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Development of new solvent system for the analysis of 2-4 D (herbicide) extracted from blood

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Abstract

2,4-Dichlorophenoxyacetic acid, more commonly referred to as 2,4-D, is an Organochlorus poison which is one of the most widely used herbicides. Several instrumental method like Gas liquid chromatography (GLC), High performance liquid chromatography (HPLC), UV-visible

Spectrophotometer, etc., are available for the separation and identification of 2,4-D, but most of them are expensive and more time consuming. So, a relatively inexpensive and less time consuming method is presented for the separation of 2,4-D i.e. Thin Layer Chromatography (TLC). This method has several advantages over the other methods such as its low cost, fewer chemicals required simultaneous analysis of large and low amount of sample. 2,4-D was extracted from the blood and then it was identified by using 20 solvent systems. The spots developed on the TLC plates were developed using Iodine vapors.

Key Words: 2,4-D, Organochloro, TLC, R_f , Spraying reagent etc.

Introduction

2,4-Dichlorophenoxyacetic acid commonly known as 2, 4-D, is a widely used herbicide and secondarily a plant growth regulator in the phenoxy class of chemicals. It effectively controls unwanted and invasive weeds across agricultural fields, lawns, public parks, lakes etc. 2, 4-D is used for broad leaf weed control in agricultural and non agricultural settings. It is registered for use in both terrestrial and aquatic environments. Major sites include pasture and rangeland, residential lawns, roadways, and cropland. Crops treated 2,4-D includes field corn, soyabeans, spring wheat, sugarcane, barley, etc. [1-5]. It is available in several chemical forms, including salts, esters, and an acid form. Structure of 2,4 D is given in Figure - 1.

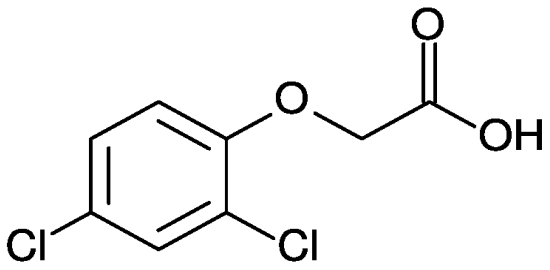


Figure 1: Structural representation of 2, 4 –D

2,4-D is a synthetic auxin, which is a class of plant hormones. It is absorbed through the leaves and is translocated to the meristems of the plant. Uncontrolled, unsustainable growth ensues, causing stem curl-over, leaf withering, and eventual plant death. 2,4-D is typically applied as an amine salt, but more potent ester versions exist as well [6-10].

2,4-D can induce a genetically programmed sequence of cellular death known as apoptosis2,4-D exposure substantially increases the risk of Non-Hodgkin's lymphoma. These effects of 2,4-D on the cellular membrane are implicated in the observed toxicity of 2,4-D to hepatocytes. So, this is a carcinogenic which affects the human body which needs to be detected if present in human body [11-15]. The pharmacokinetics and toxicity of 2,4-D are well studied in laboratory animals, volunteers, epidemiology studies and incidents involving attempted suicide. The physical and chemical properties of 2,4-D are given in table – 1.

The technique if TLC can be used to identify the traces of this insecticide if present in human body in poisoning and suicidal cases. In the present paper different solvents were to analyze 2,4D using Thin Layer Chromatography technique (TLC).

