

## Originals and Papers

### Determination Of Malathion In Blood And Urine Using Thin-Layer Chromatography

A.K.Jaiswal<sup>\*</sup>, Ashmeet Kaur<sup>\*\*</sup>, T.Millo<sup>\*\*\*</sup>, Adarsh Kumar<sup>\*\*\*</sup> & M.Gupta<sup>\*\*\*\*</sup>

#### Abstract

Malathion is a broad-spectrum Organophosphorus insecticide generally used to control a variety of insects. Routinely it is analysed by Gas Liquid Chromatography & High Performance Liquid Chromatography. Now an attempt has been made to develop a new method for analysis of Malathion in biological samples using Thin Layer Chromatography (TLC) technique, which is inexpensive, accurate and non-destructive. Malathion was extracted from blood and urine using solvent extraction methods and then identified on the TLC plates.

For chromatographic separation, various binary and tertiary solvent systems were used to demonstrate in the laboratory. For detection on developed plates, palladium chloride reagent was used which successfully increased the sensitivity without dispensing with the simplicity of the method. For the analysis, a total of 20 solvent systems were chosen. Out of these 20 solvent systems, the three best solvent systems namely Cyclohexane: Chloroform (60:40), Hexane: Acetone (80:20) and Acetone: Hexane: Toluene (40:40:20) were chosen for statistical analysis which included the calculation of mean Rf value, value of standard deviation and coefficient of variance. The method developed is simple, inexpensive, accurate and non-destructive that allows for sensitive and reproducible analysis of Malathion.

**Keywords:** *Organophosphorus compound, Malathion, palladium chloride, hRf, TLC plate*

#### Introduction

Malathion is a pesticide that is used to kill insects on agricultural crops, on stored products, in home gardens, and in outdoor sites. It is also used to kill mosquitoes in large outdoor areas. Additionally, Malathion is used to kill fleas on pets and to treat head lice on human beings. Malathion is available in the market in two forms: a pure form of a colourless liquid and a technical-grade solution (brownish-yellow liquid), which contains malathion (greater than 90%) and impurities in a solvent. Commercially it is available as kill bug, bugsolin etc<sup>1-5</sup>. It belongs to alkyl group of phosphate comprising of diethyl (dimethoxythiophosphorythio) succinate.

Its molecular formula is C<sub>10</sub>H<sub>19</sub>O<sub>6</sub>PS<sub>2</sub> and molecular weight is 330.3. Technical grade is 95% pure, melting point -2.85°C, boiling point- 156-157°C, vapour density-5.3 mPa (30°C), density- 1.23 (25°C).

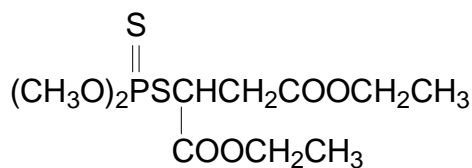
It is readily soluble in most organic solvents, e.g. alcohols, esters, ketones, ethers, aromatic hydrocarbons; slightly soluble in petroleum ether and some types of mineral oil. It does not mix well with water so it is usually mixed with oily liquids before it is applied to crops or animals. Formulation for malathion include emulsifiable concentrate, dust, flowable, granular wettable power<sup>6-12</sup>.

\* **Corresponding author** & Chemist, Dept. of Forensic Medicine & Toxicology, AIIMS, New-Delhi.

\*\* M. Sc. Student, AIBHAS, Amity University, UP.

\*\*\* Assistant Professor, Dept. of Forensic Medicine & Toxicology, AIIMS, New-Delhi-29.

\*\*\*\* Research Associate, Shriram Institute of Industrial Research, New Delhi.



**Figure-1:Structure of Malathion**

The present study involves separation of Malathion from blood and urine using different extraction methods and then identifying them on the TLC plates. For chromatographic separation various binary and tertiary solvent systems were used to demonstrate in the laboratory. The most effective solvent systems are described here. For detection on developed plates, palladium chloride reagent was used which successfully increased the sensitivity without dispensing with the simplicity of the method<sup>13-20</sup>.

## Material and Methods

### (A) Reagents and Equipment:

- a) Solvent mixture: All reagents of analytical grades were used.
- b) Visualising Reagent: Palladium chloride (Merck limited, Worli, Mumbai)
- c) Equipment: TLC Aluminium sheet Silica gel 60 F<sub>254</sub>, Merck KGaA, Germany.
- d) Chromatographic chamber: Glass chromatographic chamber were used so that a TLC plate is placed in a slightly inclined position.
- e) Sample application: Fine glass capillary was used for spotting the sample on TLC plate.

