A New Chromogenic Spray Reagent for the Detection and Identification of Oxyfluorfen Herbicide in Biological Material by High-Performance Thin-Layer Chromatography

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1 Introduction

Oxyfluorfen (molecular formula $C_{15}H_{11}ClF_3NO_4$) **(Figure 1)** is a diphenyl ether herbicide used for broad-spectrum pre- and post-emergent control of annual broad leaf and grassy weeds in a variety of tree fruits, nuts, vines, and field crops. The largest agricultural markets in terms of total pounds of active ingredient are wine grapes and almonds. There are also nonagricultural ornamental and forestry uses. Oxyfluorfen is also used for weed control in landscapes, patios, driveways, and similar areas in residential sites [1]. Both light and oxygen play an important role in the activity of oxyfluorfen. It has been reported that oxyfluorfen becomes more active as light intensity increases, and hyperbolic relationship between the two has been found [2]. Oxyfluorfen is acting as a protoporphyrinogen oxidase inhibitor [3–5] and is used for pre- or post-emergence to control monocotyledonous and broad leaved weeds. In human toxicity, the database of oxyfluorfen is not clearly known. Oxyfluorfen is known to inhibit protoporphyrinogen oxidase (also known as protoporphyrinogen IX), resulting in the inhibition of heme biosynthesis and induction of symptoms in rodents consistent with the expression of human variegate porphyria (*i.e.,* effects on the liver, blood, and blood-forming tissue). Oxyfluorfen is of a low-order acute oral toxicity and is a mild eye and skin irritant [6]. As oxyfluorfen is not metabolized in plants and is subjected to very little translocation, photo-transformation is suggested as a possible abiotic degradation process. Oxyfluorfen is white to orange or deep red brown crystalline solid with a melting point of 65–84°C, density of 1.49 g mL^{-1} [7]. Solubility in water is 0.1 mg L^{-1}. Oxyfluorfen is stable under normal temperatures and pressures but may decompose if exposed to UV light [8]. In routine forensic toxicology, herbicides are analyzed by thin-layer chromatography (TLC) because of its simplicity and speed. Several chromogenic reagents have been used for the detection and identification of oxyfluorfen. These reagents are either of low sensitivity or are not specific. A selective and sensitive reagent is therefore required for the detection and identification of oxyfluorfen in TLC and high-performance thin-layer chromatography (HPTLC) analysis. The use of a reducing reagent followed by a coupling reagent used as a spray reagent for the detection and identification of oxyfluorfen by HPTLC is reported in this paper.

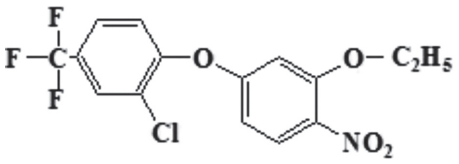

Figure 1

The structure of oxyfluorfen.

2 Experimental

2.1 Chemicals and Reagents

All reagents and chemicals were of analytical grade. Distilled water was used throughout. An accurately weighed amount of 10.00 g of oxyfluorfen standard was dissolved in absolute ethanol and transferred to a 1000 mL volumetric flask, diluted to the mark with absolute ethanol and mixed well. Five concentration levels of oxyfluorfen were also prepared in absolute ethanol (0.1 µg, 0.5 µg, 1.0 µg, 1.5 µg, and 2.0 µg). An aqueous solution of stannous chloride 5% *w/v* was prepared by dissolving 5 g of stannous chloride in 100 mL distilled water. Five-millimeter concentrated HCl–β-napthol (5%, *w/v*) was prepared by dissolving 5 g of β-napthol in 100 mL distilled water, and 5% NaOH solution and nitrating mixture were freshly prepared.

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2.2 High-Performance Thin-Layer Chromatography

Chromatography was performed on 20 cm × 10 cm silica gel 60 F_{254} HPTLC glass plate (Merck KGaA, Darmstadt, Germany). HPTLC plates were activated by heating in an oven at 60°C for 1 h. Extracts from visceral tissue and stock solution of oxyfluorfen were spotted using a specified Hamilton (Bonaduz, Switzerland) TLC glass syringe (25 µL capacity) on HPTLC plate. A 10 µL volume of visceral tissue extract and each level (0.1 µg, 0.5 µg, 1.0 µg, 1.5 µg, and 2.0 µg) were applied on HPTLC plate. The plates were allowed to develop to the distance of 10 cm in a previously prepared saturated TLC chamber with hexane–acetone (8:2) as the mobile phase. After development, the plates were removed from the chamber and dried in air at room temperature for 20 min. The dried plates were sprayed with 5% stannous chloride solution in HCl, and then, the plates were heated in an oven at 110°C for 20 min and allowed to cool at room temperature. After cooling, the plates were sprayed with freshly prepared cooled (0–5°C) nitrating mixture of $NaNO_2$ in HCl followed by a solution of β-napthol in NaOH. A brick-red-colored spot was observed at 6.1 cm on HPTLC plate with a retardation factor (R_F) value of 0.61 **(Figure 2).**

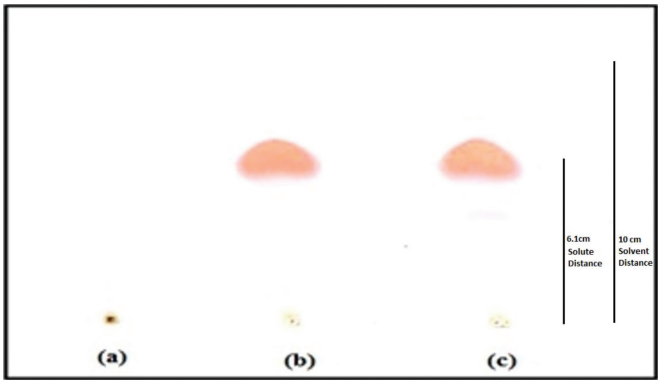

Figure 2

High-performance thin-layer chromatograms obtained from (a) blank viscera, (b) oxyfluorfen from extract of viscera, and (c) standard oxyfluorfen.

