Original Paper

Thin Layer Chromatographic Analysis of Bromadiolone

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ABSTRACT

Rats are among the most destructive of pests, ravaging crops and prepared food material. A variety of rodenticides are available in India for killing these pests, and among them, the most common compounds include zinc phosphide and bromadiolone. While there is little doubt that they are effective rodenticides, the flip side is that they are increasingly being employed for committing suicide, and even homicide. Thin layer chromatography, an inexpensive and relatively simple method for detecting various poisons has rarely been employed in detecting bromadiolone in biological materials. An attempt has therefore been made in this study to identify bromadiolone in biological samples. This can be employed in clinical and forensic cases as a preliminary test, which if necessary can subsequently be confirmed with more sophisticated analyses.

Key Words: Rodenticide, Rat poison, Bromadiolone, Thin layer chromatography

Introduction

Forensic science laboratories receive a variety of samples for toxicological evaluation. Identification (and sometimes quantification) of the exact toxic agent involved is often a difficult and challenging task for the forensic scientist. Rodenticide is a type of pesticide that is used in urban and rural areas to control all kinds of rodent pests, especially rats. These pesticides play an important role in promoting public health by destroying mice and rats around the home, as well as outdoors, but the current marketing and usage practices have led to increasing employment of these toxic agents for committing suicide, and even homicide. Some of the commonly available rodenticides in India include bromadiolone, zinc phosphide, and phosphorus. Bromadiolone is one of the most commonly used rodenticides all over the country. The relatively easy availability and inexpensive nature of this compound makes it attractive in suicidal or homicidal cases.

The IUPAC name of Bromadiolone is 3-[3-(4'-bromobiphenyl-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxycoumarin (**Fig 1**). It is available as bait, concentrate, tracking powder or impregnated in paraffin blocks. Formulations include dusts (0.1%), solutions (0.25%), pellets, and particulates and solids (0.005%). It is also available as a mixed formulation with sulphaquinoxaline. Bromadiolone is a white to off-white powder. Its solubility in water is very low (less than 20 mg/litre at 20°C), while it is slightly more soluble in ethanol, ethyl acetate, and dimethylformamide.

Bromadiolone can be absorbed via the gastrointestinal tract, skin, and respiratory system. It acts by disrupting the normal blood clotting mechanisms, causing an increased bleeding tendency in rodents. The liver is the main organ of accumulation and storage, while the major route of elimination is via the faeces. Bromadiolone has been detected in the liver as the unchanged parent compound. It has high, acute oral toxicity (LD_{50} of 1-3 mg/kg for various species including rodents and non-rodents). The dermal toxicity is also high (LD_{50} of 9.4 mg/

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kg in rabbits). Poisoning mainly produces manifestations relating to abnormal bleeding. Bromadiolone is non-irritant to the skin, but is a slight irritant of the eye.[1]

Detection and assay of the compound and its residues has been attempted with a variety of chromatographic and fluorescence methods.[2-5] For the preliminary examination of samples, thin layer chromatography (TLC) has always been popular in forensic science laboratories. This method was employed in the present study in the forensic examination of bromadiolone, as a possible screening method.

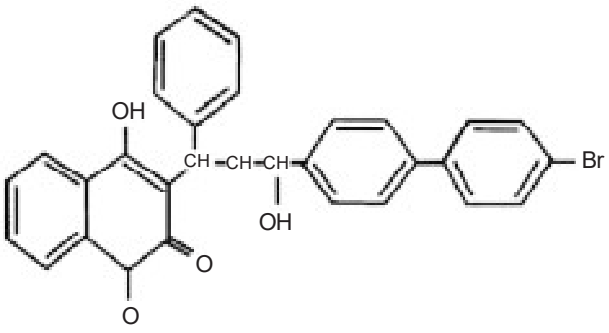

Fig. 1 Chemical Structure of Bromadiolone

Materials and Methods

Samples of bromadiolone (Brand name-Ratkill®) were purchased from the market. These are available in solid-cake form (25g), encased in yellow plastic wrapping. Before analyzing them, the samples were finely minced and dissolved in normal hexane, and kept overnight to facilitate proper extraction. A filtrate of this solution was then collected in a clean and sterilized porcelain basin, and kept undisturbed for a few hours. After drying the filtrate at room temperature, a few millilitres of acetone was added to the residue. This solution was then spotted on a TLC plate. The developing chamber was properly saturated with the solvent system, and the TLC plate was placed vertically in it. The plate was removed from the chamber after making a solvent run of 10cm from the point of spotting. This developed plate was then dried at room temperature. The details of the experimental conditions have been given in **Table 1**. Observations were made under sunlight, ultraviolet light, and iodine fuming.

