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Original Article

Detection of Various Poison in Biological Sample Using High Performance Thin Layer Chromatography

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ABSTRACT

Background: Poisoning is a major public health issue in many countries, particularly in India, where exposures to pesticides, household agents, drugs, industrial chemicals, and animal toxins are common. Contributing factors include rapid industrialization, the easy availability of over-the-counter medications, and extensive pesticide usage in agriculture. Rapid detection of poisons in biological samples is critical for timely medical intervention. **Materials and Methods:** This study involved the preparation of standard solutions of five pesticides: cypermethrin, lambda-cyhalothrin, malathion, monocrotophos, and quinalphos. Saliva samples were artificially spiked with these compounds and then processed for analysis using High Performance Thin Layer Chromatography (HPTLC). Specific chromatographic conditions were optimized for each poison using mobile phases like n-hexane and acetone to ensure effective separation and detection. **Results:** The analysis demonstrated the presence of all five pesticides in saliva samples. The Rf values observed for each spiked sample closely matched those of the respective standard solutions, confirming the successful identification of each poison. This validated the sensitivity and specificity of the HPTLC method for detecting these toxic substances in biological matrices. **Conclusion:** HPTLC is a reliable, efficient, and cost-effective analytical method for detecting common poisons in biological samples such as saliva. The technique offers significant clinical value by enabling timely diagnosis and management of poisoning cases, potentially improving patient survival rates.

Keywords: Biological Samples, Pesticides, High Performance Thin Layer Chromatography (HPTLC), Poison.

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INTRODUCTION

Poisoning is a major health problem in many developed countries, including India (Batra *et al*., 2003). There are more than 9 million natural and synthetic chemicals worldwide, and the list keeps growing. The incidence of poisoning in India is estimated to be around 18.7 per 100,000 population per year in 2019 (Fernando, 2002).

According to World Health Organization (WHO), approximately 3 million poisonings occur annually worldwide. Developing countries like Sri Lanka and India report high rates of toxicity and death due to poisoning (Thomas *et al*., 2000). In India, Household agents (Pyrethroids, rodenticides, carbamates, phenyl, detergents, corrosives, etc.,) had the highest incidence of poisoning, followed by drugs, pesticides, industrial chemicals, plants, animal bites and stings (Srivastava *et al*., 2005). According to the National Crime Records Bureau reports, poisoning was estimated to contribute 4.6% of the 451,757 accidental deaths recorded in India 2014 and 6.3% of the 413,457 accidental deaths in 2015 (Aggarwal, 2014). Food poisoning and snakebites caused an estimated 1624 and 8554 deaths, respectively, in 2015. Among modes adopted to end life by suicide victims, poisons contributed to 26.0% (34,254) and 27.9% (37,232) of total cases in 2014 and 2015, respectively, with another 0.5% in both years being contributed by sleeping pills (Aggarwal, 2015). The prevalence of poisoning has increased as a result of rapid industrialization, easy availability of over-the-counter medication and the widespread use of pesticides in agriculture (Maharani and Vijayakumari, 2013).

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Chromatography is commonly used in the pharmaceutical industry for the quantification of drugs and other pharmaceuticals. Among the various chromatographic techniques, High Performance Thin Layer Chromatography (HPTLC) has gained popularity to identify many chemicals due to its speed, versatility, applicability, and ability to analyze more than 100 samples simultaneously (Futagami *et al*., 1997). Analyzing blood samples for the presence of drugs and other poison, using High-Performance Thin-Layer Chromatography (HPTLC) has emerged as a crucial approach in forensic toxicology and clinical settings (Nair *et al*., 2021).

HPTLC, is a chromatographic technique used to separate, identify, and quantify components in a mixture. It offers higher resolution and sensitivity compared to traditional TLC (Thin-Layer Chromatography) (Tomsia *et al*., 2022). HPTLC can be used to separate and quantify the various components present in a biological sample, including poisons (Amir *et al*., 2022). Hence, we proposed to estimate various poison in sample using HPTLC method.

MATERIALS AND METHODS

HPTLC provide a simple, rapid and reliable method for detecting and identifying poisons in biological sample. The combination of advanced instrumentation, high-quality stationary phase, and sophisticated software in the HPTLC system ensured the successful separation and analysis of compounds in complex samples, making it a powerful technique in analytical chemistry.