Experiment and methodology

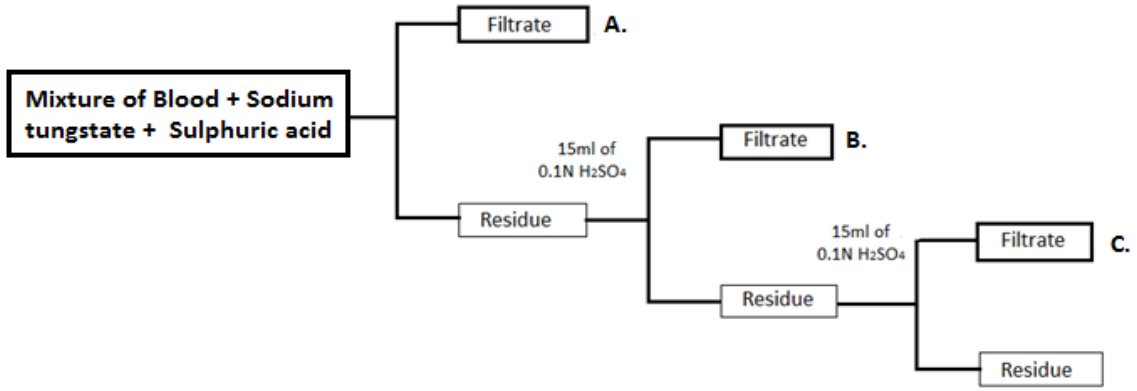
1. *Chemical Reagents*
   - Silica gel G, methanol, ammonium from Glaxo India Limited Mumbai.
   - n-Hexane, conc. hydrochloric acid, sulphuric acid, chloroform, cyclohexane, acetone, dioxane ,petroleum ether, xylene from Merck India.
   - Diethyl ether, and ethanol from Merck Germany .
   - Ethyl acetate from Glaxo Smithkline Pharmaceuticals Mumbai.
2. *Glassware:* Beakers, conical flasks, TLC glass plates, glass rod, chromatographic chamber, separating funnel, volumetric flask & fine capillary tubes of borosil made were used.
3. *Spiking of the blood sample*: 5ml of blood sample was spiked by 1ml of 1000 ppm 2,4-D standard.

4. *Preparation of Standard solution:* The 1000 ppm solution of 2, 4-D was prepared by dissolving 0.1 gm of 2, 4-D in 100 ml of acetone.
5. *Spraying Reagent:* The solid crystals of the iodine are used as visualizing agent.
6. *Extraction of 2, 4 D Poison from Blood:* The poison was extracted from blood by solvent extraction method. The protocol of experiment is given in Figure 2.

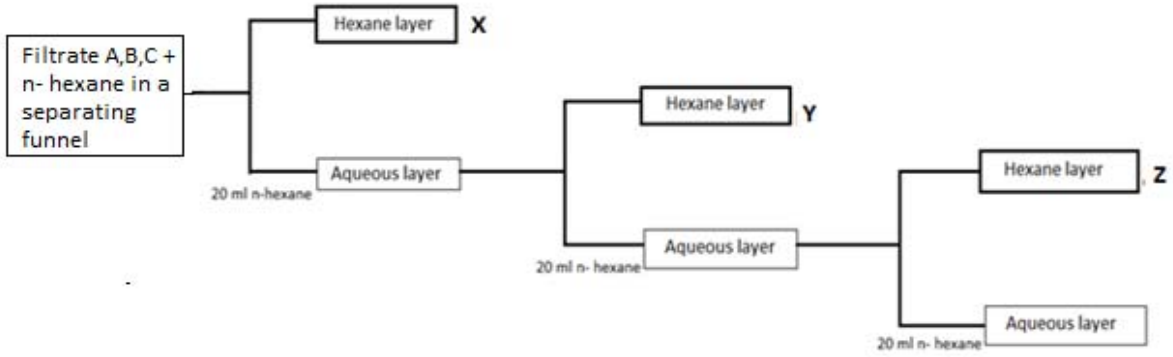
Table 1. Physical and Chemical Properties of 2, 4- D

Properties	Comments
Form	White to brown crystalline solid
Vapor pressure	1.9×10^{-5} Pa, 1.4×10^{-7} mmHg
Henry's constant	8.6×10^{-6} atm·m^3/mol
Melting point	140.5^{0}C
Molecular Weight	221
Solubility in water (mg/L)	pH 5: 29,934 ± 2957 pH 7: 44,558 ± 674 pH 9: 43,134 ± 336
Molecular formula	$C_8H_6Cl_2O_3$
Log $K_{o/w}$	0.001 M solution pH 5: 2.14 pH 7: 0.177 pH 9: 0.102
$K_{o/c}$	20-136

1 ml of blood + 10ml of 10 % of sodium tungstate solution followed by the addition of 15 ml of 0.1N sulphuric acid. Shake the mixture for 2 minutes.



Filtrate A, B and C were collected in a separating funnel and treated with the 20 ml of n-Hexane and shaken for 2 minutes and the two layers were separated.



Hexane layer X, Y and Z were collected and passed through anhydrous sodium sulphate and evaporated till dryness followed by dissolution in 1 ml of acetone solution.

Figure 2: Steps for the extraction of 2,4D from blood

7. *Preparation of TLC plates:* 25 gm of silica Gel was dissolved in 50 ml of distilled water and mixed to make slurry. Slurry was applied on the glass slide so that a continuous uniform

layer can be formed. A temperature of 80^0C is provided by placing the glass slide in a hot air oven for about 1 hour to make the slurry completely dry (Figure 3).

