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Research article

CHLORPYRIFOS IN BIOLOGICAL SAMPLES BY HPTLC

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ABSTRACT: Chlorpyrifos, an organophosphorous compound, is widely used as an insecticide to improve the yield of agricultural produce in India and world. The present study was conducted to screen biological samples received in forensic science laboratories for residual chlorpyrifos. Detection of chlorpyrifos in viscera is usually done by thin layer chromatography using Hexane: acetone (8: 2) as solvent system. Mercurous nitrate, mercuric nitrate ($HgNO_{3)}$ followed by diphenyl carbazone and mercuric nitrate (HgNO3) followed by potassium ferrocynide (K_4Fe (CN) $_6$) are used as chromogenic reagents. Due to presence of chlorine, the identification was supported by applying flame test. Routinely used solvent system the hexane: acetone (8: 2) separates the compounds at the solvent front and creates confusion in the identification. Various proportions of same solvent system where tried and a good separation of chlorpyrifos was achieved by using 9:1 ratio. The Rf value in this system was found to be 0.75. The system is specifically useful for separating the insecticide in biological samples. In non biological samples 8:2 ratio also serve the purpose. The detection limit for the insecticide in this system is found to be 5 µg.
Keywords: Chlorpyrifos, HPTLC, Chromogenic, Biological samples

INTRODUCTION

Chlorpyrifos and rogor are used as pesticides for controlling agricultural pest; they are organophosphourus insecticide. This is frequently used for suicidal purpose. Chloropyrifos is practically insoluble in water, readily soluble in organic solvents like acetone (6500 g/Kg at 25 ^{o}C) and methanol (450 g/Kg at 25 ^{o}C). It is readily absorbed into the bloodstream from gastrointestinal tract through the lungs and skin [1]. The major metabolites observed are 3, 5, 6-trichloro-2-pyridinol (TCP), diethyl phosphate and diethylthio- phosphate. Chlorpyrifos is very toxic and affects the central nervous, cardiovascular, and respiratory systems, dose up to 28 g may be fatal. Optimization of Chlorpyrifos degradation by *Pseudomonas putida* was done by Vijayalakshmi P. and Usha M. S [2]. Effect of Pesticide (Chlorpyrifos) on Soil Microbial Flora and Pesticide Degradation by Strains Isolated from Contaminated Soil was done by [3]. Determination of chlorpyrifos residues in buffalo meat samples using high performance liquid chromatography [4].

Chemicals and reagents

Mercurous nitrate solution: 1.0040 g of mercurous nitrate dissolved in20ml of water and 4 drops of Conc. nitric acid was added and diluted to 100 ml with milli q water.

Potassium ferrocynide solution: 5.3 gm of potassium ferro cyanide dissolved and diluted to 100 ml of milli Q water.

Diphenyl carbazone solution: 0.1015 gm of diphenylcarbazone dissolved and diluted to 100 ml with acetone.

EXPERIMENTAL

Extraction

100 grams viscera sample from viscera 1 (stomach, intestine with contents) and Viscera 2 (lungs, liver, kidney, spleen) was taken, chopped, extracted in ether (neutral extract). Control chlorpyrifos was also extracted in ether, the extract was kept overnight the extract was dried and taken in methanol.

High performance thin layer chromatography (HPTLC) [6]

The HPTLC glass plate of the size 20 cm X 20 cm and TLC chamber (30 cm X 25 cm X 15 cm) were used The HPTLC plates were coated with slurry of silica gel G (20 µm), air dried, activated for one hour in an oven at 110 ^{o}C. The extract obtained was spotted on four different plates by using same capillaries. Plate (A), plate (B), plate (C) and plate (D) were run in different solvent systems. Plate (A) was run in hexane: acetone (8: 2) solvent system and. Plate (B), (C) and (D) were run in hexane: acetone (9: 1) solvent systems. They were removed, developed up to 10 cms, dried in air and sprayed plate (A) and plate (B) with mercurous nitrate, mercuric nitrate followed by diphenyl carbazone and mercuric nitrate followed by potassium ferrocynide.