Chemicals and Reagent

Acetone, n-hexane, toluene, methanol, and ethanol were utilized. These solvents played crucial roles in sample preparation, purification, and the development of chromatographic separations

Apparatus

HPTLC aluminum plates precoated with silica gel [20x20 cm] 0.2 mm thickness; micro syringe 100 µL, glass chamber UV Lamp short wavelength 287 nm and, HPTLC Densitometric scanner with software.

Standard Solutions

Cypermethrin

Stock solution

Weigh 1 mg of cypermethrin was dissolve in 1 mL of acetone slowly until it dissolves completely. To obtain 1mg/mL of cypermethrin stock solution. Using a micropipette, carefully measure 50 µL from the 1 mg/mL cypermethrin stock solution and transfer it to the appropriately labelled microcentrifuge tube and add 950 µL of acetone to the respective microcentrifuge tube and it was kept in vortex sorter for 15-30 min to ensure the attain homogeneity. The final concentration was used for HPTLC was 50 µL/mL.

Sample preparation

- Sample concentration: (50 mg/mL, 1:10 dilution).
- Sample Prepared In: Acetone.
- Sample Description: Cypermethrin.
- Stationary phase: Silica gel GF 254.
- Mobile phase: Toluene: Acetone (8:2).
- Scanning wavelength: 254 nm.
- Applied volume: Sample: Track 1 (4 µL) Track 2 (8 µL).
- Development mode: Ascending mod.

Lambda-cyhalothrin

stock solution

Weigh 1 mg of Lambda-cyhalothrin was dissolved in 1 mL of acetone slowly until it dissolves completely. To obtain 1 mg/mL of Lambda-cyhalothrin stock solution. Using a micropipette, carefully measure 50 µL from the 1 mg/mL Lambda-cyhalothrin stock solution and transfer it to the appropriately labelled microcentrifuge tube and add 950 µL of acetone to the respective microcentrifuge tube and it was kept in vortex sorter for 15-30 min to ensure the attain homogeneity. The final concentration was used for HPTLC was 50 µL/mL.

Sample preparation

- Sample concentration: (50 mg/mL, 1:10 dilution).
- Sample Prepared In: Acetone.
- Sample Description: Lamda Cyhalothrin.
- Stationary phase: Silica gel GF 254.
- Mobile phase: Toluene: Acetone (8:2).
- Scanning wavelength: 254 nm.
- Applied volume: Sample: Track 1 (4 µL) Track 2 (8 µL).
- Development mode: Ascending mode.

Malathion

Stock solution

Weigh 1 mg of malathion was dissolved in 1 mL of acetone slowly until it dissolves completely. To obtain 1 mg/mL of malathion stock solution. Using a micropipette, carefully measure 50 µL from the 1 mg/mL malathion stock solution and transfer it to the appropriately labelled microcentrifuge tube and add 950 µL of acetone to the respective microcentrifuge tube and it was kept in vortex sorter for 15-30 min to ensure the attain homogeneity. The final concentration was used for HPTLC was 50 µL/mL.

Sample preparation

Sample concentration: (50 mg/mL,1:10 Dilution).

- Sample Prepared In: Acetone.
- Sample Description: Malathion.
- Stationary phase: Silica gel GF 254.
- Mobile phase: n-Hexane: Acetone (8:2).
- Scanning wavelength: 254 nm.
- Applied volume: Sample: Track 1 (4 µL) Track 2 (8 µL).
- Development mode: Ascending mode.

Monocrotophos

Stock solution

Weigh 1 mg of monocrotophos was dissolved in 1 mL of acetone slowly until it dissolves completely. To obtain 1 mg/mL of monocrotophos stock solution. Using a micropipette, carefully measure 50 µL from the 1 mg/mL monocrotophos stock solution and transfer it to the appropriately labelled microcentrifuge tube and add 950 µL of acetone to the respective microcentrifuge tube and it was kept in vortex sorter for 15-30 min to ensure the attain homogeneity. The final concentration was used for HPTLC was 50 µL/mL.

Sample preparation

- Sample concentration: (50 mg/m, 1:10 dilution).
- Sample Prepared In: Acetone.
- Sample Description: Monocrotophos.
- Stationary phase: Silica gel GF 254.
- Mobile phase: Toluene: Acetone (8:2).
- Scanning wavelength: 254 nm.
- Applied volume: Sample: Track 1 (4 µL) Track 2 (8 µL).
- Development mode: Ascending mode.