Results and Discussion

Samples of bromadiolone were subjected to TLC analysis for the separation of its constituents. Hexane was found to be the best solvent for the extraction of these samples. Nine different solvent systems were tried for the separation of this sample (**Table 2**). Out of these nine solvent systems, three produced noticeable and significant results. The best solvent system was found to be hexane: benzene: rectified spirit: acetic acid (6:2:2:1). The other two solvent systems tried included one that comprised hexane: benzene: ethyl alcohol: acetic acid (6:2:23:1), and another that consisted of hexane: benzene: ethyl alcohol: acetic acid: ammonia (5:2:2:0.5:0.5). These were studied under iodine fumes. Two spots (blue and brown) were usually visible in sunlight, while UV- results could not produce any notable observation. It can be deduced from **Table 3** that the solvent system-9 was the best solvent system for the separation of this poison.

Conclusion

Rodenticides are among the most commonly used pesticides today, and are widely available in the market all over the country. This study was conducted on one of the most popular ingredients of Indian rodenticides - bromadiolone. Thin layer chromatography (TLC) was employed, and the results indicate that it could be very helpful as a screening procedure to detect this poison fairly easily and reliably in biological samples, involving minimum expenditure.

Table 1 Experimental Conditions of the Study

Solvent system-9	Hexane: Benzene: Rectified spirit: Acetic acid (6:2:1:1)
Room temperature	26°C
Saturation time	25 min
Time of run	47 min
Solvent system-8	Hexane: Benzene: Ethyl alcohol: Acetic acid (6:2:1:1)
Room temperature	25°C
Saturation time	25 min
Time of run	41 min
Solvent system-7	Hexane: Benzene: Ethyl alcohol: Acetic acid: Ammonia (5:2:2:0.5:0.5)
Room temperature	28°C
Saturation time	25 min
Time of run	36 min

Table 2 List of Solvent Systems

S.No.	Solvents System	Ratio
1.	Hexane	
2.	Hexane: Acetone	9:1
3.	Hexane: Ethyl alcohol	8:2
4.	Hexane: Benzene	1:1
5.	Hexane: Benzene: Ethyl alcohol	8:1:1
6.	Hexane: Benzene: Ethyl alcohol	6:3:1
7.	Hexane: Benzene: Ethyl alcohol: Acetic acid: Ammonia	5:2:2:0.5: 0.5
8.	Hexane: Benzene: Ethyl alcohol: Acetic acid	6:2:1:1
9.	Hexane: Benzene: Rectified spirit: Acetic acid	6:2:1:1

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Table 3 Results – hRf Values

Solvent system-9	Hexane: Benzene: Rectified spirit: Acetic acid (6:2:1:1)		
Observation taken with	Sun light	UV light	Iodine Fuming
Number of Spots	02	NIL	07
hRf values	71 (blue) 92 (brown)	NIL	92, 76, 67, 53, 39, 32, 24, 11
Solvent system-8	Hexane: Benzene: Ethyl alcohol: Acetic acid (6:2:1:1)		
Observation taken with	Sun light	UV light	Iodine Fuming
Number of Spots	02	NIL	07
hRf values	95 (blue) 97 (brown)	NIL	39, 47, 49, 53, 95, 97
Solvent system-7	Hexane: Benzene: Ethyl alcohol: Acetic acid: Ammonia (5: 2: 2: 0.5: 0.5)		
Observation taken with	Sun light	UV light	Iodine Fuming
Number of Spots	02	NIL	05
hRf values	90 (blue) 97 (brown)	NIL	10, 47, 52, 90, 97