A SPECIFIC SPRAY REAGENT FOR IDENTIFICATION AND DETECTION OF CARBARYL IN BIOLOGICAL MATERIAL

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ABSTRACT:

Although efforts have been made to reduce the use of carbamate insecticides, they are still widely used in certain parts of India. Carbamate insecticides are used to control a wide variety of pests, including moths, cockroaches, ants, ticks and mosquitoes. The easy availability of these insecticides results in their being frequently encountered in forensic casework. Hence its selective characterisation is therefore necessary. Presently a number of chromogenic reagents have been used for its detection by thin layer chromatographyfrom biological samples. We develop a new, specific, sensitive chromogenic reagent for detection and identification of carbaryl in biological materials. We use 10% sodium hydroxide, mixture of copper chloride and sodium bromide as spray reagents. Carbaryl on 10 % sodium hydroxide undergoes hydrolysis, forming corresponding phenol. This hydrolysed product reacts with sodium bromide in presence of copper chloride and forms bluish-violet complex on HPTLC. Other carbamate insecticides failed to give colourspot. Limit of detection of this reagent is 5 µg

Keywords: Insecticide, HPTLC

Introduction:

Carbaryl (1-naptal-N Methyl carbamate) belongs to a family of chemicals that kill or control insect, known as carbamate. These insecticides are widely used against a broad spectrum of insects on field crops, fruits and vegetables and against household flies and mosquitoes[1]. A number of reagents have been used for its detection and identification .Viz. diazophenol (after alkaline hydrolysis)[,] alkaline fast blue-B [2];Tollen's reagent [3] has been widely used for the detection of carbamate insecticides. The use of alkaline phenythydrazine hydrochloride [4], ammonium cerium nitrate [5,] copper chloride (III) then ammonium

metavanadate [6] diazotized p-amino-1 naphthol-3 sulfonic acid then NaOH is reported to be specific for carbaryl only. However, it is not very sensitive; the spots are ill-defined for low Concentration of insecticides and they cannot be easily located, possible because of biological impurities.

It is therefore necessary to have a sensitive reagent to detect carbaryl. In this paper; we report use of 10 % NaOH followed by mixture of sodium bromide and copper chloride for HPTLC detection and identification of carbaryl insecticide with a solvent system Hexane and Ethyl acetate (9:1).

Materials and Method:

Chemicals and reagents:

All the chemicals were of analytical grade Distilled water was used throughout the analysis.10 % (v/v) Sodium Hydroxide was prepared by dissolving 10 gm. of Sodium Hydroxide pellets in 100 ml of distilled water.

a) 5 gm. of Sodium bromide dissolved in 50 ml of distilled water. b) 5 gm. of Copper chloride dissolve in 50 ml of distilled water.Standard solution of carbaryl (1mg ml^{-1}) was prepared in ethanol. Similarly all the standard (profenopos, thiodan, cypermetherin, began, carbofuran) were also prepared in ethanol.Accelerated solvent Extractor ASE 200(Dionex) was used for extraction of carbaryl

Extraction procedure:

It is an automated system for extracting organic compounds from variety of solid and semisolid samples. If the sample contains water than diatomaceous earth is added to absorb the water contents and get a solid and semisolid sample for extraction. The ASE 200 acceraletes the traditional extraction process by using solvent at elevated temp. Pressure is applied to the sample extraction cell. To maintain the heated solvent in a liquid state during the extraction. After heating, the extract is flushed into the collection vials and ready for analysis. Approximately 20 gm. of visceral sample such as stomach, intestine, liver, spleen, kidney having history of consumption of carbaryl insecticides, cut into fine pieces along with diatomaceous earth and transferred into extraction cell. The extract were collected in a clean collection vial, diethyl ether was used for extraction at 50 ^{0}C and 1000 psi pressure in two

cycles. The extract obtained were transferred into a steel capsule and evaporated to dryness at room temp. The residue were dissolved in 2ml of ethanol and processed further by HPTLC.

High performance thin layer chromatography:

Chromatography was performed on 20 cm x 20 cm silica gel 60 F_{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 carbaryl,extract of viscera having history of death due to consumption of carbaryl, blank viscera,carbofuran,baygon ,profenopos(organophosporous),Thiodan(organochloro),cypermetherin(pyrethroids), 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 ethyl acetate: Hexane (9:1) v/v as mobile phase. The plate was removed from the chamber dried in air and sprayed with 10 % NaOH solution followed by mixture of (a) and (b) solution by using glass sprayer. Successively blue-violet spots were observed at RF value 0.51 for std. carbaryl and viscera having history of death due to carbaryl. Other carbamates such as carbofuran, baygon did not give colour spot.

Fig No. 1 HPTLC chromatograph

1 standard carbaryl
2 viscera
3 blank viscera
4 carbofuran
5 baygon

Recovery Experiments:

Carbaryl (1 mg in ethanol) was separately added to the minced visceral tissue (50 gm.) mixed well and left for 24 hours. The tissue samples were then

processed as above (Extraction procedure)except that the residue from extraction of the tissue were dissolved in 1 ml of ethanol .this solution 10µl) was spotted in separate activated plate with the respective standard solution10 ul of carbaryl containing 7,8,9,9.5,10 mg in 10 ml ethanol. The plates were then developed and processed as described above. The intensity of the spot obtained for the extracts of visceral tissue were compared with those from the standard and found to be most similar to the spot resulting from the 8 mg. (10 ml) $^{-1}$ std.solution of carbaryl. Hence the recovery for carbaryl was 80%

Result and discussion:

This reagent is selective for carbaryl. Other carbamates such as carbofuran, baygon and organ phosphorous insecticides such as profenofos, organochloro such as thiodan, pyrithroids such as cypermethrin do not give colour spot. More over constituents of viscera (aminoacids, peptides and proteins) which are generally co-extracted withthe insecticide do not interfere. The sensitivity of reagent is ca0.5µg per spot observed after development.

On alkaline hydrolysis carbaryl yields 1-napthol which reacts with copper chloride and sodium bromide to give violet bluish complex. The colour of spot is stable for couple of days. The reagent described here is very sensitive and specific for carbaryl and hence it can be used routinely for detection and identification of carbaryl and its breakdown product 1-napthol in biological and non-biological material in forensic toxicology.

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