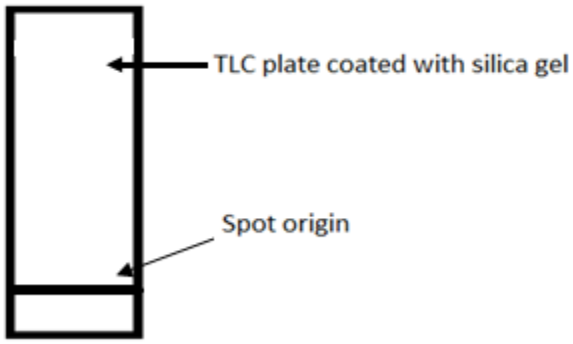


Figure 3: Preparation of TLC plate

8. *Spotting of Sample and Standard on TLC Plates:* The sample extracted from the blood was spotted on the plate using very fine capillary tube along with the standard at a distance of 0.5 cm from bottom of TLC plate. (Figure 4).

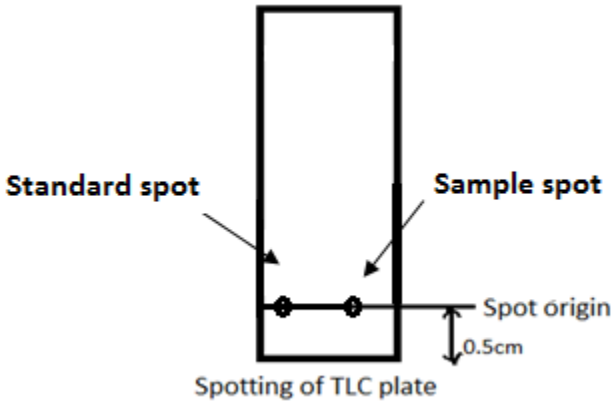


Figure 4: Spotting of the standard and sample on TLC plate.

9. *Developing of TLC Plates:* spotted plate was placed in chromatographic chamber.Care must be taken while placing the plate in development chamber the origin spots should not be

below the solvent level. If the spots are submerged in the solvent, it will be washed off from the plate and lost. Different solvents with the different ratio were used and a development time of twenty minutes is provided for 10 cm TLC plate . TLC plate was removed from the chamber when the plate has run far enough. The process of development of TLC process is given in (Figure 5 & 6).

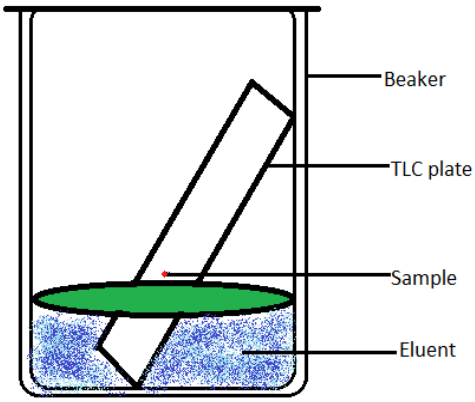


Figure 5: TLC plate placed in beaker with solvent system

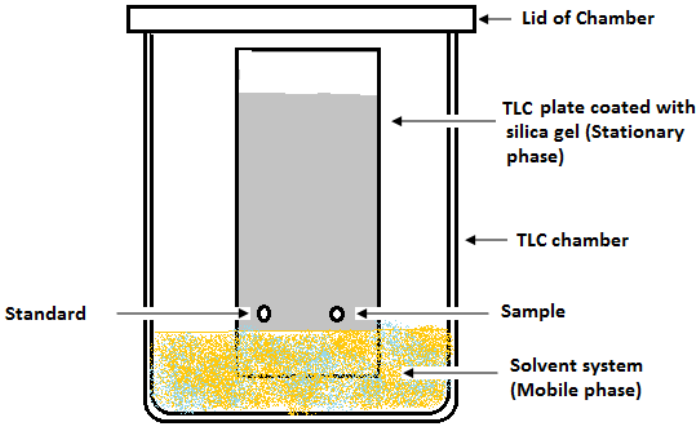


Figure 6: TLC plate showing position of Mobile phase and spots

10. *Visualization of TLC Plates:* After development plate were exposed to Iodine Vapors for visualization. Yellow colored spots were observed which disappeared within 5 minutes (Figure 7).