2.3 Recovery and Experiment

Oxyfluorfen (10 mg) in absolute ethanol was separately added to 100-g-minced visceral tissue (stomach, intestine, liver, spleen, and kidney), mixed well with water kept for 24 h, and then extracted with ethyl acetate using a separating funnel. The upper layer of ethyl acetate was extracted. The ethyl acetate extract was transferred to an evaporating dish, and the lower aqueous phase was re-extracted with ethyl acetate (3 × 50 mL). The combined ethyl acetate extracts were evaporated at room temperature. The residual remains obtained after evaporation were dissolved in absolute ethanol (10 mL) and spotted (10 µL) on an activated HPTLC plate along with standard solution of oxyfluorfen, and this plate was then developed as per the process described above.

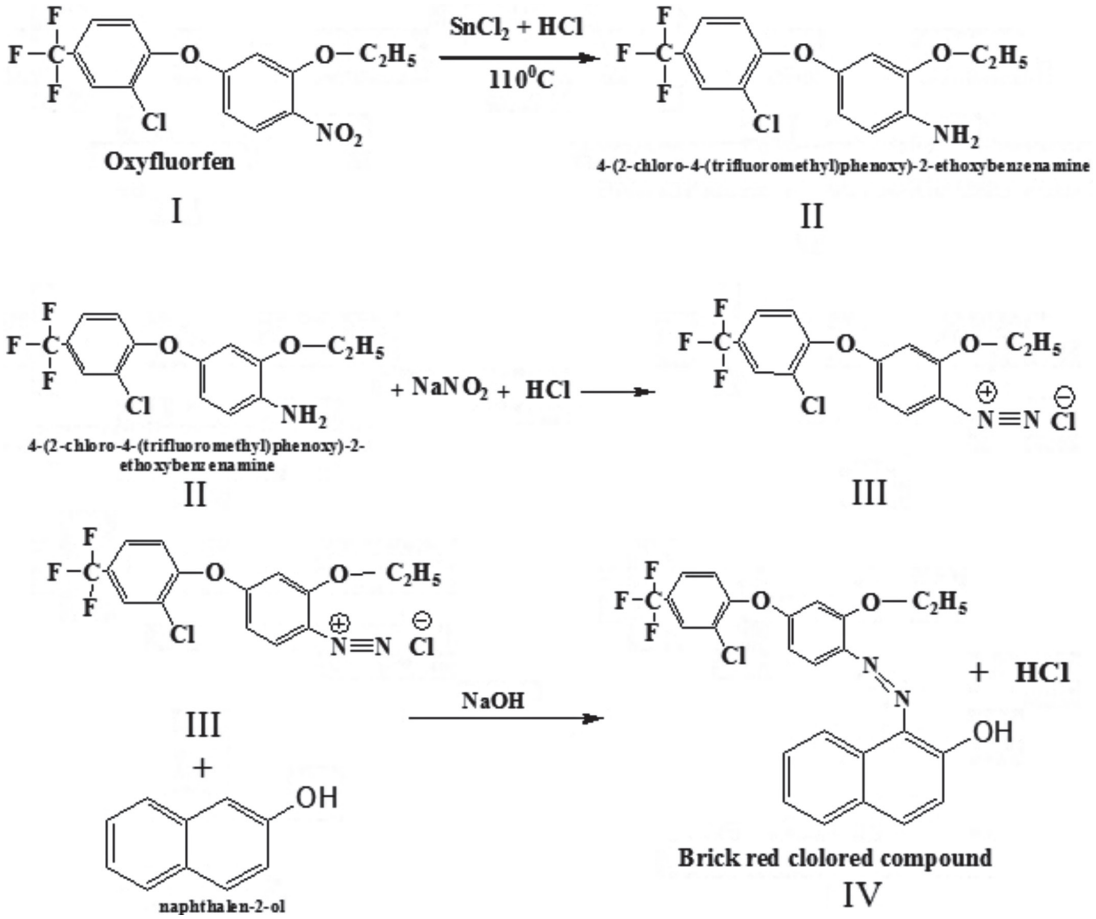

Figure 3

Schematic representation of reactions involved in the process.

3 Results and Discussion

HPTLC is used in insecticidal analysis when many samples have to be analyzed. Here, an attempt was made to detect oxyfluorfen. Oxyfluorfen was firstly reduced with the help of stannous chloride and hydrochloric acid to form a compound of structure II. Then, compound II was diazotized with the help of using sodium nitrite in hydrochloric acid to form a compound of structure III. Afterward, compound III reacted with β-napthol in sodium hydroxide to form a brick-red-colored compound of structure IV (schematic reactions shown in **Figure 3**). The diazotization is a known reaction, but this is the first time applied to detect herbicide oxyfluorfen in biological material. This reaction is specific for oxyfluorfen herbicide and other organocholoro and organophosphorus and carbamate insecticides do not give a brick-red-colored spot or interfere. We observed brick-red-colored spot from 0.5 μg and above concentrations of oxyfluorfen. The detection limit was 0.5 μg. The color of spot was stable for many days.

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