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Research article

THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF BOOM FLOWER (NITROBENZENE) IN BIOLOGICAL MATERIALS

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ABSTRACT: A sensitive and selective thin layer chromatographic method for the detection and identification of nitrobenzene using furfural as a spray reagent is described. The reduction product of nitrobenzene gives aniline. Which react with furfural reagent to give a red color spot. The reagent does not react with other organo phosphorous (except ethyl parathion), organochloro, carbamate and synthetic pyrethriod insecticides. The reagent is specific and selective only for nitrobenzene. The constituents of viscera (amino acids, peptides, proteins etc.) and plant materials do not interfere with the test. The detection limit for nitrobenzene is about 0.5 µg.
Keywords: Biological materials, Forensic Analysis, Extraction, Nitrobenzene, TLC, Furfural

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INTRODUCTION

The agriculture use of organophosphorous, organochlorine, carbamate, pyrethroid and nitrogen-based insecticides to protect crops from insects has increased enormously in recent years. Homicidal, suicidal, and accidental use of insecticides in India is very common because of to their ready availability. Nitrobenzene (essence of mirbane, oil of mirbane) is a colorless to pale yellow oily liquid. It is also known as Boom Flower. It has an odor of volatile oil almonds and is freely soluble in ethanol, ether and benzene [1]. Nitrobenzene insecticides (20 % formulation) are widely used in agriculture sector. LD_{50} of nitrobenzene orally in rats is 600 mg/kg. During 2014 the Regional Forensic Science Laboratory, Aurangabad, Maharashtra, India, detected 22 cases of nitrobenzene poisoning in biological samples. The increasing number of biological samples for poison detection needs versatile, sensitive, and selective reagents. Thin layer chromatography (TLC) is the method of choice because of their speed, low cost and versatility.

The literature survey reveals few reports on detection of nitrobenzene by TLC [2], GC [3] and HPLC [4]. However there are limitations to their use in routine forensic work owing to the complex matrix which may damage the columns. TLC can moreover be used to screen many samples in the time taken by these instrumental methods to screen one sample only. TLC is therefore the method of choice for screening biological samples. In this communication we have discussed reduction of nitrobenzene followed by coupling with furfural in acidic condition to form a colored Schiff's base.

Chemical and reagents

All reagents and chemicals used were of analytical-reagent grade. Glass-distilled water was used throughout the experiment. A standard solution of nitrobenzene (1 mg ml^{-1}) was prepared by dissolving technical grade nitrobenzene (Sainath Agro-Vet, Pvt. Ltd. Kopargaon) in 10 ml ethanol. Reducing agent is prepared by dissolving 5g stannous chloride in 100 ml conc.HCl. Furfural reagent (5 %) was prepared by dissolving 5 ml furfural and 5ml acetic acid in 100 ml distilled water.

EXPERIMENTAL

Extraction

Ammonium sulfate (10g) was added to visceral tissue (stomach, intestine, liver, spleen and kidney; 100g) containing nitrobenzene and the samples was individually minced in water. Each sample was then extracted with 150 ml ether in a separating funnel, by shaking for 2-3 min. The ether extracts was transferred to an evaporating dish and the aqueous phase was re-extracted with ether (2 or 3 X 50 ml). The solvent of the combined ether extract was then evaporated at room temp. The residue was then dissolved in a minimum volume of ethanol (5 ml). This solution was treated as stock sample solution.

Thin-Layer Chromatography (TLC)

TLC was performed on glass plate coated with slurry of silica gel G (Acme, India) in water (1:2) to a thickness of 0.25 mm. The plates were activated by heating at 110^0 C for in an oven.

Standard stock solution of nitrobenzene (1mg ml^{-1}).

Standard solution of other organophosphorous insecticides, for example, dimethoate, dichlorvos and thimet.

Standard solutions of organochloro insecticides, for example, endosulfan, DDT and BHC.

Standard solutions of carbamate insecticides, for example, carbaryl, carbofuran and carbosulfan.

Standard solutions of pyrethroid insecticides, for example, fenvalrate, cypermethrin, and deltamethrin and solutions of nitrobenzene extracted from viscera.

These solutions are spotted on the plate, which was then developed with Chloroform: Acetone (7:3) as mobile phase to a distance of 10 cm in a previously saturated TLC chamber. The plate was removed from the chamber, dried in air, and sprayed uniformly with reducing agent. Then plate was heated in an oven at 110^0 C for about 20 min. The plate was then removed and kept for attaining room temperature and sprayed uniformly with 5 % furfural solution. Immediately a cherry red color was appeared (Figure 2). The spots are stable for an hour and then turns to brownish in color.

