg/l at 20°C),(Bowman and sans ,1979) and also soluble in most of the commonly used organic solvents such as dichloromethane, 2-propanol, hexane, toluene etc. However it is slightly soluble in aliphatic hydrocarbons, petroleum ether and mineral oils. Its molecular formula is $C_8H_{10}NO_5PS$ and molecular weight is 263.23 units. (Clark and Moffat, 1986).

The pesticide residue analysis comprises of extraction of pesticide residue from the sample matrix, removal of interfering co-extractives, identification and estimation. There is variety of analytical methods that are vailable for detecting, measuring, and/or monitoring methyl parathion, its metabolites, and other biomarkers of xposure and effect to methyl parathion as Gas chromatography (GC) coupled with electron capture (ECD), flame photometric (FPD), or flame ionization detection (FID) ,HPLC, Colourmetry and Thin layer chromatography.
The objective of our study is to develop new alternative, cost effective and environment friendly method for identification of pesticides. Thin layer chromatography has been found to be an integral and important method in modern pesticide residue analysis. This is simple, sensitive, precise, versatile, rapid, and inexpensive technique.(Das,1981) Residues of methyl parathion in rats and chickens (Beck and Sherman, 1968) and in citrus fruit juices (Rippel and Kovac ,1968))have been detected by TLC and the limit of detection was 0.2 ppm. TLC complement gas chromatography (GC) and high- performance column liquidchromatography (HPLC) for pesticide separation, detection, identification, and quantification because of its following unique advantages over column chromatography: single use of the layer simplifies sample preparation procedure; simple equipment; high sample throughput with low operating cost because multiple samples can be run simultaneously with standard on a single plate using a very low volume of solvent; high resolution through multiple development or two-dimensional (2D) development on a plate with a single adsorbent or dual adsorbents; selective and sensitive post chromatographic detection and identification with a very wide variety of chromogenic, fluorogenic, and biological reagents and coupled spectrometric techniques. (Sherma ,2005), (Chatwal and anand 2002).

The present paper reports the extraction of methyl parathion 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 methyl parathion on developed plates.

MATERIAL AND METHODS

Reagents and Equipment

i. Standard Solution: 1000 ppm solution of methyl paratyion in acetone was prepared.
ii. Sample preparation: 5ml of blood sample was spiked with 1.5 ml of standard solution of methyl parathion and was kept in incubator at 37°C for overnight. Then it was extracted by hexane thrice and all the hexane layers together were passed through anhydrous sodium sulphate and the remaining solvent was removed by evaporation. (Ansari,et al .,1997), (Soomro ,et al., 2008), (Solomon and Subramaniam ,2006).
iii. Solvent mixture: Analytical grade solvents were used for preparing various solvent systems.

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iv. Equipment: TLC aluminium sheet silica gel 60 F254, Merck KGaA, Germany and glass chromatographic chamber (Madhurai, India) were used for the experiment. A glass chamber of suitable size with an airtight lid was equilibrated with respective solvent system for 20-30 min prior to each experiment. For each of the solvent systems separate cleaned chamber was used. Fine glass capillaries were used for spotting the samples on TLC plates.

v. Visualising reagent : 0.5 gm of palladium chloride was dissolved in 100 ml of water. Concentrated hydrochloric acid was added gradually for maintaining the pH of the solution. (Laboratory manual ,2005)

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 20 cm 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 were calculated by ratio of distance travelled by sample and distance travelled by solvent.

The following binary and ternary solvent systems with varying composition were used in the present study-

Binary solvent systems

1. Benzene: Chloroform (80:20)
2. Benzene:Chloroform(70:30)
3. Benzene:Chloroform(60:40)
4. Benzene:Chloroform (50:50)
5. Hexane:Cabontetrachloride(10:90)
6. Hexane:Cabontetrachloride (20:80)
7. Hexane:Cabontetrachloride(50:50)
8. Hexane:Cabontetrachloride (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 Systems

15. Hexane: Chloroform: dioxane(20:40:40)
16. Hexane: Chloroform: dioxane (40:30:30)
17. Hexane: Chloroform: dioxane (50:25:25)
18. Hexane: Chloroform: dioxane (70:15:15)
19. Hexane: Chloroform: dioxane (80:10:10)
20. Hexane: Isoamyalcohol: Ethylacetate (70:15:15)

RESULT AND DISCUSSION

Twenty binary and ternary solvent systems were undertaken for research work. Table 1 and 2 emvisages that the R_f values of parathion extracted from blood is nearly equal to that of standard. Statistical analysis was performed on four solvent systems namely benzene: chloroform (80:20), hexane: carbontetrachloride (50:50), hexane: carbontetrachloride (20:80), hexane: propanol (20:80) out of twenty solvent systems (Table 3, 4, 5, 6). The mean R_f values, standard deviation, co-efficient of variance were calculated. All the solvent systems under study cover approximate 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 68.75% items. $X \pm 2\sigma$ covers 95.45%. $X \pm 3\sigma$ covers 99.73%). (Arora and Mallan ,2010).

