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USE OF UV FLUORESCENCE AND DRAGENDORFFS REAGENT FOR THIN LAYER CHROMATOGRAPHIC DETECTION OF TRIAZINE CLASS HERBICIDE ATRAZINE

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Abstract
Atrazine is a member of triazine family and belong to class of herbicide compounds. It is the noblest important herbicide and has a wide diversity role of uses mainly in the field of agriculture. During the last few years, Forensic Science Laboratories of Maharashtra State, India, detected a large number of human poisoning cases with Atrazine. Since a large number of biological samples were received for toxicological chemical analysis. Thin-layer chromatography (TLC) was the method of choice for their estimation. This study reports that dragendorffs reagent was found to be a selective and sensitive spray reagent for identification of atrazine by TLC analysis. Chemically atrazine reacts with dragendorfs reagent to obtain orange spot before UV. In UV at shorted wave length of 254*nm* it gives intense blue-colored compound. The dragendorffs reagent does not react with the Organochlorine insecticides, Organo phosphorus insecticides, and Pyrethroids insecticide. Visceral constituents (amino acids, peptides, proteins, fats etc.) do not interfere with these results.
Keywords **Atrazine, Forensic science, Dragendorffs reagent, Triazine class herbicide, Thin-layer chromatography, UV Florescence.**

1 Introduction:

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-triazine), is probably the most commonly used chlorinated herbicide in the world [1]. It is a highly effective herbicide that causes human/animal primarily involved endocrine system disrupter causes hormone imbalance acetylcholine receptors. Easy availability of this compound is frequently encountered in

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forensic casework, since insecticides are frequently misused in homicidal, accidental, and suicidal poisoning cases [1, 2]. In 2014, the Regional Forensic Science Laboratory, Aurangbad, India, detected several cases of human poisoning by atrazine. In routine forensic toxicology, insecticides are generally analyzed by thin-layer chromatography (TLC).

Few chromogenic reagents have been encountered in literature for the detection of nitrogen based insecticide by TLC, including Dragendorff's reagent [3], Iodoplatinate [4], and *p*-Dimethyl aminobenzaldehyde [5] and Cobalt thiocyanate [6]. This study reports a new method for the analytical determination of atrazine in visceral samples by TLC. Sensitive and selective detection of atrazine after TLC is possible by use of dragendorffs as spray reagent. Atrazine reacts with dragendorffs to produce an intense orange-colored compound and before spraying we observed UV fluorescence short wave 254nm blue color compound.

2 Experimental:

2.1 Chemicals and Reagents

All reagents were of analytical-reagent grade. Standard atrazine (Rallis India, Mumbai, M.S., India) solution was prepared in methanol (2 mg mL^{-1}).

Dragendorffs reagent =

Solution [A] 17 gm of basic bismuth sub nitrate in 80 mL distil water and 20 gm Tartaric acid. Solution [B] 16 gm of potassium iodide in 80 mL distil water (S.D. Fine-Chem Ltd., Mumbai, India).

Stock working solution= A+B [5+5] mL each and add 20mL acetic acid in 70 mL distilled water.

2.2 Extraction of Atrazine from Biological Materials

About 50 g viscera [(I) pieces of stomach and intestine with contents, (II) pieces of liver, spleen, kidney, and lungs] containing atrazine was taken. Material was cut into fine pieces and minced carefully; 50 mL methanol was added. The contents were kept for 2 h and then filtered, and the solvent was allowed to evaporate. The residue was re dissolved in 1 mL of methanol and was used for thin-layer chromatography.

2.3 Thin-Layer Chromatography

For the detection of atrazine residues, precoated TLC plates (silica gel 60 F254, Merck Ltd., Germany) were used. Chloroform: Acetone (7:3, by volume) mixture was used as solvent system for atrazine residues. The samples were spotted on TLC plates with fine capillary tubes along with pure atrazine as the standard. The plates were dried, and the chromatogram was developed in a previously saturated tank containing the solvent system as mentioned above. After developing the plates, the solvent front (distance travelled by the solvent) was immediately marked and the extra solvent was evaporated (dried) in fume hood. then we observed the plate at short wave length UV at 254*nm*, we saw dark blue spot with faint blue background gives 3 spot was clearly visible at *Rf* 0.26,0.61and 0.91, as shown in [figure **1**]

The plates were then sprayed with dragendorffs reagent. A faint orange spot with yallowish background was clearly visible at *Rf* 0.61 as shown in [figure **2**]

