Short Communication

Detection of Butachlor in Viscera by Thin Layer Chromatography

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ABSTRACT

Butachlor, a selective systemic herbicide, was isolated, analyzed and detected in viscera obtained from medicolegal autopsy. Butachlor was extracted by using solvent extraction methods and then identified by Thin Layer Chromatography (TLC). For chromatographic separation, various solvent systems were used. Bromophenol blue was used as chromogenic reagent on developed TLC plates which successfully increased the sensitivity without dispensing with the simplicity of the method. For the study, a total of 15 solvent systems in different ratios were chosen. Of these, the best two solvent systems, namely, Benzene:Diethyl ether (8.5:1.5) and Hexane: Acetone (9:1) were chosen for statistical analysis, which included the calculation of mean Rf value and value of standard deviation and coefficient of variance.

Key Words: Butachlor; Thin layer chromatography (TLC); Rf value, Bromophenol blue

Introduction

Butachlor is a chloroacetanilide compound of carbamate group, and is a selective systemic herbicide which is widely used by farmers. It is absorbed primarily by the germinating shoots and secondarily by roots, with translocation throughout the plant, giving higher concentrations in vegetative parts than in reproductive parts. It is effectively used for the control of broad-leaved annual grasses. Its higher concentration in the vegetative parts inhibits protein synthesis. Butachlor has been reported to be an indirect mutagen and carcinogen in various *in vitro* assay systems. It is a moderate skin irritant and mild eye irritant. It is stable up to 165°C and also in UV light. Butachlor is an amber coloured liquid having molecular weight 311.9, molecular formula $C_{17}H_{26}ClNO_2$, and IUPAC name N-butoxymethyl-2-chloro-2,6-diethylacetanilide.[1]

Butachlor shows selectivity for barley, cotton, peanuts, sugar beet, wheat and several brassica crops. Effective rates range from 1.0-4.5 kg/ha. Activity is also dependent on water availability such as rainfall.[2-5] Butachlor is misused sometimes, and a few poisoning cases (homicide/suicide) have been reported. The review of literature on analytical methods for analysis of butachlor reveals that gas liquid chromatography and high performance liquid chromatography have been extensively used, but these methods are not only costly but time consuming. An attempt has been made in this study to analyse butachlor by using thin layer chromatography (TLC) which is relatively cheap and takes very less time for analysis.[6-9]

Materials and Methods

Collection and Preparation of Sample: 50 gm of biological material (viscera) was collected from the mortuary and spiked with 5 ml of 100 ppm of butachlor and incubated up to 48 hours.

Chemicals and Reagents: diethyl ether, benzene, n-hexane, acetonitrile, anhydrous sodium sulphate, glass wool, and acetone. All chemicals used were of analytical grade (Merck).

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Apparatus: separating funnel, volumetric flask, conical flask, pipettes, sprayer, sample applicator, TLC plates precoated with silica gel 60 F256 aluminium (Merck).

Preparation of Spray Reagent: 0.05g of pure bromophenol blue was dissolved in 35 ml acetone and diluted with distilled water in 100 ml volumetric flask.

Preparation of Standard Solution: The solutions of reference herbicide (>99% pure) of 50, 100 and 1000 ppm concentration were prepared in n-hexane (HPLC grade).

Preparation of TLC Plates: TLC plates were prepared by dissolving 25 gm of silica gel G in 50 ml of distilled water by making a slurry. This slurry was poured on the applicator and the applicator was then moved over the plate in one motion. Plates were allowed to dry at room temperature and then kept in hot air oven at 80ºC for one hour.

Extraction Procedure[10,11]: Butachlor was extracted from tissue by solvent extraction method as outlined below:

25 gm tissue with 15-20 gm anhydrous sodium sulphate and 50 ml n-hexane were taken in a round bottomed flask fitted with air condenser, and heated in a water bath for one hour. The contents were cooled and filtered. The three hexane layers were pooled together. 50 ml of acetonitrile was added and saturated with n-hexane in a separating funnel and shaken vigorously for 5 minutes. The acetonitrile phases were pooled together. To this, 50 ml of water and 15 ml of hexane was added in a separating funnel and was shaken vigorously for 2-3 minutes. The collected hexane layers were pooled and passed through anhydrous sodium sulphate. The extract was concentrated in a water bath up to 1 ml. Clean-up was done as per standard procedure.

