

**DEVELOPMENT OF NEW SPRAY REAGENT TO FIND A MYSTERY
BEHIND THE DEATHS OF CHILDREN`S OF A SINGLE FAMILY–
MURDER OR SUICIDE**

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ABSTRACT

An 11 years village girl from joint family cooked noodles in a earthen pot and served to her two younger sisters and one brother with herself. After sometime they started vomiting and giddiness and all of them became serious. Out of four, three children`s were died during hospitalization within half an hour. MO collected stomach wash and viscera of three children`s and IO collected the vomit mix food particles from crime scene then they send to laboratory for analysis. Analysis to prove the presence of organochloro insecticide Thiodan (Endosulfan) by TLC method I.

KEY WORDS: Human viscera, Organochloro insecticide, Endosulfan, TLC.

INTRODUCTION

Various kinds of substances are used in agriculture for crop protection and pest control. Owing to Their easy availability these substances are misused in criminal poisoning cases. In such poisoning cases medical officer preserves the postmortem samples for routine toxicological examination and submit it to forensic science laboratory. In such situation toxicologist play a vital role in identification of the toxic substances at low concentration.

Number of chromogenic reagents is reported in the literature for detection of different class of Insecticides.^[4-5] Various analytical and advance instrumental methods viz. gas chromatography^[6-8] and mass spectroscopy^[7] are also reported in the literature. Since in toxicology, generally no clue about the nature of poison is available and the poison extracted from the biological material is only in microgram level, TLC screening using numbers of reagents are in use. This may lose the quantity of poison. Hence, it has been taken to develop unique chromogenic reagent for all class of insecticides by reacting them in alkaline media with O-Tolidinemixed with Acetone on thin layer chromatography plate. Here we study the interesting case in Marathwada region.

In a village, the deceased girl collected noodles from earthen pot and boiled in utensil. Noodles were consumed by her two sisters and one brother with herself. All sisters and brothers felt giddiness in 10 to 15 minutes after consumption of noodles. All were admitted in hospital within 15 minutes. Girl who cooked the noodles and one of her sister and brother died within 30 minutes. Doctor had collected stomach wash and viscera from two sisters and brothers of which only one sister survive. Police seize boiled noodles with the pot and vomit mixed food particles. All viscera of deceased and other food material were submitted forensic science laboratory. As per postmortem doctor opinion asorganochloroinsecticide like smell. So it is confirmed by forensic department that food that have consumed is having organochloro compound as a poison that causes death of 3 members. The organochloro compound analysis by TLC method which is confirmed by alkaline media with O-Tolidinemixed with Acetone on thin layer chromatography plate. This case is raised out in Maharashtra state assembly period during question hour.

MATERIAL AND METHOD

Chemical and Reagents

All the chemicals were of analytical grade Distilled water was used throughout the analysis. 20 % (v/v) Sodium Hydroxide was prepared by dissolving 20 gm. of Sodium Hydroxide pellets in 100 ml of distilled water.

Followed by 5 gm. of O-Tolidine dissolved in 50 ml of Acetone. Further plate will be put in ultraviolet (UV) chamber for 20 minutes. Standard solution of Endosulphan ($1\text{mg}/\text{ml}^{-1}$) was prepared in ethanol. Similarly the entire standard (profenopos, cypermetherin, begon, carbofuran) were also prepared in ethanol.

It is therefore necessary to have a sensitive reagent to detect endosulfan. In this paper we report use of 10 % NaOH followed by mixture O-Tolidide 1.0% in acetone for HPTLC detection and identification of endosulfan insecticide with a solvent system Hexane: Acetone (8:2). All the poisons develop on TLC at 0.3 to 0.8 RT. So it is easy to identify which poison is there by TLC.

Reagents used

All the chemicals were of analytical grade Distilled water was used throughout the analysis. Haxane, Diethylether, Sodioum Hydroxide, O-tolidine, and acetone and silica gel-G.Endosulfan (Thiodan) 35% EC from rallies India limited was used as standard.

Thin Layer Chromatography

A standard glass TLC plate (10 x 20 cm) was coated with 0.25 mm layer of silica gel-G slurry in water (1:2). The plate was activated at 110° C for about 1 hour before use. The standardsolutions ofendosulfan were procured (Thiodan) 35% EC from rallies India limited (1 mg/ml) ware prepared the sample spotted on the activated TLC plate. The plate was developed up to the distance of 10 cm in a presaturated TLC chamber using Haxane: Acetone (8:2) as mobile phase. After development, the plate was dried in air and uniformly sprayed with 10% Sodium Hydroxide and then followed by Nickel amine in Ammonia solution^[1] spray reagent. A grayish black colored spot was immediately observed at Rf 0.48 and 0.77. Further spraying with o-tolidine in Acetone then observe in UV chamber after 10min greenish colored spot was detected at same rf value.

Recovery of Insecticides from Biological samples

Extraction Method

Ammonium sulphate (10 gm) was added to samples of biological tissue (stomach, intestine, liver-spleen and kidney (100gm), containing Endosulfan insecticides. The sample were then minced individually with water and then extracted in separating funnel with diethyl ether (100ml). The ether extract was transferred to an evaporating dish and the aqueous phase was re-extracted with diethyl ether (2 x 50ml). The extracts were combined and the solvent was evaporated at room temperature. The residue was dissolved in ethanol (2ml) and the solution was used for further analysis. About 60 gm of visceral sampal tissue was macerated in homogenizer and homogenized tissues were subjected to detection of pesticide. Similarly gastric lavage sample and vomit mixed with food particles were extracted with diethyl ether. spotted on activated TLC plate with 2.0 µg, 4.0 µg, 6.0 µg, 8.0 µg and 10 µg. The plates were

developed and sprayed with chromogenic reagent as described above. The intensity of the spots developed for the visceral extract was compared with standards.

RESULTS AND DISCUSSION

Three sisters and one brother consumed noodles. Two sisters and one brother died within 30 minutes and one were seriously hospitalized but not saved. Instant deaths of Two sisters and one brother itself revealed that some strong poison might have been consumed because any other reasons cannot affect all persons in similar manner. Endosulfan was detected in all autopsy tissue, stomach wash, vomit mix food particles and clue articles i.e. boiled noodles recovered from crime seenplace. Thin layer chromatography (TLC) (Fig 1) shows the presence of endosulfan at Rf 0.48,0.77 but no other insecticide was observed. Endosulfan containing cyclic sulphite in its structure is readily hydrolyzed by alkali. The sulphite (characteristic formation from tetravalent sulphur compound by action of alkali^[3] in turns react with nickel (II) amine to give grayish black nickel (IV) oxyhydrate NiO(OH)₂. Endosulfan gives two black color spots at different Rf values, Due to the presence of alpha and beta isomer.^[2] The color of spot is stable for more than 24 hours.

CONCLUSION

Endosulfan mixed with noodles was chemically confirmed. On the basis of Forensic report investigation was carried out and it was established that girl had made noodles which was poisoned by endosulfan earlier by accident. The girl has no knowledge of endosulfan and its toxicity as she was only 10 years old. Therefore the cause of death was endosulfan but no other insecticide. We has developed simple and convenient method which is useful for identification of poison very easily.

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