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# HPTLC Detection of Pyrethroids in Autopsy Tissues

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**Abstract** Pyrethroids use as an insecticide has been increasing in recent years as a replacement for organophosphate insecticides that are being phased out because of water-quality concerns (California Department of Pesticide Regulation, 2005). Pyrethroids are used in both agricultural and urban (commercial and residential) areas. The occurrence of pyrethroids is of concern because pyrethroids are known to be highly toxic to aquatic organisms,. Due to their easy availability, Pyrethroids insecticides are often misused in homicidal and suicidal cases, requiring toxicological examination. Forensic toxicologists need to be able to characterize these insecticides.

As pyrethroid use continues to increase in both urban and agricultural settings, it is important to have robust, sensitive, rapid methods that are capable of detecting measuring these compounds in autopsy tissues with relevant concentrations (below acute toxicity levels) in both blood and viscera. HPTLC detection of Pyrethroids from autopsy tissues is best method for forensic case work. This method will also help scientists to understand pyrethroid behavior in the environment.

**Keywords** Insecticide organophosphate, toxicity, HPTLC

## Introduction

Pyrethroid use as an insecticide has been increasing in recent years as a replacement for organophosphate insecticides that are being phased out because of water-quality concerns (California Department of Pesticide Regulation, 2005). Pyrethroids are used in both agricultural and urban (commercial and residential) areas [1]. The occurrence of pyrethroids is of concern because pyrethroids are known to be highly toxic to aquatic organisms. Due to their easy availability, Pyrethroids insecticides are often misused in homicidal and suicidal cases, requiring toxicological examination. Forensic toxicologists need to be able to characterize these insecticides.

Cypermetherian, Deltametherin, Fevolarate are the synthetic pyrethroids having insecticidal activity against a wide range of pests with low mammalian toxicity. Highly potent Pyrethroids like cypermetherin, Deltametherin,Fenvolarate,are the esters of 2,2 dimethylcyclopropare carboxylic acid with 2,2-dihalovinyl side chain, these Pyrethroids have a cyanide group attached at the α-position to the carboxyl ate group.

As pyrethroid use continues to increase in both urban and agricultural settings, it is important to have robust, sensitive, rapid methods that are capable of detecting and measuring these compounds in autopsy tissues with relevant concentrations (below acute toxicity levels) in both blood and viscera. HPTLC detection of Pyrethroids from autopsy tissues is best method for forensic case work, where more than thousand autopsy samples are tested every month. This method will also help scientists to understand pyrethroid behavior in the environment.

Very few TLC methods have been utilized for the presence of pyrithroid insecticide from autopsy tissues. The literature survey reveals that, the reagents include Phosphomolybdic acid [2], ulta-voilet (UV) irridation [3] and silver nitrate, and irridation with UV light [4] palladium Chloride [5] etc. None of above mention chromogenic reagent is specific for α-cyno ester compound. However, Copper-acetate and O-tolidine [6] and P-nitrobenzaldehyde followed by P-dinitrobenzene [7] reagents have been recently reported. In the present with the slight modification for very much similar compound is reported. We reports, alkaline hydrolysis of p-nitrobenzaldehyde as a specific spray reagent for α cyno ester by High Performance Thin Layer Chromatography. The basic of this reagent underlines on the formation of well-known chemical reaction of Benzoin condensation. This reagent produces violet spots relatively with synthetic Pyrethroids containing cyno group.

## Material and Method

### Chemicals and Reagents

All the chemicals and reagents used were of analytical grade.

1) Sodium hydroxide solution: 10%-10 gm of sodium hydroxide pallets in distilled water and dilute to 100 ml.
2) P-Nitrobenzaldehyde reagent: 1%-1 gm of P-Nitrobenzaldehyde dissolved in 100 ml of ethanol
3) Reference Standard of insecticides:: 1mg/ml $^{-1}$ prepared of –Pyrethroids in Ethanol
   A) Cypermetherin in Ethanol
   B) Deltametherin in Ethanol
   C) Fenvolerate in Ethanol

- Solvent: Hexane, acetone, Tolune, Cyclohexane

### Method

### High Performance thin Layer Chromatography

Chromatography was performed on 20 cm x 20 cm silica gel $60F_{254}$ HPTLC glass plate(Merck),A camag(Switzerland),linomat IV applicator was used to apply 10 µl in ethanol equivalent to 10 µg along with std cypermetherin, Deltametherin,Fenvolerate and extract of negative control tissue of autopsy were also applied on HPTLC plate. The plate was then developed in pre-saturated 24 cm x 8 cm x 22.5 cm camag twin through TLC chamber to a distance of 10 cm using hexane: acetone (8:2) and Cyclohexane: Tolune (5:5) v/v as mobile phase. The plate was removed from the chamber, dried in air and sprayed with sodium hydroxide solution and P-Nitrobenzaldehyde reagent by using glass sprayer. Successively blue-violet spots were observed at RF values shown in table 1