UV Spectrophotometry

UV VIS spectrophotometer SPECORD S 100 B was used for analysis. The portion near Rf 0.75 from plate (C) was removed by scrapping, dissolved in methanol and filtered through Whatmann filter paper no 42,The filtrate was concentrated on water bath. The residue was taken in aq. acid, UV reading was noted.

GC-MS

Make: Perkins Elmer Clarus 600.

Column: Capillary column Ellit 5 ms, length 30 m, 0.25 mm ID and width 0.25 mm.

Operating conditions for GCMS (5)

Carrier gas: Helium flow rate – 1 ml/min

Injector: Split mode, temperature 240^{o}C

Oven temperature: Initial temperature: 130^{o}C with 2 minutes hold

Ramp rate: 5^{o}C per minute

Final temperature: 280^{o}C with 5 minutes hold

Mass spectrophotometer conditions:

Source Temperature: 150^{o}C.

Interface temperature: 50^{o}C.

Electron Ionization mode with energy 70eV**.**

RESULTS AND DISCUSSIONS

The post-mortem samples i.e. viscera is received in poisoning cases. It is great challenge to extract poison from biological sample as biological samples are very complex and it contains large amount of fatty material. Along with poison, fatty material also get extracted in solvent. Here we have used ether as solvent for extraction, ether is universal solvent and highly volatile also it reduces the cost of analysis .Chlorfyrofos and Rogor was separated from biological samples by HPTLC.. For spotting instead of ether here we have used acetone as a solvent for spotting to reduce interference of fatty material. For development of HPTLC plate instead of hexane: acetone (8: 2) we have change the ratio to Hexane: acetone (9: 1). For development of Chorpyrofos and Rogor mercurous nitrate was used as a chromogenic spray reagent. mercurous nitrate is one of the specific spray reagent for organophosphorus insecticides. In hexane: acetone (8: 2) solvent system, Chlorpyrifos and rogor observed with low Rf value, Black spot with mercurous nitrate is often confused with Thimet and Rogor as shown in figure 1. In Hexane: acetone (9: 1) solvent system Chlorpyrifos observed at Rf 0.75 and Rogor gives spots at Rf 0.20,0.40,0.60,0.85. as shown in figure 2. After obtaining the separation pattern as shown in figure 2, the portion at Rf 0.75 was extracted in methanol as a solvent and evaporated to dryness and UV chromatogram was plotted in aqueous acid. Uv Chromatogram shows λ max at 230nm and 289nm tallies with standard Chlorpyrifos Chromatogram as shown in figure 3. Dimethoate does not give any distinct absorption maxima for UV Chromatogram.

Concentrated methanol extract was used for hyphenated technique GC- MS for further identification and confirmation. M.S spectrogram gives major mass fraction at 97 and 197 as shown in figure 4.Library search of spectrogram was done and it exactly tallies with Chlorpyrifos as shown in Figure 4 and 5.

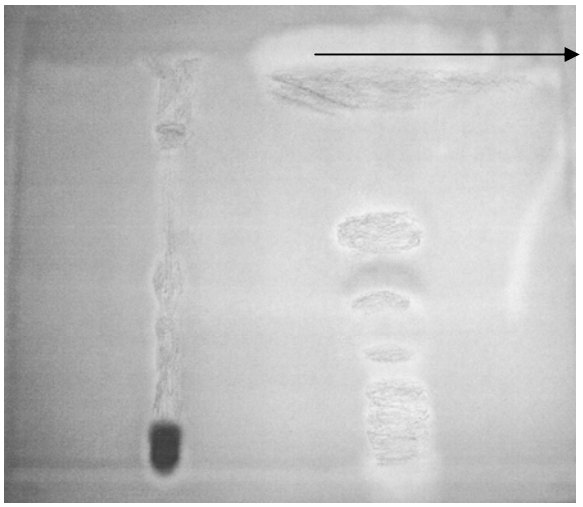

Figure 1. HPTLC pattern of Sample in solvent system hexane: acetone (8:2)
1:-Control rogor
2:-Control chlorpyrifos

