International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 14 Issue 1 ǁ January 2026 ǁ PP. 212-214

Diazotization-Based Chromogenic Detection of Pendimethalin on TLC Plates

Sachin R. Golhe*[ac], Dadasaheb. M. Bansode [a], Charansigh. B. Ghoti[a], Vijay J Thakare[b], Anil B. Naik[c].

a-Regional Forensic Science Laboratory, Amravati, MS, India.
b-Directorate of Forensic Science Laboratories, Mumbai, MS, India.
c-Department of Chemical Technology, Sant Gadge Baba Amravati University, Amravati, MS, India.

Abstract
Pendimethalin is a widely used dinitroaniline herbicide, frequently encountered in agricultural, environmental and forensic investigations. Conventional thin layer chromatographic (TLC) detection of pendimethalin relies predominantly on ultraviolet (UV) visualization, which often lacks selectivity and can be compromised by co-extractives present in real samples. In the present study, a selective post-chromatographic derivatization strategy based on nitro group reduction followed by diazotization to develop colour which is useful for the qualitative identification of pendimethalin on TLC plates. The sequential chemical transformation results into formation of a stable, intensely coloured azo-type chromophore, allowing direct visual detection under daylight. Reaction parameters such as reagent concentration, acidity, coupling time, and plate drying conditions were systematically optimized to obtain maximum colour intensity and stability. 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 fast, specific and selective technique for the qualitative identification of Pendimethalin in Visceral samples.

Keywords
Pendimethalin, Planar Chromatography, TLC Derivatization, Diazotization, Nitro Reduction, Chromogenic Detection.

Date of Submission: 13-01-2026 Date of acceptance: 29-01-2026

I. Introduction

Pendimethalin [N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine] belongs to the dinitroaniline class of herbicides and is extensively applied as a pre-emergent weed control agent in cereals, legumes and vegetable crops. Due to its large-scale use and moderate persistence, pendimethalin residues are commonly detected in soil, water and agricultural commodities. Its improper uses have consequential problems on living beings, necessitating reliable analytical screening methods [1]. Advanced instrumental techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) provide sensitive and quantitative determination of pendimethalin however these approaches require costly instrumentation and skilled operation [2-4]. In contrast planar chromatographic techniques such as thin layer chromatography (TLC) widely used for preliminary screening because of their simplicity, low operational cost and capability for parallel analysis [5]. Pendimethalin exhibits only weak UV absorption and is typically visualized as a quenching spot under UV254 nm which may be obscured by matrix components.[6] Pendimethalin contains aromatic nitro groups that can undergo chemical reduction to amines, which may subsequently be transformed into diazonium salts. Diazotization reactions are well established in analytical chemistry for converting aromatic amines into highly reactive intermediates capable of forming intensely coloured azo compounds [7]. Such chemistry has been successfully applied in planar chromatographic detection of pesticides and other nitrogen containing compounds [8-9]. Based on these principles, the present study develops a diazotization–alkaline coupling sequence tailored for pendimethalin detection on TLC.

II. Materials and Methods

2.1 Chemicals and Reagents

Pendimethalin was purchased from Crop Life Science Limited (Gujarat, India), silica gel 60 F_{254} TLC plates, Toluene, Acetone, Stannous Chloride ($SnCl_2$), Conc. Hydrochloric acid (HCl), Sodium nitrite ($NaNO_2$) and Sodium hydroxide (2N NaOH) was used. All reagents were of analytical reagent grade.

2.1.1. Preparation of reagent

a) Standard solution of pendimethalin was prepared in methanol (2 mg/mL).

b) Spray reagents -

i) 10% v/v Hydrochloric Acid (aq).

ii) Aqueous solution of 2% w/v Sodium Nitrite,

iii) 5% w/v solution of Stannous Chloride in Conc. HC1

iv) 10% β -Naphthol in 2 N Sodium hydroxide were prepared.

2.2 Thin layer Chromatography

A Silica gel-G (0.25 mm) was prepared, Dried and activated at 110 °C for 30 min prior to use. A 10 µl aliquot from blank Viscera, Spiked Viscera and standard pendimethalin was spotted as narrow spot using glass capillaries. 5% Stannous Chloride/Conc. HCl applied using spraying assembly and plate were heated at 100°C for 10 min for reduction. After cooling to room temperature plate was developed in Benzene:Acetone:Etanol (8:1:1) up to 10 cm, then sprayed with 2% Sodium Nitrite and 10% alkaline β-Naphthol. A stable Brick red coloured spot at Rf- 0.55 confirmed Pendimethalin. [Fig.1.]

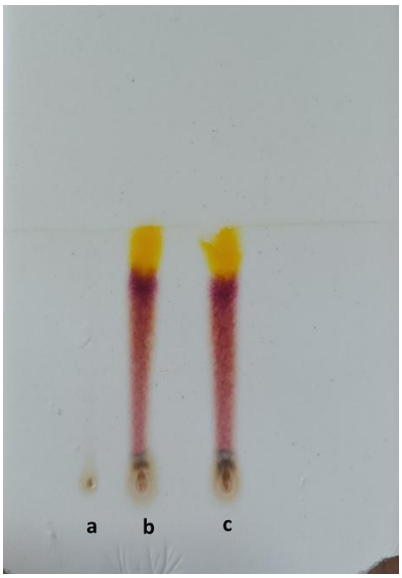

Fig.1: Thin layer Chromatography showing spot a) Blank viscera extract, b) spiked Viscera extract and c) commercial standard of Pendimethalin.