Quinalophos

Stock solution

Weigh 1 mg of quinalophos was dissolved in 1 mL of acetone slowly until it dissolves completely. To obtain 1mg/mL of quinalophos stock solution. Using a micropipette, carefully measure 50 µL from the 1 mg/mL quinalophos stock solution and transfer it to the appropriately labelled microcentrifuge tube and add 950 µL of acetone to the respective microcentrifuge tube and it was kept in vortex sorter for 15-30 min to ensure the attain homogeneity. The final concentration was used for HPTLC was 50 µL/mL.

Sample preparation

- Sample concentration: (50 mg/mL, 1:10 dilution).
- Sample Prepared In: Acetone.
- Sample Description: Quinalophos.
- Stationary phase: Silica gel GF 254.
- Mobile phase: n-Hexane: Acetone (8:2).
- Scanning wavelength: 254 nm.
- Applied volume: Sample: Track 1 (4 µL) Track 2 (8 µL).
- Development mode: Ascending mode.

Sample preparation

The preparation of saliva samples for HPTLC analysis involves by taking 0.5 mL of saliva and adding 25 µL or 50 µL of a standard solution derived from the stock solution; to facilitate the extraction of target compounds, 1.5 mL of methanol is added to the mixture. The sample is then vortex sorted thoroughly for 5 min, ensuring complete mixing and effective extraction. Following this, the mixture is centrifuged at 5000 rpm for 10 min, which separates the supernatant from the precipitate. The supernatant, containing the extracted compounds, is carefully collected and then concentrated by evaporating the solvent using a nitrogen (N_2) evaporator. Once evaporation was completed, the residue was reconstituted with 1 mL of acetone, stabilizing the sample for injection. Finally, the reconstituted sample was injected into the HPTLC system.

HPTLC plate preparation

The HPTLC plate was cut into (10×20 cm) and Prior to sample application. The prewashing process involved immersing the plates twice in 10 mL of methanol each time, effectively removing any contaminants or residues that could interfere with the separation process. After the methanol wash, the plates were activated by heating them at 80°C for 5 min.

Procedure for Determining the poison

Using a 100 µL Hamilton syringe and a Linomat V applicator with an 8 mm band width, the sample solutions (cypermethrin, lambda-cyhalothrin, malathion, monocrotophos and quinalophos) (50 µL/mL) and standard solutions (cypermethrin, lambda-cyhalothrin, malathion, monocrotophos and quinalophos) (50 µL/mL), were applied to pre-coated Silica gel GF 254 plates (20-10 cm with 250 µm thickness, E. Merck) the mobile phase of Toluene: Acetone (8:2) for LaMDA cyhalothrin, cypermethrin and Monocrotophos, n-Hexane : Acetone (8:2) for Malathion and Quinalophos, (30 min at ambient temperature saturated in a CAMAG glass twin trough chamber) mobile phases were carefully optimized before analysis. the plate was inserted in the twin chamber and allowed to run for 8cm. Following the development, the plates were dried and scanned in absorbance mode using a CAMAG TLC scanner III with a deuterium source at 287 um.

The reflection mode slit dimension setting was set to 6 mm for length and 0.45 mm for width, and the scanning rate was 20 mm sec. The Win CAT (Catenary Analysis software) software

was used to identify the chromatograms peaks. The validation of the developed HPTLC method was conducted following the guidelines outlined by the International Conference on Harmonization (ICH) in Q2 (R1). The method was rigorously evaluated for its specificity, sensitivity, accuracy, precision, repeatability, and robustness to ensure it meets the required standard

RESULTS

HPTLC Results

The HPTLC (High-Performance Thin-Layer Chromatography) analysis was done to identify the specific pesticide poisons, including cypermethrin, lambda-cyhalothrin, Malathion, Monocrotophos and Quinalophos in biological samples such as saliva.

Cypermethrin

The qualitative analysis of saliva sample containing cypermethrin poison and standard cypermethrin was performed using HPTLC. The optimized mobile phase used was Toluene: acetone in the ratio of 8:2, the peak area of specific poison in the saliva sample corresponded with standard poison was determined using Win CAT software. The Rf values obtained in saliva sample contain cypermethrin was 0.64 and standard poison cypermethrin was 0.66. Figure 1 represents Chromatogram of standard and sample cypermethrin, Figure 2 represents HPTLC band chromatogram of standard and sample cypermethrin.