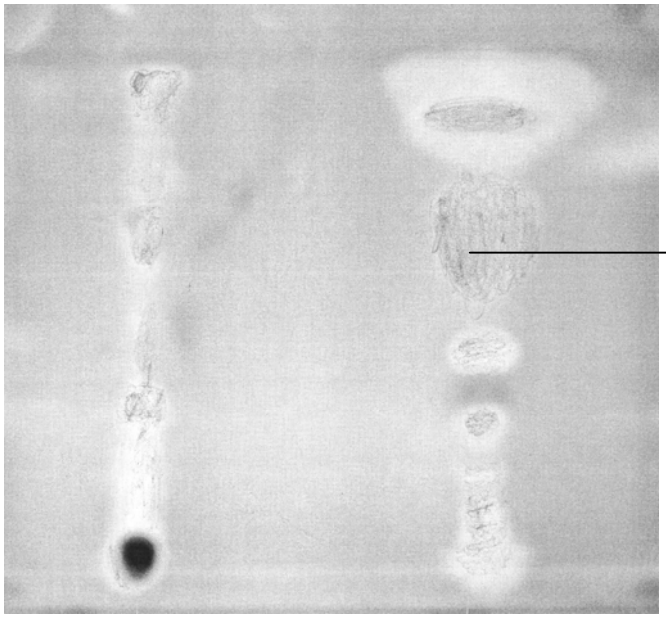

Figure 2. HPTLC pattern of Sample in solvent system hexane: acetone (9:1)
1:- Control rogor
2:-Control chlorpyrifos

