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A New chromogenic reagent for carbamate insecticides

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Abstract:

Carbamate insecticides are increasingly being used in agriculture on daily basis. New varieties of these insecticides are easily available. Due to their easy availability insecticides are often misused in homicidal and suicidal cases, requiring toxicological examination. Forensic toxicologists need to be able to characterise these insecticides. A number of analytical and advance instrumental methods, i. e. Gas chromatography, and Mass sprectoscopyeasely have been reported for detection of carbamates from biological and non-biological materials. Though these tech rapid, specific and sensitive bur they cannot be always used for detection of insecticides which are extracted from biological materials, as the purity of samples is in question, Hence in routine forensic toxicological examination, high performance thin layer chromatography is the best technique for identification and detection of insecticides from biological materials. In present paper, Author has made efforts to use new chromogenic spray reagent for HPTLC detection of carbamates We use 5% of toluidine followed by 10 % of aq. Sodium nitrate and 10 % aq. Sodium hydroxide which gives orange and violet spots.

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Introduction:

Carbamates (carbaryl, baygon, carbofuran) belong 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 5 % o-Toluidine reagent followed by 10% sodium nitrate and then 10% sodium hydroxide for HPTLC detection and identification of carbamate l 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.

a) 10% aq. Sodium hydroxide: 10 % (v/v) Sodium Hydroxide was prepared by dissolving 10 gm. of Sodium Hydroxide pellets in 100 ml of distilled water

b) o-Toluidine reagent: 5 gm. of o-toluidine in 14 ml HCL fill up to 100 ml distilled water

c) 10% aq. Sodium nitrate

Standard solution of carbaryl (1mg ml $^{-1}$), carbofuran (1mg ml $^{-1}$), baygon (1mg ml $^{-1}$), were prepared in ethanol. Similarly all the standard (profenopos, thiodan, cypermetherin, were also prepared in ethanol.

Accelerated solvent Extractor ASE 200(Dionex) was used for carbaryl, carbofuran, baygon

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, carbofuran,baygon and extract of viscera having history of death due to consumption of carbamate, blank viscera,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 hexane: ethyl acetate (9:1) v/v as mobile phase. The plate was removed from the chamber dried in air and sprayed with o-toluidine reagent followed by 10% sodium nitrate and 10% sodium hydroxide Solution by using glass sprayer. Successively orange and blue-violet spots were observed at RF values shown in table 1

Table 1:

No	Name of insecticide	Colour	Rf value
1	Baygon	Orange Faint violet	0.48 0.51
2	carbaryl	orange	0.46
3	carbofuran	Violet Violet	0.46 0.49

Recovery Experiments:

Carbaryl, baygon, carbofuran (each 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 carbamate other organ phosphorous insecticides such as profenofos, organochloro such as thiodan, pyrithroids such as cypermethrin do not give colour spot. More over constituents of viscera (amino acids, peptides and proteins) which are generally co-extracted with the insecticide do not interfere. The sensitivity of reagent is ca 0.2 µg per spot observed after development.

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