### (B) Sample preparation

- i. Standard Solution: 1000-ppm solution of Malathion in acetone was prepared by dissolving 0.1 gm of known Malathion standard in 100 ml acetone.
- ii. Visualising Reagent: 0.5 gm of palladium chloride was dissolved in 100 ml of water & then acidified upto the solution was pH 3, by using drops of concentrated Hydrochloric acid.
- iii. Preparation of development tank: A glass chamber of suitable size with an airtight lid was perfectly saturated with vapours of solvent system for 20-30 min. For each solvent system separate cleaned chambers were used. The spotted plate was inserted and sealed to maintain airtight environment.

### a) Extraction from blood<sup>9</sup>:

10ml of blood was spiked with 3 ml of malathion and kept in incubator overnight. It was mixed with 10ml of 10% sodium tungstate solution and 15ml of 1 N. Sulphuric acid, shaken for two minutes and then filtered. The residue was washed with 2 X 15ml of 0.1 N sulphuric acid. The washings were collected and mixed with the filtrate, transferred into a separating funnel and extracted thrice with 20ml portions of n-Hexane. The hexane layers were combined, passed through anhydrous sodium sulphate and the solvent was removed by evaporation.

### b) Extraction from urine<sup>9</sup>:

10 ml of urine was spiked with 3 ml of Malathion and kept in incubator overnight. This is taken in a conical flask. To it 25 ml of n-Hexane was added and contents were refluxed on a warm water bath for half an hour. After cooling, the liquid was filtered & mixed with 10ml n-Hexane and taken in a separating funnel. The n-Hexane layer was separated, passed through anhydrous sodium sulphate and evaporated to dryness.

### c) Procedure

The sample which was extracted from blood and urine along with the standard sample was spotted on the TLC plate. The spots were allowed to dry and spotted plate was developed with different solvent systems. The approximate development time for a ten-centimetre TLC plate was fifteen to twenty minutes. The plate was allowed to dry and then spots were viewed using the palladium chloride, visualising reagent.

The solvent systems used in the study included the following:

1. Cyclohexane:Chloroform (40: 60)
2. Cyclohexane:Chloroform (50: 50)
3. Cyclohexane:Chloroform (60: 40)
4. Cyclohexane: Toluene (50: 50)
5. Benzene (100)
6. Benzene: Methanol (40: 60)
7. Benzene: Methanol (50: 50)

8. Acetone: Benzene (50: 50)
9. Acetone: Benzene (60: 40)
10. Acetone: Benzene (70: 30)
11. Acetone: Benzene (80: 20)
12. Hexane: Acetone (80: 20)
13. Hexane: Acetone (70: 30)
14. Hexane: Acetone (60: 40)
15. Hexane: Acetone (50: 50)
16. Hexane: Chloroform (50: 50)
17. Hexane: Chloroform (60: 40)
18. 18.Hexane: Chloroform (70: 30)
19. Acetone: Hexane: Toluene (40: 40: 20)
20. Hexane: Acetone: Cyclohexane (50: 20: 30)

**TABLE 1: hRf VALUE OF MALATHION IN DIFFERENT SOLVENT SYSTEMS**

<b>SOLVENT SYSTEM</b>	<b>STANDARD</b>	<b>BLOOD EXTRACT</b>	<b>URINE EXTRACT</b>
Cyclohexane: Chloroform (40: 60)	37	37	36
Cyclohexane: Chloroform (50: 50)	22	22	22
Cyclohexane: Chloroform (60: 40)	20	21	21
Cyclohexane: Toluene (50: 50)	12	11	12
Benzene (100)	17	17	17
Benzene: Methanol (40: 60)	30	30	31
Benzene: Methanol (50: 50)	05	05	05
Acetone: Benzene (50: 50)	93	93	92
Acetone: Benzene (60: 40)	95	94	94
Acetone: Benzene (70: 30)	96	96	96
Acetone: Benzene (80: 20)	97	97	97
Hexane: Acetone (80: 20)	46	46	46
Hexane: Acetone (70: 30)	50	50	51
Hexane: Acetone (60: 40)	92	92	92
Hexane: Acetone (50: 50)	96	96	96
Hexane: Chloroform (50: 50)	24	25	24
Hexane: Chloroform (60: 40)	37	37	36
Hexane: Chloroform (70: 30)	52	50	51
Acetone: Hexane: Toluene (40: 40: 20)	77	76	77
Hexane: Acetone: Cyclohexane (50: 20: 30)	50	50	50

## Results and Discussion

After the TLC plates were sprayed with the palladium chloride, Malathion appeared as yellowish-orange spot surrounded by brown on a white background. The reaction was instantaneous. Separation using different solvent systems gave different Rf values (as shown in Table 1) for Malathion. Colour formation was permanent. The method described above was sensitive to Rf value of Malathion extracted from blood and urine under experimental conditions and was found nearly equal to that of standard used. The results are presented in Table 1. Values of hRf (distance travelled by the sample divided by the distance travelled by the solvent times 100) are presented in the table as well.