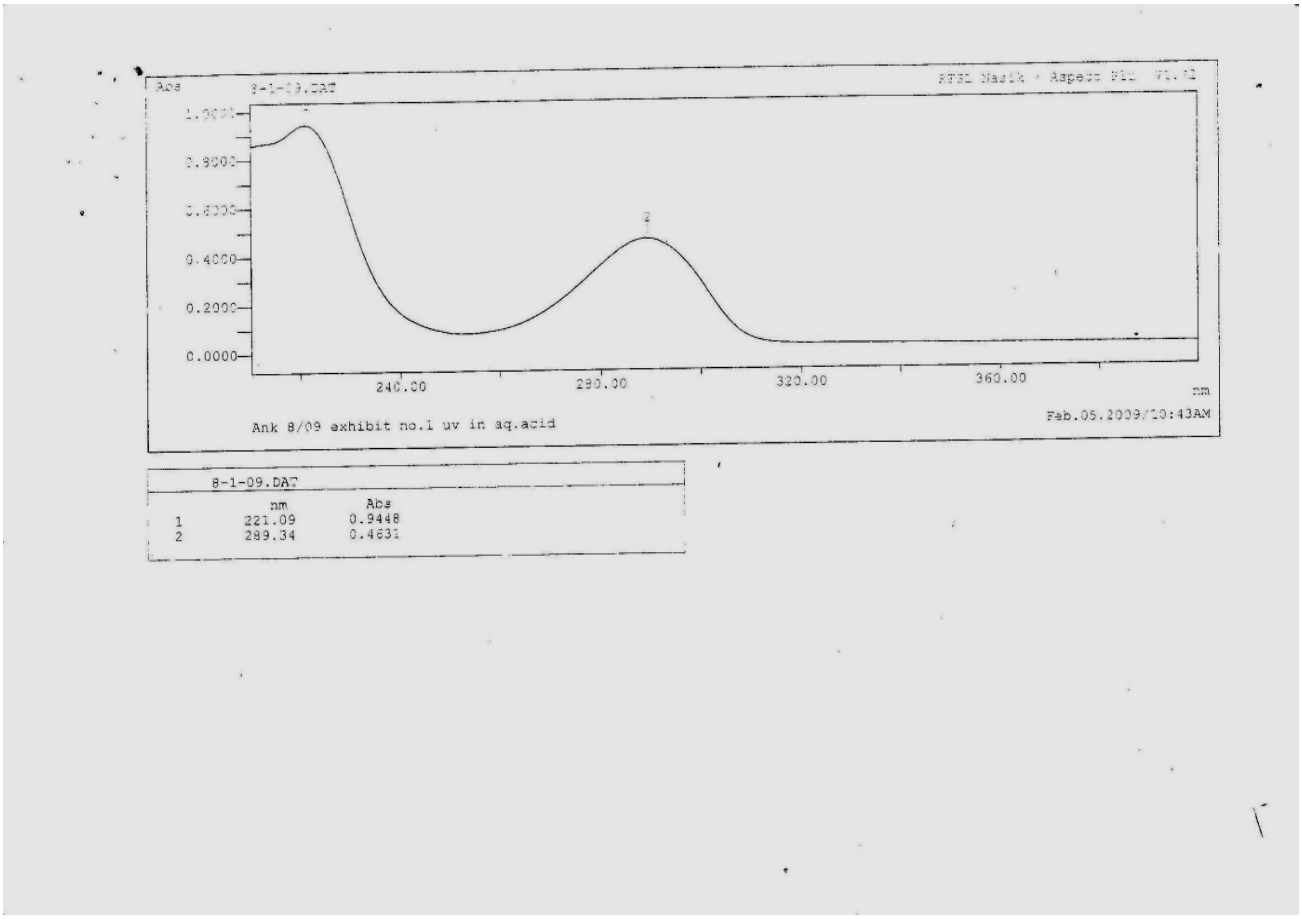

Figure 3:- UV spectra of sample in 0.1 N HCL

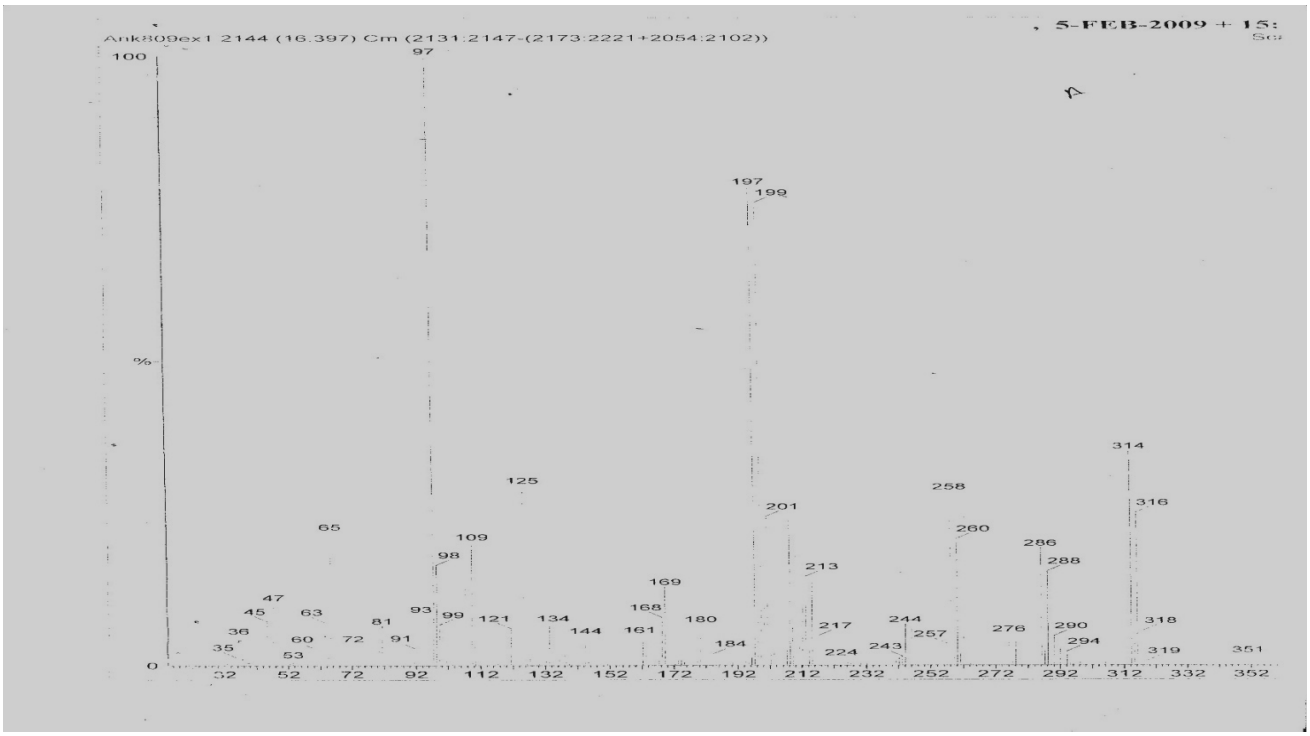

Figure 4:- TIC of sample run on GC-MC