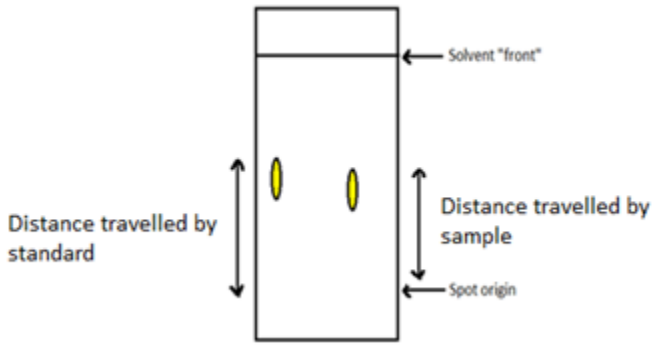


Figure 7: Visualization of standard and sample spots

Calculation of R_f: After visualization the R_f value can be calculated by using following formula.The value of R_f is always less than 1 as distance travelled by solute is always higher than the distance travelled by solvent. R_f value of sample and standard is given in table 2.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Result and discussion

After development, the TLC plates were exposed with Iodine crystal. Yellow colour spot were observed for both sample and standard which disappeared within five minutes. The R_f value sample and standard were calculated and value so obtained with different solution is given in Table 2. Out of 20 samples positive results were obtained in only 5 solvent system such as Hexane: Benzene: Ethyl acetate: Acetic acid (6:2.5:1:0.5), Hexane: Chloroform: Benzene: Acetic acid(6:3:0.5:0.5), Hexane: Ethyl acetate: Chloroform: Acetone (5:3:1:1), Hexane: Ethyl acetate(9:1), Hexane: Benzene: Ethyl acetate(6:3:1).

Conclusion

Although different methods has been employed for the identification of 2, 4-D from the blood such as High Performance liquid Chromatography (HPLC), Gas Chromatography(GC) etc. As in comparison with these methods, Thin layer Chromatography (TLC) is less expensive and also this method does not require any sophisticated instruments .

Table 2. Different Solvent systems with their ratios and R_f values

S.No.	Solvent system	System Ratio	R_f of Sample	R_f of Standard
1.	Hexane : Benzene : Ethyl acetate : Acetic acid	6:2.5:1:0.5	0.54	0.66
2.	Hexane : Chloroform :Benzene: Acetic acid	6:3:0.5:0.5	0.26	0.30
3.	Hexane : Ethyl acetate : Chloroform : Acetone	5:3:1:1	0.48	0.51
4.	Hexane : Ethyl acetate	9:1	0.9	1.0
5.	Hexane : Benzene : Ethyl acetate	6:3:1	0.9	1.0
6.	Hexane : Acetone	9:1	NIL	NIL
7.	Hexane : Benzene : Methanol: Acetic acid	5:3:1.9:0.1	NIL	NIL
8.	Benzene : Chloroform : Methanol : Acetic acid	70:20:9.5:0.5	NIL	NIL
9.	Benzene : Chloroform : Acetic acid	6:3:1	NIL	NIL
10.	Hexane : Benzene : Chloroform : Acetic acid	6:2:1.5:0.5	NIL	NIL
11.	Benzene : Hexane : Chloroform : Methanol : Acetic acid	50:30:15:2.5:2.5	NIL	NIL
12.	Hexane : Benzene: Chloroform	7:2:1	NIL	NIL
13.	Hexane : Acetone	9:1	NIL	NIL
14.	Hexane : Acetone	7:3	NIL	NIL
15.	Hexane : Ether	9:1	NIL	NIL
16.	Petroleum ether : Liquid Paraffin	8:1.5:0.5	NIL	NIL
17.	nHexane : Acetone	9.0.5:0.5	NIL	NIL
18.	Cyclohexane : Liquid Paraffin	8:0.5:1.5	NIL	NIL
19.	Cyclohexane : Methylenedichloride	9:1	NIL	NIL
20.	Petroleum	10	NIL	NIL

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He has also received several awards for his research work such as Prof. K.A. Thaker award in 209 and Dr. P.D. Sethi annual certificate of merit award in 2008. He has received fellow membership from different Academic/Scientific bodies such as Fellow Institutions of Chemists, Fellow Academy of Sciences for Animal Welfare, Fellow International Science Congress Association. He is on board of referees of the Indian Police Journal, Associate Editor of International Journal of Medical Toxicology and Legal Medicine and Member of Scientific Council of Journal of Forensic Medicine and Toxicology.

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