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Original Research Paper

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CHROMOGENIC SPRAY FOR DETECTION AND IDENTIFICATION OF PARAPHENYLENEDIAMINE FROM BIOLOGICAL MATERIAL

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ABSTRACT Thin layer chromatography (TLC) is a simple, rapid and reliable technique usually used in forensic science laboratory for detection of poison in biological material. It can separate many complex mixtures in a short period of time. In this study an effort has been taken to determine Paraphenylenediamine by using Thin Layer chromatography. A specific chromogenic reagent Ninhydrin in ethanol has been used for the detection of Paraphenylenediamine with Chloroform: Acetone (1:1) as a solvent system for the separation.

KEYWORDS : Paraphenylenediamine, Ninhydrin, Hair Dyes, Forensic Science, Biological Material.

INTRODUCTION:

The Paraphenylenediamine (PPD) is a synthetic compound [C6C6H4(NH2)2] belonging to the class of aromatic amine derived from paranitroaniline. It is a white colour powder, but it can darken due to air oxidation. PPD poisoning is amongst one of the causes of poisoning in India. It is a main constituent of hair dye formulation marked with the brand name of Kala Pathar, Indica, Godrej expert rich cream, Kaveri easy fast, Mayuri Henna etc. in India and easily available in market at a low cost. Paraphenylenediamine is commonly used in hair dyes and many other dye formulations [1]. The compound is easily soluble in organic solvents like ethanol, acetone, etc. PPD is a kind of aromatic amine is widely used for cosmetic purpose and it is also used in dying, photochemical and rubber vulcanizing industry [2]. Use of theses dyes leads to exposure to PPD and causes various local and systemic complications.PPD is an important constituent of hair dyes and it is an oxidative chemical that can cause hypersensitivity reactions and systemic side effects of poisoning [3]. Due to their easy availability and inadvertent knowledge their ingestion either intentional or accidental is frequently reported in India. In such poisoning cases medical officers preserve proper biological samples for process of toxicological analysis. In routine forensic toxicology, poison is generally analysed by thin layer chromatography in biological samples. In this paper, we describe a TLC method for detection and Identification of PPD using Ninhydrin spraying reagent. Ninhydrin spraying reagent is specific for amines [4].

EXPERIMENTAL:

CHEMICALS, REAGENTS AND SOLUTIONS:

All reagents used were of analytical-reagent grade. Standard Paraphenylenediamine (High Purity Laboratory Chemicals PVT. Ltd., Mumbai, India) solution was prepared in Diethyl ether. Ninhydrin (S.D.Fine-Chem Limited, Mumbai, India) solution was prepared for spraying by dissolving 2 grams of Ninhydrin in 100 ml of ethanol.

EXTRACTION OF PPD FROM BIOLOGICAL MATERIALS:

A portion of about 100 g each of different types of biological tissues (pieces of stomach, intestine, liver, spleen, lungs and kidneys) containing PPD was taken. Viscera was cut into fine pieces and minced carefully, 100 ml Diethyl Ether was added and left for about 1 hour, then filtered and extracted. The ext ract was transferred to an evaporating dish and the aqueous layer was re-extracted with 100 ml Diethyl Ether. The Extracts combined together in evaporating dish and the solvent was evaporated at room temperature. The residue was dissolved in 1 ml Diethyl Ether and the solution was used for spotting.

THIN LAYER CHROMATOGRAPHY:

Chromatography was performed on pre-coated Aluminum TLC plate (silica gel 60 $F_{254,}$ Merck Ltd. Darmstadt. Germany) for detection of PPD. The extract of blank viscera and PPD containing viscera were spotted on TLC plate along with the spot of Paraphenylenediamine standard with fine capillary tubes. The plate was dried and developed in a presaturated tank containing the Chloroform: Acetone (1:1) as solvent system. After development the plate was removed from chamber, dried at room temperature and then sprayed with Ninhydrin spraying reagent. A Red-Violet colour spot was developed at R_F = 0.54(Figure 1) after 4-5 minutes and stable for long time.

RESULT AND DISCUSSION:

Paraphenylenediamine is an aromatic organic compound which reacts with 2%Ninhydrin in Ethanol which gives Red-Violet colour (Figure 1). The colour of spot remains stable. The limit of detection with this reagent is approximately 5μg. The chemical reaction of PPD with Ninhydrin reagent is shown in Figure 2. This spray reagent is highly sensitive, stable, easily available and specific for the detection of Parapheny lene diamine (PPD) from biological material. This spray is specific for amines [4]. Hence, no spots were observed for Endosulfan (Organochloro insecticide), Monocrotophos, Chlorpyriphos, Triazophos, Quinolphos (Organophosphorus insectcide), Cypermethrin, Deltamethrin (pyrethroid), Carbofuron (carbamate). This spray method is economic, single step spray, reproducible and does not involve in any critical reaction condition. This reagent can also be used for the quantitative estimation of PPD in biological samples using spectrophotometric techniques (strong absorbance range between 400-600nm due to extended system of π electrons). Hence, this reagent can be used routinely for detection of PPD in biological samples.

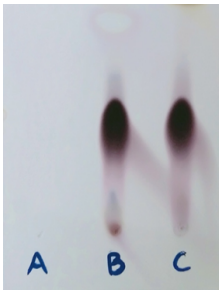

Figure 1. TLC showing spot of PPD using Ninhydrin spray reagent.

A) Blank Viscera Extract
B) PPD poisoning Viscera Extract
C) PPD standard

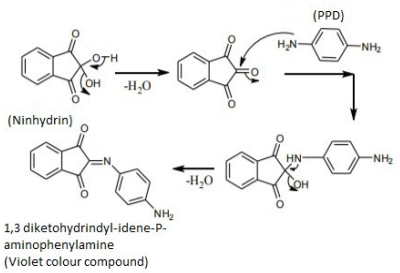

Figure 2. Reaction of p-Phenylenediamine with Ninhydrin

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