Lamda Cyhalothrin

The qualitative analysis of saliva sample containing Lamda cyhalothrin poison and standard Lamda cyhalothrin was performed using HPTLC. The optimized mobile phase used was Toluene: acetone in the ratio of 8:2, the peak area of specific poison in the saliva sample corresponded with standard poison was determined using Win CAT software. The Rf values obtained in saliva sample contain Lamda cyhalothrin was 0.63 and standard poison Lamda cyhalothrin was 0.65. Figure 3 represents chromatogram of standard and sample Lamda cyhalothrin, Figure 4 represents HPTLC band chromatogram of standard and sample Lamda cyhalothrin.

The qualitative analysis of saliva sample containing malathion poison and standard malathion was performed using HPTLC. The optimized mobile phase used was n-Hexane: Acetone (8:2), the peak area of specific poison in the saliva sample corresponded with standard poison was determined using Win CAT software. The R_f values obtained in saliva sample contain malathion was 0.47 and standard poison malathion was 0.47. Figure 5 represents chromatogram of standard and sample malathion, Figure 6 represents HPTLC band chromatogram of standard and sample malathion.

Quinalophos

The qualitative analysis of saliva sample containing quinalophos poison and standard quinalophos was performed using HPTLC. The optimized mobile phase used was n-Hexane: Toluene: Acetone (8:2), the peak area of specific poison in the saliva sample corresponded with standard poison was determined using Win CAT software. The R*f* values obtained in saliva sample contain quinalophos was 0.70 and standard poison quinalophos was 0.71. Figure 7 represents chromatogram of standard and sample quinalophos, Figure 8 represents HPTLC band chromatogram of standard and sample quinalophos.

Monocrotophos

The qualitative analysis of saliva sample containing Monocrotophos poison and standard Monocrotophos was performed using

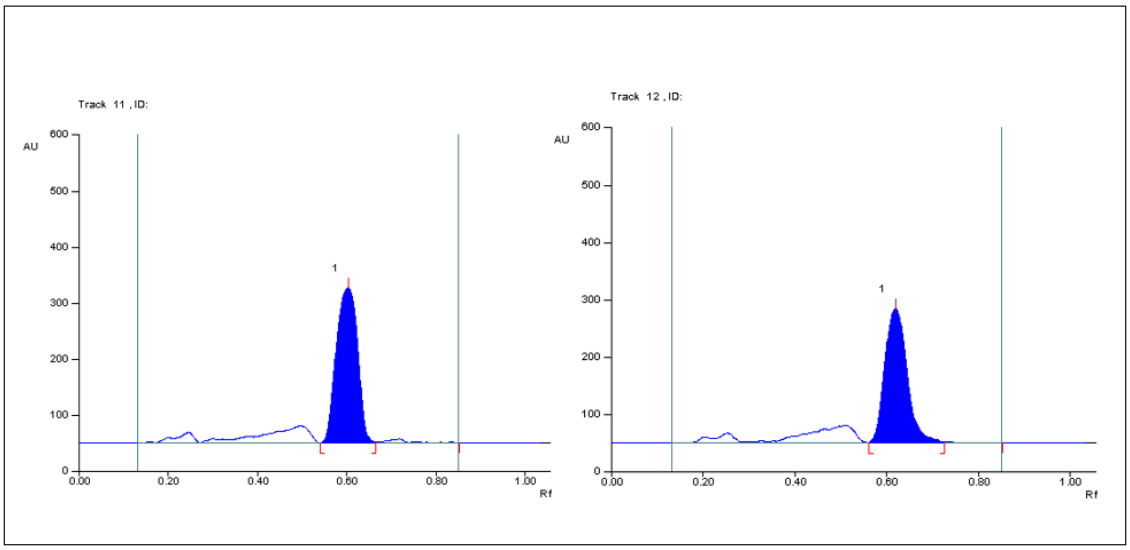

Figure 1: Chromatogram of standard and sample Cypermethrin.

Figure 2: HPTLC band Chromatogram of standard and sample cypermethrin.