**Table 1**

| Control Insecticides | Solvent system Hexane: acetone {8:2) | Solvent system Cyclohexane:Tolune(5:5) |
|---|---|---|
| 1. Cypermetherin | 0.53 | 0.60 |
| 2. Deltametherin | 0.60 | 0.93 |
| 3. fenvolerate | 0.66 | 0.64 |

### Extraction of Pyrethroids in Autopsy tissues

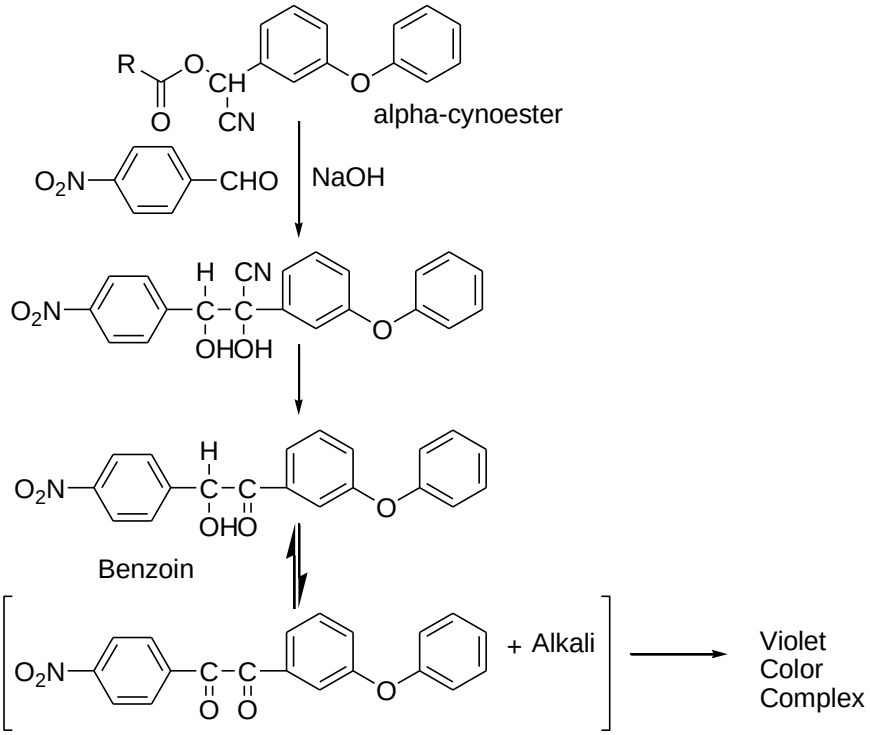
100 gm of stomach, Intestine, Liver, Spleen or Kidney tissue (having a death history of Cypermetherin poisoning) was chopped to fine pieces in a beaker. About 20 gm of ammonium sulphate was added and it was kept for two hours for precipitation of proteins and amino acids. About 100 ml of diethyl ether or hexane was added to the beaker, stirred well and watery part was transferred to a 500 ml of glass separating funnel. After waiting for few minutes the organic solvent layer was separated in glass capsule and evaporated in air. The procedure was repeated for at least two times with enough quantity (2*100 ml) of the solvent for complete extraction of the Pyrithroid, the Concentrated residue was dried over sodium sulphate and then it was applied to the HPTLC plate for screening along with a reference standards

**Result and Discussion**

The result of HPTLC screening is tabulated in Table 1. Which shows RF values of Cypermetherin, Deltametherin, Fenvolerate insecticides?

The developing solvent mixtures were: 1) Hexane: Acetone 2) Cyclohexane: Tolune (5:5)

The reaction mechanism of pyrethroids shows—alkaline hydrolysis of α-cyno ester produce HCN and corresponding benzaldehyde derivative [8]. Sodium Hydroxide present in the reaction mixture readily reacts with pyrethroid compound to produce HCN. Which reacts with p-Nitrobenzaldehyde reagent: to give Benzoin Condensation product. The Benzoin condensation is reversible in alcoholic alkaline solution through tautomerisn. A characteristic color reaction is observed on addition of aqueous alkali to a solution of Benzoin in the presence of air (autoxidation) [9]. After a certain induction period the solution acquires a blue-violet colour, which disappears when the solution is oxygenated by keeping for some time.

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