2.3. Recovery of Pendimethalin from Biological Materials

To perform quantitative analysis of Pendimethalin, 10 mg of its standard solution in ethanol 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 diethyl ether (3×50 ml) as described above. After evaporation of ether, residual Pendimethalin then spotted on thin layer chromatographic plate against the different concentrations namely 7, 7.5, 8, 8.5, 9.0, 9.5 and 10 mg/ml . All spot were of volume 10 µl. The chromatogram was developed by utilizing mentioned procedure. Colour intensity of spot in corelation of visceral sample closely matched with the intensity of the standard spot of 9.5 mg/mL , suggesting that about 95% of the compound was successfully recovered.

III. Results and Discussion

Pendimethalin showed reproducible migration with a constant Rf value under the optimized chromatographic conditions. Before derivatization, the compound could be detected only as a quenching spot under UV light and no visible spot was observed in daylight.

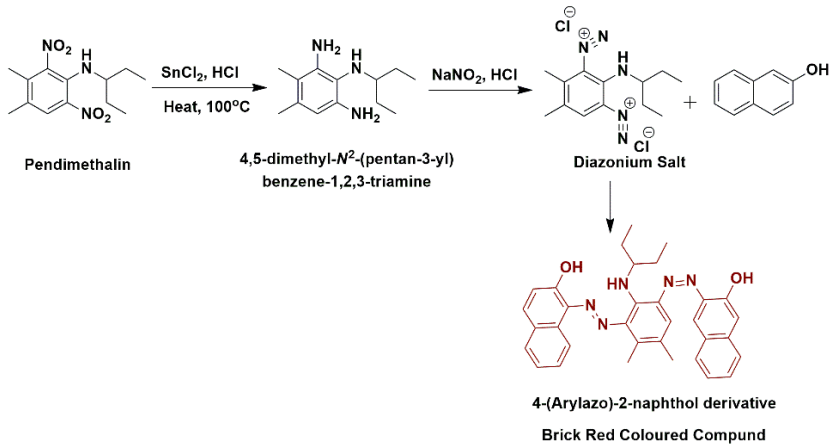

Fig.2: Reaction mechanism for the Reduction followed by Diazotization of Pendimethalin.

The systematic derivatization involved reduction of the nitro groups of pendimethalin to aromatic amines using Conc. HCl/ $SnCl_2$, followed by diazotization with $NaNO_2$/HCl which produced a distinct reddish coloured zone at the Rf position of pendimethalin. This color development enabled easy visual identification of pendimethalin on the TLC plate. [Fig.2.] The intensity and stability of the colour facilitated clear visual identification without the need for UV illumination. The colour remained stable for sufficient duration. Selectivity testing using pesticides lacking Nitroaromatic functionality (e.g., pyrethroids) did not yield comparable coloration, indicating that the derivatization sequence is functionally selective for nitro-containing aromatic compounds such as pendimethalin.

IV. Conclusion

A selective and visually interpretable post-chromatographic derivatization method has been developed for the identification of pendimethalin on TLC plates. The method exploits nitro reduction followed by diazotization and alkaline colour development to generate a stable chromophore. Its simplicity, low cost and compliance with established planar chromatographic principles make it suitable for routine forensic, environmental and agricultural screening applications.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

Author would like to express his sincere gratitude to Smt. Vaishali Mahajan, I/c Deputy Director, Regional Forensic Science Laboratory, Amravati, Home Department, Maharashtra state for their continuous support, valuable guidance and encouragement throughout the course of study.

References

[1]. P. C. Abhilash and N. Singh, "Pesticide use and application: An Indian scenario," Journal of Hazardous Materials, vol. 165, pp. 1–12, 2009.
[2]. A. Paul, K. Banerjee, S. Dasgupta, and S. H. Patil, "An improved GC–MS–SIM analytical method for determination of pendimethalin residue in commercial crops and soils and its validation," PLoS ONE, vol. 20, no. 8.
[3]. X. Zhang, Y. Li, H. Wang, and J. Chen, "Determination of pendimethalin dynamic residual distribution in crucian carp tissues and associated risk assessment," International Journal of Analytical Chemistry, vol. 2021, Art. no. 9984230, 2021.
[4]. A. Saha, I. Mukherjee, and M. Gopal, "Simultaneous analysis of herbicides pendimethalin, oxyfluorfen, imazethapyr, and quizalofop-p-ethyl by LC–MS/MS and safety evaluation of their harvest time residues in peanut (Arachis hypogaea L.)," Journal of Food Science and Technology, vol. 52, no. 7, pp. 4001–4014, 2015.
[5]. E. Stahl, Thin-Layer Chromatography: A Laboratory Handbook, Springer, 1969.
[6]. N. Sauret et al., "Near-UV molar absorptivities of alachlor, mecroprop-p, pendimethalin, propanil and trifluralin in methanol," Journal of Photochemistry and Photobiology A: Chemistry, vol. 193–194, pp. 237–244, 2008
[7]. P. E. Wall, Encyclopedia of Separation Science, "Thin-Layer Chromatography: Diazotization," Academic Press, 2000.
[8]. J. Sherma, "Post-chromatographic derivatization in planar chromatography," Journal of AOAC International, vol. 90, no. 4, pp. 1109–1124, 2007.
[9]. A. S. Patil et al., "A new chromogenic spray reagent for the detection and identification of oxyfluorfen herbicide in biological material by high-performance thin-layer chromatography," Journal of Planar Chromatography – Modern TLC, vol. 32, no. 1, pp. 69–71, 2019.