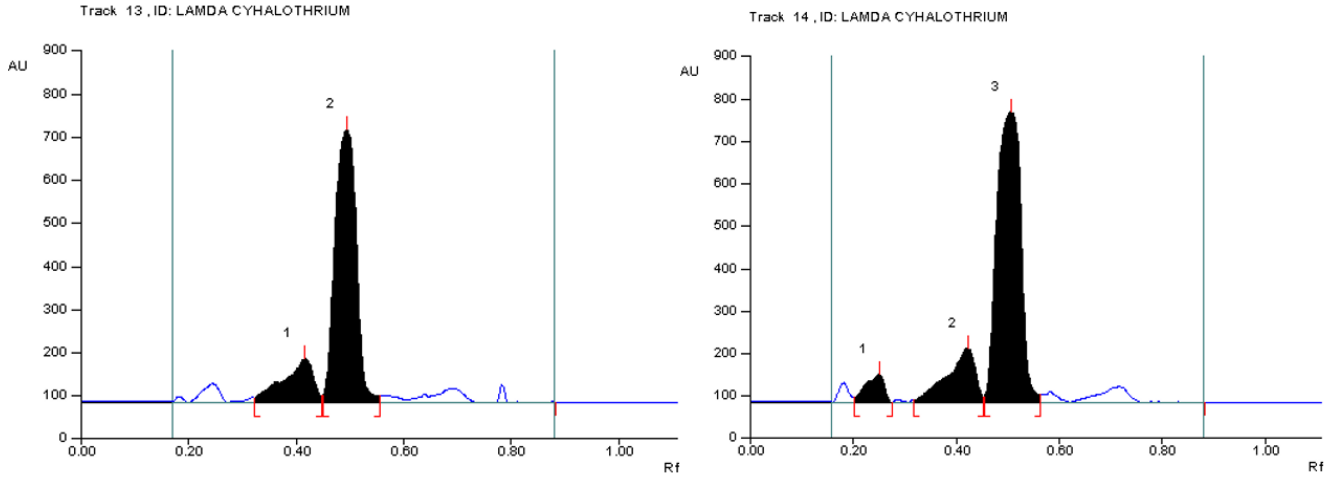

Figure 3: Chromatogram of standard and sample Lamda cyhalothrin.

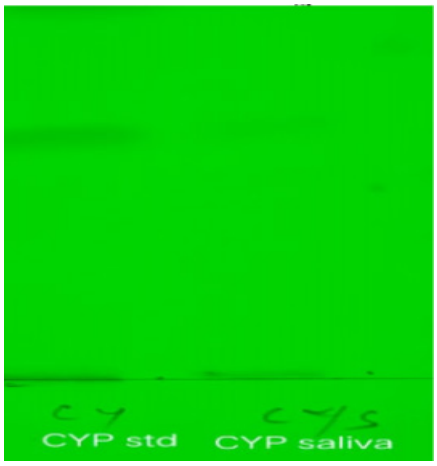

Figure 4: HPTLC band Chromatogram of standard and sample Lamda Cyhalothrin.