All in all the analysis was done on a total of 20 solvent systems, out of which the three best solvent systems namely Cyclohexane: Chloroform (60: 40), Hexane: Acetone (80:20) and Acetone: Hexane: Toluene (40:40:20) were chosen and statistical analysis was performed on these systems. The performed statistical analysis included calculation of mean Rf value, value of standard deviation and coefficient of variance whose results are presented in tables 2, 3 and 4. In case these three best solvent systems are not available, other mentioned solvent systems can also be used for the analysis of Malathion.

**TABLE 2**

**REPLICATE Rf VALUES OF MALATHION IN SOLVENT SYSTEM CYCLOHEXANE: CHLOROFORM (60:40)**

Trial	Rf (Standard)	Rf (Blood Extract)	Rf (Urine Extract)
1	0.20	0.21	0.21
2	0.19	0.20	0.19
3	0.21	0.21	0.20
4	0.20	0.20	0.21
5	0.20	0.21	0.20
6	0.21	0.20	0.20
7	0.19	0.19	0.20
8	0.21	0.20	0.21
9	0.20	0.20	0.20
10	0.21	0.21	0.20
	<b>Mean Rf Value:</b> <b>0.20</b>	<b>Mean Rf Value:</b> <b>0.20</b>	<b>Mean Rf Value:</b> <b>0.20</b>
	<b>Standard Deviation:</b> <b>0.0078</b>	<b>Standard Deviation:</b> <b>0.0067</b>	<b>Standard Deviation:</b> <b>0.0063</b>
	<b>Coefficient of Variance:</b> <b>3.90%</b>	<b>Coefficient of Variance:</b> <b>3.35%</b>	<b>Coefficient of Variance:</b> <b>3.15%</b>

**Conclusion**

Due to the time involved in screening malathion with other analytical methods, such as GC (gas chromatography), alternative methods were sought. TLC proved to be an excellent choice because it is both fast and economical. Malathion is easily visualised with spray reagents such as Palladium chloride. Since many factors affect hRf values, standard sample should always be used in conjunction when screening malathion with TLC to avoid misidentification.

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**TABLE 3: REPLICATE Rf VALUES OF MALATHION IN SOLVENT SYSTEM**

**HEXANE: ACETONE (80:20)**

Trial	Rf (Standard)	Rf (Blood Extract)	Rf (Urine Extract)
1	0.46	0.46	0.46
2	0.47	0.46	0.45
3	0.46	0.46	0.45
4	0.45	0.47	0.47
5	0.46	0.47	0.46
6	0.46	0.45	0.46
7	0.45	0.46	0.46
8	0.47	0.46	0.46
9	0.46	0.47	0.47
10	0.47	0.45	0.47
	<b>Mean Rf Value:</b> <b>0.46</b>	<b>Mean Rf Value:</b> <b>0.46</b>	<b>Mean Rf Value:</b> <b>0.46</b>
	<b>Standard Deviation:</b> <b>0.0074</b>	<b>Standard Deviation:</b> <b>0.0073</b>	<b>Standard Deviation:</b> <b>0.0074</b>
	<b>Coefficient of Variance:</b> <b>1.61%</b>	<b>Coefficient of Variance:</b> <b>1.59%</b>	<b>Coefficient of Variance:</b> <b>1.61%</b>

TABLE 4 : REPLICATE Rf VALUES OF MALATHION IN SOLVENT SYSTEM

ACETONE: HEXANE: TOLUENE (40:40:20)

Trial	Rf (Standard)	Rf (Blood Extract)	Rf (Urine Extract)
1	0.77	0.77	0.76
2	0.79	0.77	0.78
3	0.79	0.76	0.76
4	0.77	0.76	0.77
5	0.76	0.78	0.77
6	0.78	0.79	0.76
7	0.76	0.76	0.78
8	0.76	0.75	0.77
9	0.76	0.76	0.76
10	0.77	0.76	0.78
	<b>Mean Rf Value:</b> 0.77	<b>Mean Rf Value:</b> 0.76	<b>Mean Rf Value:</b> 0.77
	<b>Standard Deviation:</b> 0.0120	<b>Standard Deviation:</b> 0.0117	<b>Standard Deviation:</b> 0.0087
	<b>Coefficient of Variance:</b> 1.56%	<b>Coefficient of Variance:</b> 1.54%	<b>Coefficient of Variance:</b> 1.13%

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