Chromogenic Visualization of Amitraz on TLC Plates Using Cobalt Thiocyanate Spray Reagent.

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Abstract:

Amitraz is widely used non-systematic acaricide and insecticide. Due to excessive or improper uses, it has consequential problems on living beings**.** In this paper, successful attempt is made to develop an easy to apply and effective thin layer chromatographic (TLC) technique, employing Cobalt thiocyanate as a chromogenic reagent for the identification of amitraz from human visceral samples. After spraying Cobalt thiocyanate on developed TLC plate of Amitraz, it produces a distinct blue coloured spot. The method exploits the ability of amitraz to form a stable Co-ordination complex with cobalt ions through its nitrogen containing functional group. The reagents do not react with the other pesticides like Monocrotophos, Chlorpyriphos, Profenophos, Carbosulfan, Carbofuran, Cypermethrin, Deltamethrin etc. The complex visceral matrix constituent such as fatty acids, lipids, peptides, proteins and other organic compounds does not react with the reagent. This approach provides a rapid preliminary technique for the semi- qualitative identification of amitraz in Visceral samples. This method serves as a rapid preliminary screening approach and is not intended to substitute confirmatory instrumental methods such as LC–MS/MS.

Keywords: Chromogenic reagent, Amitraz, Forensic Toxicology, Thin layer chromatography.

1. Introduction

Amitraz chemically known as N,N'-[(Methylimino)dimethylidyne]di-2,4-xylidine,which is extensively used as an insecticide in farming industry and horticulture. Its broad-spectrum acaricidal and insecticidal properties make it valuable in livestock treatment, particularly against ticks and mites [1]. However, excessive or improper use of amitraz has raised significant concerns regarding the persistence of its residues in food products, biological

systems, and the environment, potentially leading to toxicological effects in both humans and animals. Several poisoning incidents associated with amitraz exposure have been reported globally [2-3]. Amitraz poisoning characterized by bradycardia, hypotension and central nervous system depression [4]. Forensic Toxicology mainly deals with identification and detection of poisons. Recently numerous cases involving amitraz poisoning came across, so there is need of simple, rapid and specific method of its detection. Advanced analytical techniques for amitraz detection like GC-MS, LC-MS etc. are available [5-8]. Mentioned methods not only offer high sensitivity, specificity and accuracy but also limited by high operational costs, complex sample preparation steps and the requirement of skilled personnel. On the other hand, Thin-layer chromatography (TLC) is a low-cost analytical technique requiring minimal instrumentation making it suitable for routine analysis especially in limited-technology setups [9]. In this work, a straightforward thin-layer chromatography (TLC) technique was established for the Preliminary identification of amitraz from visceral samples which are commonly encountered in forensic toxicology investigations. Firstly here, extraction of Amitraz was carried out by employing liquid- liquid extraction using Diethyl Ether, then Amitraz were separated over thin layer chromatographic plate using Hexane: Acetone (8:2) solvent system. Further Blue coloured spot was observed by spraying cobalt thiocyanate reagent over TLC. Amitraz yielded a distinct blue-coloured complex, facilitating its preliminary identification without the need for expensive instrumentation. This method provides a convenient and effective means for the rapid presumptive detection of amitraz. This technique can be applied in forensic laboratories as an initial screening method for the detection of amitraz in biological samples associated with poisoning cases or criminal investigations.

2. Experimental

2.1. Chemicals and Reagents

The study was conducted using reagents of analytical grade to ensure accuracy and reliability. The working standard solution of amitraz was prepared by dissolving the 1 mg (Geevet Remedies, Gujrat, India) per millilitres in acetone. Hexane and acetone were purchased from Merck (India). 1.0 g cobalt (II) chloride (HiMedia Ltd., Mumbai, India) and 2.0 g ammonium thiocyanate (HiMedia Ltd., Mumbai, India) was dissolved in 100 ml of distilled water to prepare the chromogenic reagent cobalt thiocyanate.

2.2. Extraction of Amitraz from biological material

An approximately 100 g finely minced pieces of visceral sample (Stomach, Small and large intestine, Kidney, Liver and spleen) preserved in saline was taken into beaker for the extraction of amitraz. For the extraction of amitraz from visceral samples liquid-Liquid extraction process was used. The sample was extracted three times with 50 mL of ether using a separating funnel. For each extraction, the mixture was shaken for 3 to 5 minutes. All ether portions were combined and transferred to an evaporating dish, and the solvent was allowed to evaporate at room temperature. The resulting residue was then dissolved in 2 mL of acetone for thin-layer chromatographic analysis.

2.3 Thin layer chromatography

Thin-layer plates were prepared by coating clean glass plates with a uniform layer (0.25mm) of silica gel G (Molychem, India) in water as the stationary phase. Activation of the TLC plate was carried out by oven heating at 100 °C. Sample and standard (2–3 µL) were carefully spotted on the TLC plate by using capillary tube.Chromatogram is developed by Hexane:Acetone (8:2) as a mobile phase by placing the plates into TLC chamber. The mobile phase was allowed to rise up the plate for about 10 cm, after which the plates were removed and air-dried. After development, the TLC plates were sprayed uniformly with a freshly prepared Cobalt Thiocyanate chromogenic reagent. Immediately blue spots observed as shown in Fig1.