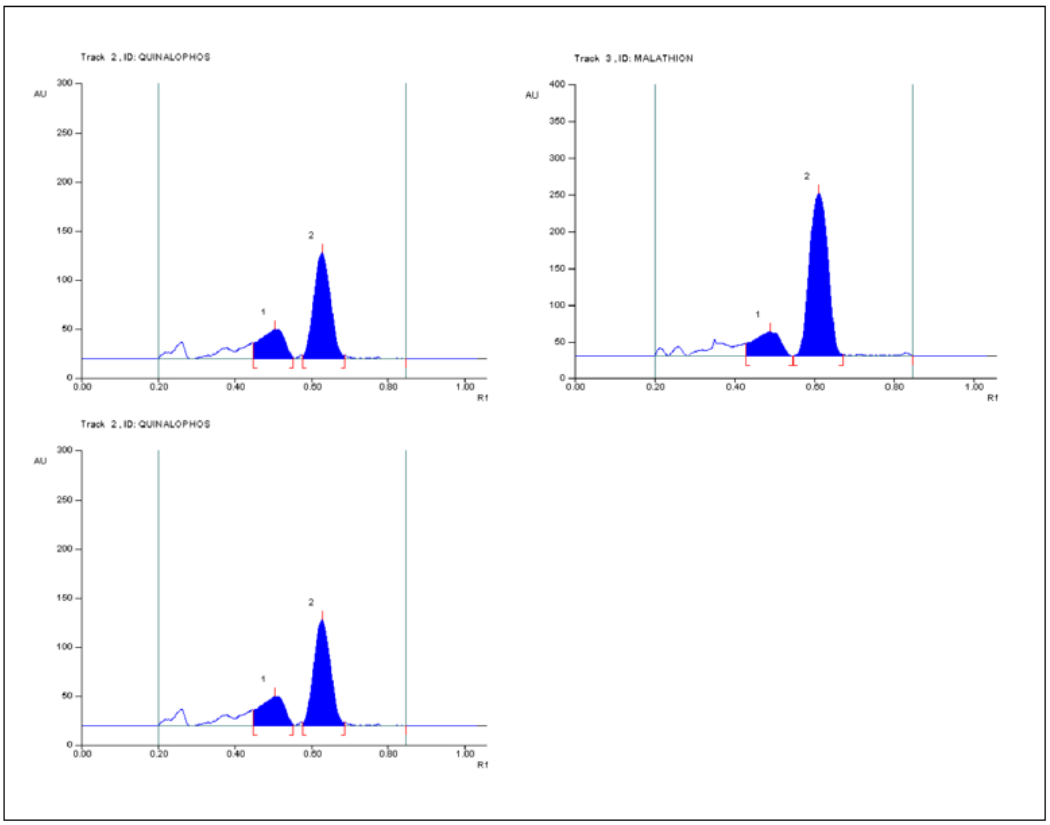

Figure 5: Chromatogram of standard and sample Malathion.

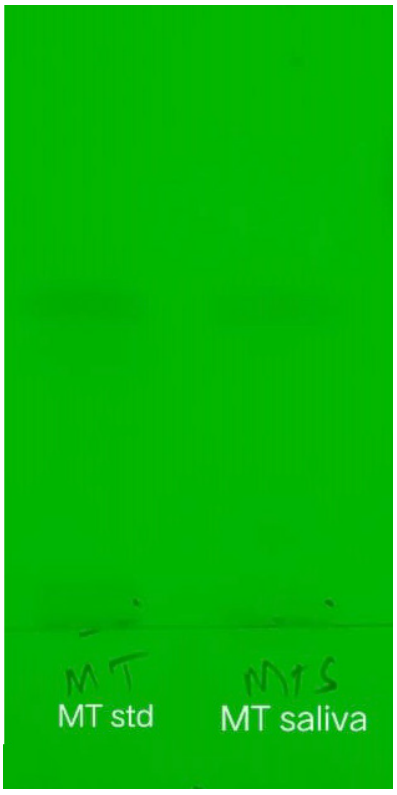

Figure 6: HPTLC band chromatogram of standard and sample malathion.

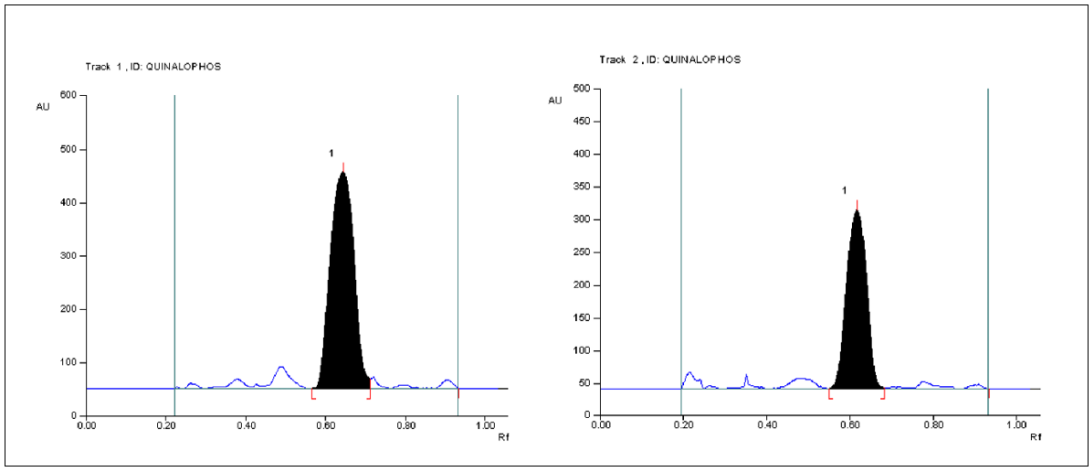

Figure 7: Chromatogram of standard and sample Quinalophos.

Figure 8: HPTLC band chromatogram of standard and sample Quinalophos.

HPTLC. The optimized mobile phase used was Toluene: Acetone (8:2), the peak area of specific poison in the saliva sample corresponded with standard poison was determined using Win CAT software. The Rf values obtained in saliva sample contain monocrotophos was 0.38 and standard poison monocrotophos was 0.39. Figure 9 represents chromatogram of standard and sample monocrotophos, Figure 10 represents HPTLC band chromatogram of standard and sample monocrotophos.