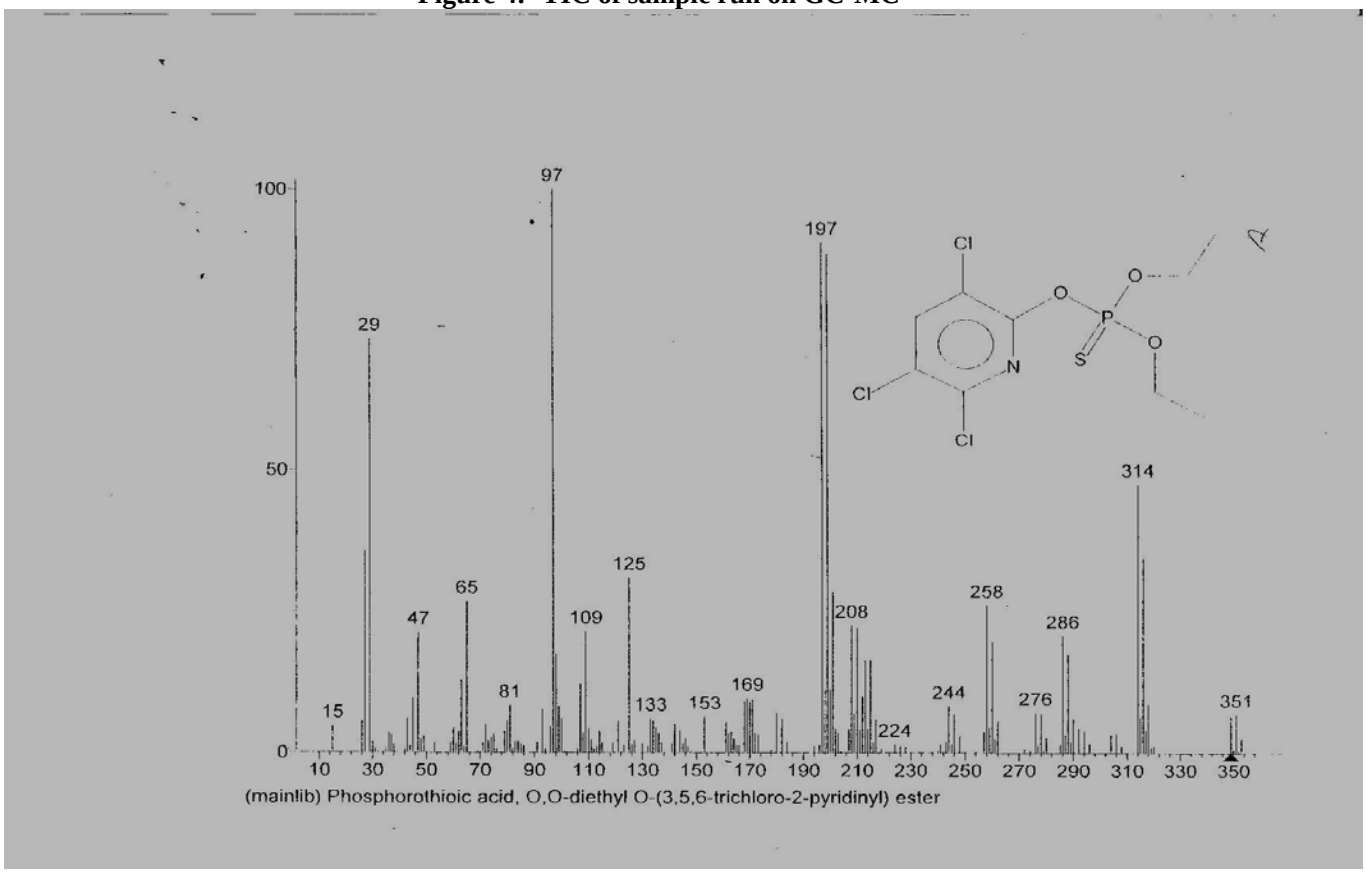

Figure 5:- Library search match for chlorpyrifos in GC-MS studies

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