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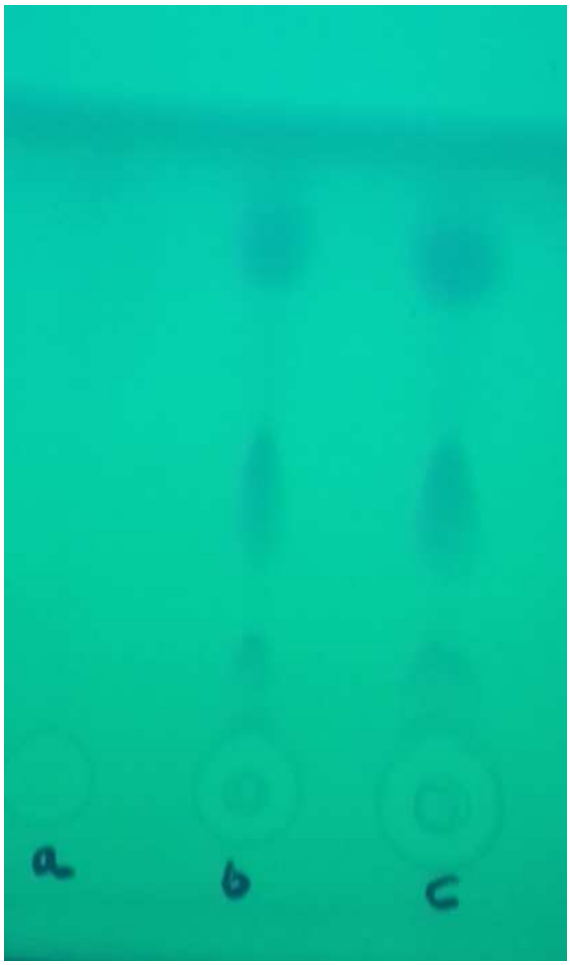
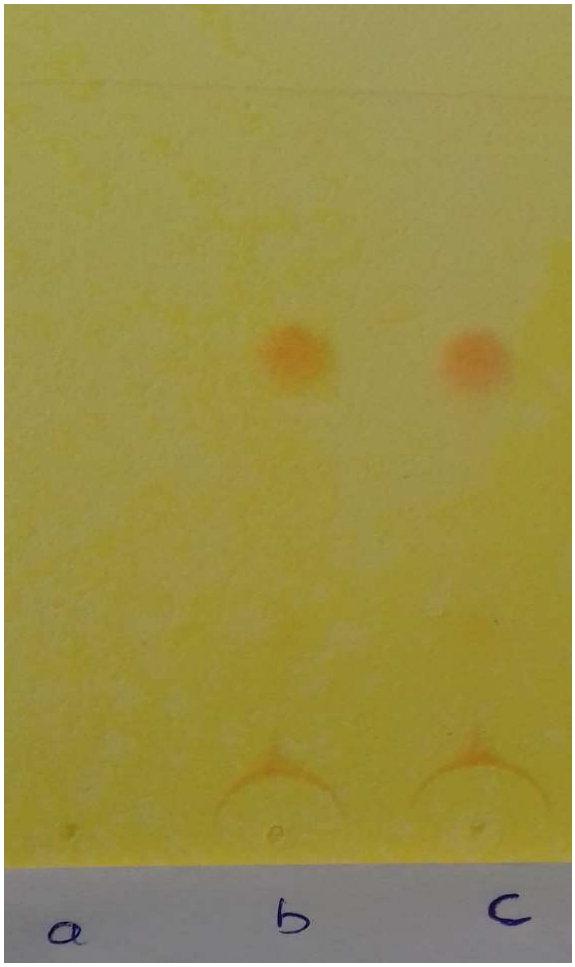


[Figure 1]



[Figure 2]

Figure 1 : TLC showing atrazine residues in UV Florescence at short wave 254*nm*: a) blank viscera, b) viscera with atrazine poisoning and c) standard atrazine.

Figure 2 : TLC showing atrazine residues using dragendorffs reagent spray reagent: a) blank viscera, b) viscera with atrazine poisoning and c) standard atrazine.

3 Results and Discussion:

Atrazine is an organic compound containing 6 chloro compound which reacts UV florescence at 254*nm* short wave shows three distinct 3 blue color spot with before

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spraying the plate and when use of dragendorffs reagent which then forms one spot intense orange-colored compound. The color species formed is the coordination complex of the atrazine and the central metal nitrogen of the reagent. The color of the spot remains stable. The limit of detection with this reagent is approximately 4 µg. The UV florescence at short wave 254 nm and dragendorffs reagent does not react with the organophosphorus insecticides dimethoate, phorate, monocrotophos, triazophos, and quinalphos; and with the synthetic pyrethroids fenvalerate, cypermethrin, and deltamethrin etc. Visceral constituents (amino acids, peptides, proteins, fats etc.) do not interfere**.** The UV Fluorescence at short wave 254nm are gives 3 blue colour spot are tally with standerd atrazine at *Rf* 0.26,0.61and 0.91**.** Although Dragendorff's reagent, iodoplatinate reagents, and *p*-dimethylaminobenzaldehyde,cobalt thiocynate are used for sensitive detection of all nitrogen-based organic compounds, the uv florescence and dragendorffs reagent utilized in the proposed method is cheap and does not involve any critical reaction condition or tedious sample preparation. Hence, this can be used routinely for the detection of atrizine in biological materials.

4 Conclusion:

To the best of our knowledge, UV fluorescence and dragendorffs reagent was used the first time for the detection and identification of atrazine in post-mortem samples (in fatal poisoning cases of atrazine). The proposed reagent is simple, sensitive, and can be used for routine analysis of triazine class of herbicide compound atrazine. Work on new spray reagents is in progress for the detection of different poisons in economical and effective way.

5 Acknowledgment:

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