DISCUSSION

This study demonstrates that HPTLC is a reliable method for the qualitative analysis of various pesticides in biological samples. We used different ratios of toluene, n-hexane and acetone as the mobile phase proved effective across the different compounds tested, ensuring accurate identification of the toxins in both saliva and plasma.

Cypermethrin

The analysis of the saliva sample containing cypermethrin revealed a R_f value of 0.64, closely matching the R_f value of 0.66 for the standard cypermethrin solution. This R_f values indicates a successful identification of cypermethrin in the sample. The mobile phase utilized (Toluene: acetone in a ratio of 8:2) was effective in achieving this resolution.

Lambda-Cyhalothrin

For lambda-cyhalothrin, the saliva sample exhibited a R_f value of 0.63, whereas the standard solution presented a R_f value of 0.65, confirming the presence of the toxin in the sample. The mobile phase utilized (Toluene: acetone in a ratio of 8:2) was effective in achieving this resolution.

Malathion

The analysis of the saliva sample containing malathion revealed a R_f value of 0.47, closely matching the R_f value of 0.47 for the standard malathion solution. This R_f values indicates a successful identification of malathion in the sample. The mobile phase n-Hexane: Acetone (8:2) utilized was effective in achieving this resolution.

Quinalophos

The analysis of the saliva sample containing Quinalophos revealed a R_f value of 0.70, closely matching the R_f value of 0.71 for the standard Quinalophos solution. This R_f values indicates a successful identification of malathion in the sample. The mobile phase n-Hexane: Acetone (8:2) utilized was effective in achieving this resolution.

Monocrotophos

For Monocrotophos, the saliva sample exhibited a R_f value of 0.38, whereas the standard solution presented a R_f value of 0.39, confirming the presence of the toxin in the sample. The mobile