Fig.1 Chromatogram a) Blank Viscera, b) Amitraz from viscera extract and c) Standard Amitraz.

Different solvent systems were tested, but the hexane: acetone (8:2) combination produced well-defined, symmetrical spots with satisfactory $R_f$values and minimal tailing, making it suitable for the detection and identification of amitraz. The procedure was performed three times on the same day and over three consecutive days to evaluate the reproducibility of visual observations. An increase in spot intensity was visually noted with increasing concentration of the analyte. The developed blue spot on the TLC plate remained visually stable for nearly 48 hours under normal ambient conditions.

2.4. Recovery of Amitraz from Biological Materials

To perform quantitative analysis of amitraz, 10 mg of its standard solution in acetone was added to 100 g of finely minced visceral tissue. The spiked biological material was thoroughly homogenized with water and stored overnight. Further, extraction was done using ether (3×50 ml) as described above. After evaporation of ether, residual amitraz then spotted by dissolving into 10 ml acetone on prepared glass thin layer chromatographic plate against the different concentrations like 8, 8.5, 9.0, 9.5, and 10 mg per 10 ml. All spot of volume 10 µl spotted on TLC. The chromatogram was developed utilizing previously mentioned procedure. Colour intensity of spot in corelation of visceral sample closely matched with the intensity of the standard spot of 9.5 mg/10 mL acetone, suggesting that about 95% of the compound was successfully recovered.

3. Result and discussions

In this study, we employed Thin-Layer Chromatography (TLC) to detect amitraz using cobalt thiocyanate as a chromogenic agent. The reaction between amitraz and cobalt thiocyanate led to the formation of a blue-coloured complex, which was indicative of a successful interaction. Upon applying cobalt thiocyanate to the TLC plate, a distinct blue spot appeared at the same Rf value (RF =0.5) as the standard amitraz, confirming the presence of the compound. The mechanism of complex formation involves the co-ordination of Co^{2+} ions with the nitrogen atoms of the imine group in amitraz. Additionally, thiocyanate ions act as secondary ligands, co-ordinating with cobalt ions through sulphur which contributes to the stabilization of the complex [10]. This interaction shifts the electronic configuration of the cobalt ion, resulting in the observed blue coloration due to d-d transitions in the metal ion Fig 2. The sensitivity of

the method was evaluated using different concentrations of amitraz and a detectable blue spot was observed at concentrations as low as 4.5 µg/mL, indicating the potential of the method for sensitive detection. Under the experimental conditions, the visual limit of detection (VLOD) was found to be 4.5 µg/mL. The intensity of the blue colour increased with higher concentrations of amitraz, suggesting a linear relationship between colour intensity and concentration. The results indicate that cobalt thiocyanate can be an effective chromogenic agent for the preliminary detection of amitraz in a variety of samples, with the TLC method offering a simple and reliable analytical tool.

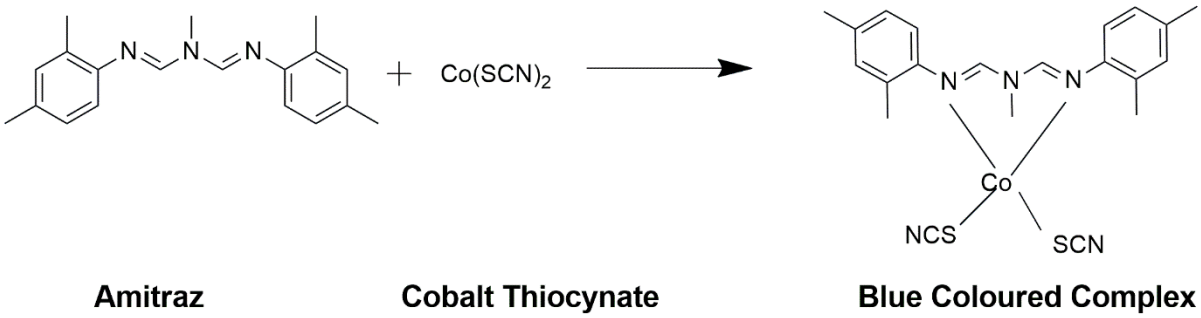

Fig.2 Probable reaction between Amitraz and Cobalt thiocyanate.

In comparison to existing methods, this approach provides a rapid and cost-effective alternative, requiring minimal sample preparation and no sophisticated equipment. Since the evaluation relies on visual detection and chromatographic comparison, the method should be regarded as a preliminary screening technique. Confirmatory instrumental analysis remains necessary for definitive identification.

4. Conclusion

This study demonstrates the effective use of thin layer chromatography with cobalt thiocyanate as chromogenic agent for the detection of Amitraz in forensic samples. The structure of blue-coloured compound forms was included. The method is simple, rapid, and applicable to cases involving the Amitraz. Its successful application to crime scene samples confirms its relevance in forensic toxicology, particularly where instrumental analysis is not readily available.

Conflict of Interest

Authors declares that there is no conflict of interest.

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