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Table 1: R_f values of methyl parathion in different binary solvent systems

S.No	Solvent System	Composition (V/V)	R_f of standard	R_f of sample
1.	Benzene:Chloroform	80:20	86	86
2.	Benzene:Chloroform	70:30	87	87
3.	Benzene:Chloroform	60:40	88	86
4.	Benzene : Chloroform	50:50	87	85
5	Hexane : Cabontetrachloride	10:90	41	42
6.	Hexane : Cabontetrachloride	20:80	39	39
7.	Hexane : Cabontetrachloride	50:50	31	31
8.	Hexane : Cabontetrachloride	60:40	32	32
9.	Hexane : propanol	30:70	92	92
10.	Hexane : propanol	40:60	89	88
11.	Hexane : propanol	50:50	88	88
12.	Hexane : propanol	60:40	85	85
13.	Hexane : propanol	70:30	83	83
14.	Hexane : propanol	80:20	80	80

Table 2 : R_f values of methyl parathion in different tertiary solvent systems

S.No	Solvent system	Composition (V/V)	Rf of standard	Rf of sample
15.	Hexane: Chloroform :dioxane	20:40:40	94	94
16.	Hexane :Chloroform: dioxane	40:30:30	83	86
17.	Hexane :Chloroform: dioxane	50:25:25	81	84
18.	Hexane :Chloroform: dioxane	70:15:15	81	82
19.	Hexane :Chloroform: dioxane	80:10:10	93	92
20	Hexane: Isoamylalcohal : Ethylacetate	70:15:15	94	94

Table 3: Replicate R_f values of methyl parathion in solvent system Benzene: Chloroform (80:20)

Trial	R_f(Standard)	R_f(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 Rf Value: 86.1 SD- 1.286 CV- 1.49%	Mean Rf Value: 86.4 SD- 1.173 CV- 1.35 %

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Table 4: Replicate R_f values of methyl parathion in solvent system Hexane: Carbontetrachloride (50:50)

Trial	Rf (standard)	R_f (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 Value 28.4 SD- 2.27 CV- 7.9%	Mean Value 28.5 SD- 2.46 CV- 8.63%

Table 5: Replicate R_f values of methyl parathion in solvents system Hexane: Carbontetrachloride (20:80)

TRIAL	Rf (Standard)	R_f (Sample)
1.	42	43
2.	44	44
3.	39	39
4.	39	39
5.	40	41
6.	43	42
7.	43	42
8.	39	39
9.	42	41
10.	40	39
	Mean Value 41.1 SD- 1.91049 CV-4.64 %	Mean Value 40.9 SD-1.84 CV- 4.498 %

TABLE 6: Replicate R_f values of methyl parathion in solvent system Hexane: Propanol (80:20)

TRIAL	Rf (Standard)	Rf (sample)
1.	86	86
2.	88	88
3.	85	87
4.	88	87
5.	88	88
6.	89	89
7.	88	89
8.	90	86
9.	87	90
10.	87	90
	Mean Rf Value 87.6 SD 1..4282 CV 1.63 %	Mean Rf Value 88.0 SD 1.49063 CV 1.69%

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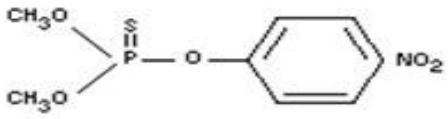

Fig 1 Structure of Methyl parathion (O,O-DimethylO-4-nitrophenyphosphorothioate)

It is also evident from the data given in the table that hexane: carbontetrachloride (50:50), hexane: carbon tetrachloride (20:80) is preferably good solvent systems for parathion identification by thin layer chromatography. Quantitative analysis of samples was also performed with these solvent systems by estimating the area of spot of control and sample. For quantitative analysis all samples should be applied to the plates as solution and as equal volumes. Although the size of spots are influenced by many factors such as composition and thickness of layer, nature of developing solvent system, condition of environment in the chromatozar, nature of solvent used for dissolving the sample. (Chatwal and Anand , 2002)

CONCLUSION

Thin Layer Chromatography is simple and fastest method in modern pesticide residue analysis. This method has been utilized with unequivocal success in different areas of biological, organic, inorganic, and forensic chemistry. Although there are many factors that may affect the R_f values such as type of adsorbent, type of solvent, temperature, vapour pressure and thickness of layer ,contamination on the glass plate, interfering substances , impurities in mobile phase ,improper thickness of gel, types of detecting or chromogenic reagent. An authentic standard should be used to avoid misidentification.

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