Recovery of Nitrobenzene from Biological Materials

10 mg quantities of nitrobenzene in ethanol were separately added to 100 g of minced visceral tissue, mixed well with water and stored for 24 h. The insecticides were then extracted by shaking for 2-3 min with ether (150 ml) in a separating funnel. The ether extract was then transferred to an evaporating dish and the aqueous phase was re-extracted with ether (50 ml). The extracts were combined, the solvent was evaporated at room temperature and the residue was dissolved in ethanol (10 ml). This solution (10 µL) was spotted on glass TLC plate with 10 µL of standard solution of nitrobenzene in ethanol of known concentrations 8, 8.5, 9.0, 9.5,10 mg per 10 ml. The plate was then developed and sprayed with reagent as described above. The intensity of the cherry red colored spot developed for the visceral extract was visually comparable with the spot corresponding to 9.0 mg per 10 ml ethanol (n=3), hence the recovery was ca 90 %.

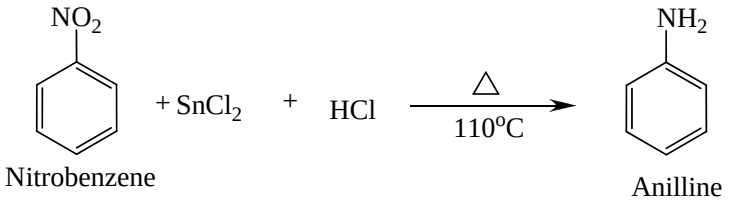
RESULTS AND DISCUSSIONS

The use of nitrobenzene as insecticide for agriculture sector dates back to 10^{th} century. Its use as an insecticide is still continued in 21^{st} century. For agriculture sectors 20 % & 40 % of its formulations are generally used. The acute oral toxicity to rats (LD_{50}) is 600 mg kg^{-1}. Due to easy availability of these insecticides they are misused by the people and death occurs.

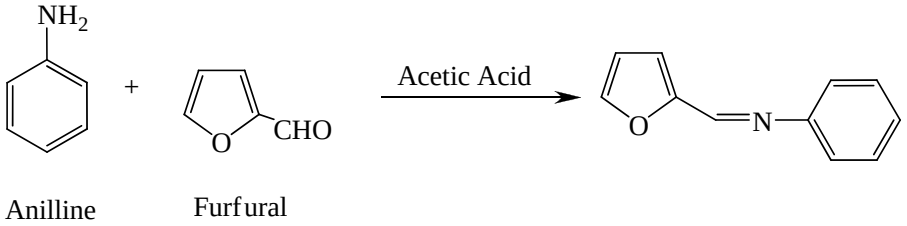
Nitrobenzene on reduction gives aniline. Aniline in acidic condition reacts with furfural reagent to form a colored Schiff base as shown in step 2 reaction. Then in step 3, rearrangements take place and the final product formed is dianyl of hydroxy glutaconic dialdehyde [5] as shown in Figure 1. Cherry red color spots from nitrobenzene standard and from nitrobenzene extracted from visceral material were observed at R_F 0.72.

From recovery experiments it was observed that the intensity of the cherry red color spot developed for the visceral extract was comparable with that of the spot corresponding to 9.0 mg nitrobenzene per 10 ml ethanol (Average from three experiments). Hence recovery was ca 90 %.

Step 1



Step 2



Step 3

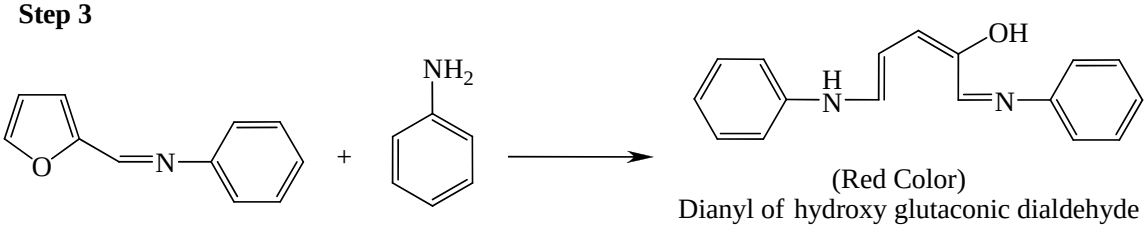


Figure 1: Reaction of nitrobenzene with Furfural.

Figure 2

Thin layer chromatogram obtained from: (a) standard solution of nitrobenzene; (b) nitrobenzene from visceral extract; (c) blank viscera; (d) dimethoate; (e) endosulfan; (f) carbaryl; (g) cypermethrin.

CONCLUSION

The reagent reported is selective and sensitive for nitrobenzene among other insecticides. Other organophosphorous (except methyl and ethyl parathion, as they contains nitro group and can be reduced to amine but these insecticides are highly poisonous and their fatal dose is very low, so now a days in India these are banned for use in agriculture sector), organochlorine, carbamate and pyrethriod insecticides do not give colored spots. Other constituents of the viscera e.g. amino acids, peptides and proteins etc. which are co-extracted with the insecticides do not interfere. The reagent described here is sensitive and selective only for nitrobenzene and hence can be routinely used for the detection and identification of residual nitrobenzene in biological materials in forensic toxicology.

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