Spotting of Sample and Standard on TLC Plate: The sample extracted from the viscera was loaded on a TLC plate along with the standard sample and dried.

Development of TLC Plate: The sample-loaded plate was placed inside the chromatographic chamber, which was previously moistened with solvents and used as mobile phase. After running the solvent system to 75 mm distance from the loaded point, the plate was removed from the TLC chamber and air-dried. The different ratios of used solvent systems were as follows:

1. Chloroform: Hexane (6:4, 5:5, 4:6, 3:7, 2:8)
2. Chloroform: Benzene (6:4, 5:5, 4:6, 3:7, 2:8)
3. Chloroform: Liquid Paraffin (9:1)
4. Benzene
5. Benzene: Ethanol (9:1)
6. Benzene: Methanol (6:4)
7. Cyclohexane: Liquid Paraffin (8.5:1.5)
8. Acetone: Benzene (1:9, 2:8, 3:7, 4:6).
9. Acetone: Hexane (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9)
10. Chloroform: H_2O: NH_3 (9: 0.5:1)
11. Chloroform: Methanol: H_2O (3:5:2).
12. Petroleum Ether
13. Petroleum Ether: Acetone (7:3)
14. Petroleum Ether: Liquid Paraffin (9:1, 2:8)
15. Diethyl Ether: Benzene (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1.5:8.5, 1:9)

Visualisation of TLC Plate: The dried and developed TLC plates were sprayed with bromophenol blue reagent.

Results and Discussion

After evaporation of solvent system, the plate was sprayed with bromophenol blue. Blue coloured spots formed immediately in the standard as well as isolated samples. The colour of spots persisted up to a few days. In both isolated and standard samples, the spot colour and Rf value were similar. Of the 15 different solvent systems used, the best two combinations of Diethyl Ether: Benzene (1.5: 8.5) and Acetone:Hexane (1:9) were chosen for statistical analysis. The results are presented in **Table 1** and **Table 2**.

This TLC method can be used for the detection of butachlor without any misidentification The standard deviation in Diethyl Ether:Benzene (1.5: 8.5) in the standard and sample respectively were 0.008650 and 0.002880, whereas in Hexane: Acetone (9:1) solvent system, it was 0.002250 and 0.0022 respectively. The migration time in the solvent system Diethyl Ether: Benzene was 23 minutes, while in Acetone:Hexane mixture, it was noted as 16 minutes. For visualization of butachlor, different spray reagents were used and the best result was obtained with bromophenol blue.

The two best solvent systems for analysis of butachlor were observed as Hexane:Acetone (1:9) and Diethyl Ether:Benzene (1.5:8.5).

Table 1 Replicate R_f Value of Butachlor in Solvent System Diethyl Ether:Benzene (1.5: 8.5)

S No.	Rf Value of Standard	Rf Value of Sample
1.	0 .71	0 .70
2.	0 .72	0 .68
3.	0.70	0 .71
4.	0 .73	0 .72
5.	0.72	0 .73
6.	0.72	0.69
7.	0.68	0.74
8.	0.74	0.71
9.	0.74	0.72
10.	0.72	0.71
	Mean Rf value = 0. 715 Standard deviation = 0.008650	Mean Rf value = 0. 711 Standard deviation = 0.002880

Table 2 Replicate R_f Value of Butachlor in Solvent System Hexane:Acetone (9: 1)

S No.	Rf Value of Standard	Rf Value of Sample
1.	0 .59	0 .59
2.	0 .60	0 .60
3.	0.60	0 .60
4.	0 .61	0 .61
5.	0.61	0 .61
6.	0.62	0.60
7.	0.58	0.60
8.	0.59	0.58
9.	0.63	0.62
10.	0.62	0.62
	Mean Rf value = 0. 605 Standard deviation = 0.002250	Mean Rf value = 0. 60 Standard deviation = 0.0022

Conclusion

Butachlor was extracted from viscera samples by solvent extraction method, and analysed and compared with standard butachlor solution on TLC plates. The retention factor (R_f) of viscera and standard samples was compared, which showed a minor difference.

The method developed for analysis of butachlor by using TLC in viscera is very cheap, time efficient, and can be performed in any laboratory.

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