

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

**Umakant D. Pawar^{*1}, Chandrakant D. Pawar², Ulka K. Kulkarni³, Devanand B.
Shinde² and Rajendra K. Pardeshi^{4*}**

¹Regional Forensic Science Laboratories Aurangabad, MS, India.

²Department Chemical Technology Dr. Babasahab Ambedkar Marathwada University
Aurangabad, Ms, India.

³Directorate Forensic Science Laboratories Mumbai, Ms, India.

⁴Sant Ramdas College Ghansawangi Jalna, Ms, India.

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***Corresponding Author**

Umakant D. Pawar

Regional Forensic Science
Laboratories Aurangabad,
MS, India.

ABSTRACT

Atrazine is a member of triazine class of herbicide compounds. It is the noblest important herbicide and has a wide diversity roll of uses: in agriculture field mainly. 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. This study reports that dragendorffs reagent was found to be a selective and sensitive spray reagent for atrazine in routine analysis by TLC. Atrazine reacts with dragendorffs reagent and dilute nitric acid which

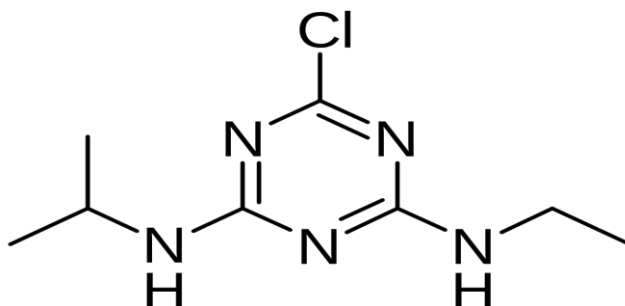
gives intense orange-colored compound. The dragendorffs reagent does not react with the organochlorine insecticides, organ ophosphorus insecticides, and pyrethroids insecticide. Visceral constituents (amino acids, peptides, proteins, fats etc.) do not interfere.

KEYWORDS: Dragendorffs reagent, Thin-layer chromatography, Triazine class herbicide, Atrazine, Forensic science.

1 INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-striazine) is probably the most commonly used chlorinated herbicide in the world.^[1] It is a highly effective herbicide that causes human animal primarily involves endocrine system disrupter causes hormone imbalance

acetylcholine receptors. Easy availability of this compound is frequently encountered in forensic casework, since insecticides are frequently misused in homicidal, accidental, and suicidal poisoning cases.^[1,2] In 2014, the Regional Forensic Science Laboratory, Aurangabad, India, detected several cases of human poisoning by atrazine. In routine forensic toxicology, insecticides are generally analyzed by thin-layer chromatography (TLC). Chemical structure of atrazine shown here.



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], 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 reagent and dilute nitric acid to produce an intense orange-colored 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] 17gm of basic bismuth sub nitrate in 80 ml distil water and 20 gm tartaric acid.

Solution [B] 16gm of potassium iodide in 80 ml distil water (S.D. Fine-Chem Ltd., Mumbai, India) and stock working solution= A+B [5+5] ml each and add 20ml acetic acid in 70 ml distilled water. The dilute nitric acid 2% concentration is used.

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

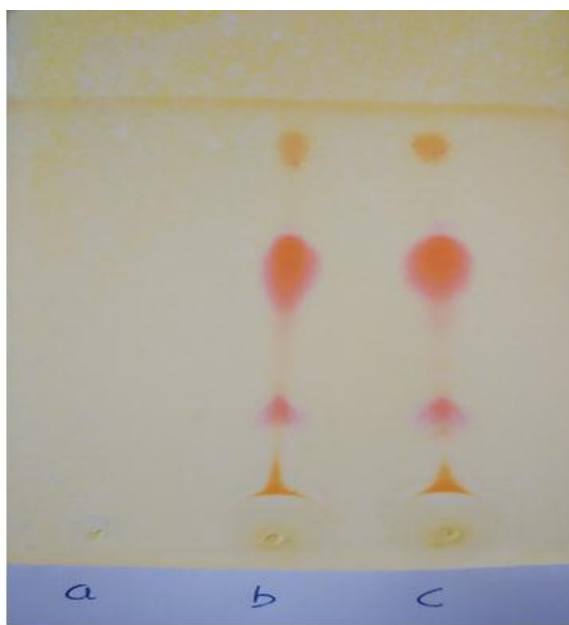
and minced carefully; 50 mL methanol was added. The contents were kept for 2 h and then filtered, and the solvent was allowed to evaporate. The residue was re dissolved in 1 mL of methanol and was used for thin-layer chromatography.

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 presaturated 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. The plates were then sprayed with dragendorffs reagent. A faint orange spot with yellowish background was clearly visible at R_f 0.61 shown in [figure 1] the another spot was appeared after spray dilute nitric acid [2%] then intense orange spot with white-faint yellow background gives 3 spot was clearly visible at R_f 0.26,0.61,0.90. shown in [figure 2]. the spot tallies with standard atrazine.

3 RESULTS AND DISCUSSION

Atrazine is an organic compound which reacts with dragendorffs reagent and dilute nitric acid to give intense orange-colored compound. The color species formed is the coordination complex of the atrazine and the nitrogen of the reagent. The color of the spot remains stable. The limit of detection with this reagent is approximately 4 μg . The dragendorffs reagent does not react with the organochlorine insecticides endosulfan, BHC, and DDT; with the organophosphorus insecticides dimethoate, phorate, monocrotophos, triazophos, and quinalphos; and with the synthetic pyrethroids fenvalerate, cypermethrin, and deltamethrin. Visceral constituents (amino acids, peptides, proteins, etc.) do not interfere. Although Dragendorff's reagent, iodoplatinate reagents, and *p*-dimethylaminobenzaldehyde are used for sensitive detection of all nitrogen-based organic compounds, the dragendorffs reagent and dilute nitric acid 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 atrazine in biological materials.

**Figure. 1.****Figure. 2.**

TLC showing atrazine residues using dragendorffs spray reagent: a) blank viscera, b) viscera with atrazine poisoning, and c) standard atrazine.in shown figure 1.

TLC showing atrazine residues using dilute nitric acid spray reagent: a) blank viscera, b) viscera with atrazine poisoning, and c) standard atrazine.in shown figure 2.

4 CONCLUSIONS

To the best of our knowledge, dragendorffs reagent and dilute nitric acid 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. Due to dilute nitric acid the 2 spot appear which tallies with standard atrazine which help us to analysis. Work on Development new spray reagents is in progress the author hope to report soon when work is over.

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