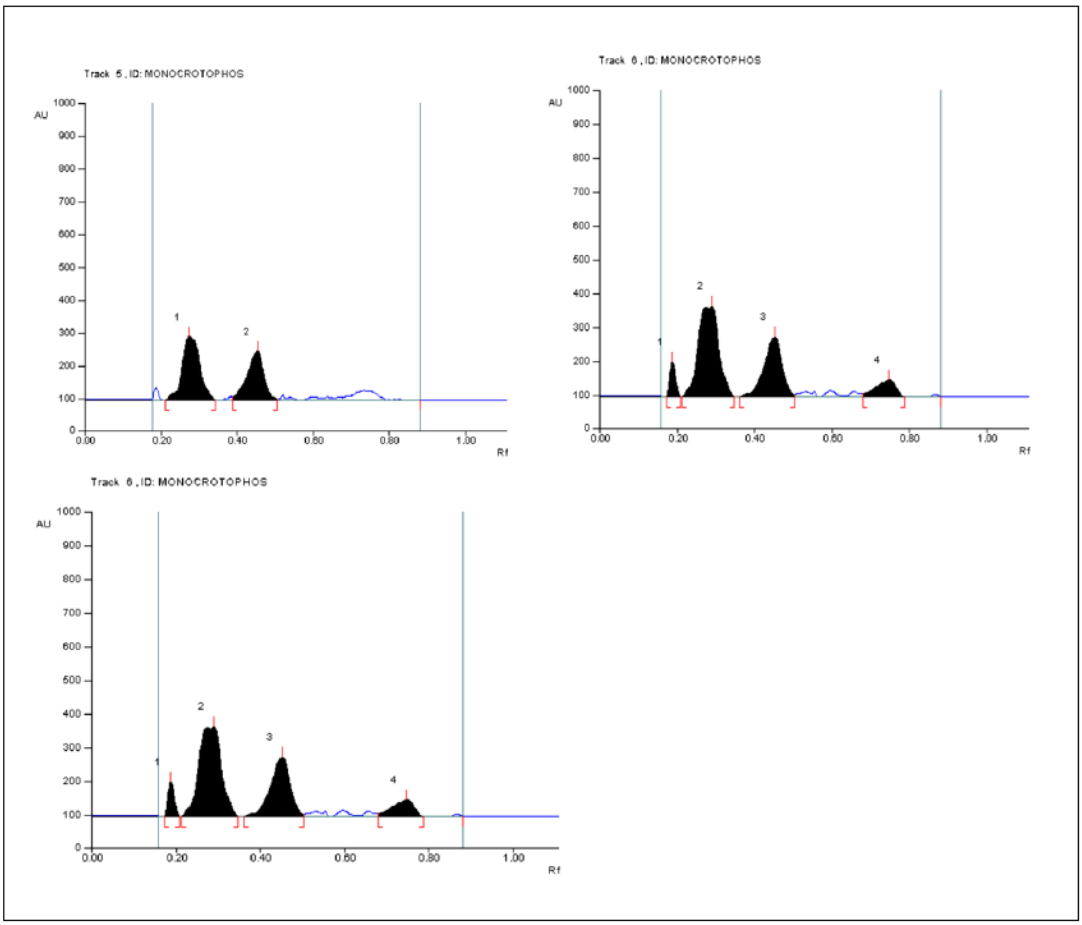

Figure 9: Chromatogram of standard and sample Monocrotophos.

Figure 10: HPTLC band chromatogram of standard and sample Monocrotophos.

phase Toluene: Acetone (8:2) was effective in achieving this resolution.

CONCLUSION

HPTLC was performed in plasma sample in comparison to standard and it confirmed the presence of cypermethrin, LaMDA cyhalothrin, Malathion, Monocrotophos and Quinalophos. Hence, HPTLC was found to be a reliable and the cost-effective approach to detect various poison in biological samples like plasma and saliva which helps health care practitioner to detect poison, which in turn save life of many patients.

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ABBREVIATIONS

HPTLC: High Performance Thin Layer Chromatography; **EDTA:** Ethylenediamine tetra-acetic acid; **Win CAT:** Catenary Analysis software; **ICH:** International Conference on Harmonization.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Aggarwal, S. (2014). Suicide in India. A report.

Aggarwal, S. (2015). Suicide in India. A report.

Amir, S. H., Zafar, L., Nasreen, F., Khalid, A., Siddiqui, S. M., & Abdullah, D. (2022). A retrospective analysis of a methanol poisoning outbreak in an Indian tertiary Care Hospital during the COVID-19 era: A pandemic within a pandemic. Asia Pacific Journal of Medical Toxicology, 11(3), 80–84.

Batra, A. K., Keoliya, A. N., & Jadhav, G. U. (2003). Poisoning: An unnatural cause of morbidity and mortality in rural India. The Journal of the Association of Physicians of India, 51, 955–959.

Fernando, R. (2002). The national poisons information centre in Sri Lanka: The first ten years. Journal of Toxicology. Clinical Toxicology, 40(5), 551–555. https://doi.org/10.1081/CLT-120014644

Futagami, K., Narazaki, C., Kataoka, Y., Shuto, H., & Oishi, R. (1997). Application of high-performance thin-layer chromatography for the detection of organophosphorus insecticides in human serum after acute poisoning. Journal of Chromatography. B, Biomedical Sciences and Applications, 704 (1–2), 369–373. https://doi.org/10.1016/S0378-4347(97)00476-3

Maharani, B., & Vijayakumari, N. (2013). Profile of poisoning cases in a tertiary care Hospital, Tamil Nadu, India. Journal of Applied Pharmaceutical Science, 3(1), 091–094. https://doi.org/10.7324/JAPS.2013.30117

Nair, S. J., Sujatha, C., Chettiar K P, S., & Sasikala, K. (2021). Toxico-epidemiology of acute poisoning; an exploratory study from a tertiary care hospital in South India along with global comparisons and solutions. Journal of Forensic and Legal Medicine, 83, Article 102247. https://doi.org/10.1016/j.jflm.2021.102247

Srivastava, A., Peshin, S. S., Kaleekal, T., & Gupta, S. K. (2005). An epidemiological study of poisoning cases reported to the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. Human and Experimental Toxicology, 24(6), 279–285. https://doi.org/10.1191/0960327105ht527oa

Thomas, M., Anandan, S., Kuruvilla, P. J., Singh, P. R., & David, S. (2000). Profile of hospital admissions following acute poisoning—Experiences from a major teaching hospital in south India. Adverse Drug Reactions and Toxicological Reviews, 19(4), 313–317.

Tomsia, M., Głaz, M., Nowicka, J., Cieśla, J., Sosnowski, M., & Chełmecka, E. (2022). Fatal methanol poisoning caused by drinking industrial alcohol: Silesia Region, Poland, April–June 2022. Toxics, 10(12), Article 800. https://doi.org/10.3390/toxics10120800

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