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The Development of The Spraying Reagent for the Detection of The Bromadiolone In Thin Layer Chromatography.

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ABSTRACT

Over the years, Forensic Science Laboratories receives cases of accidental as well as intentional bromadiolone intoxication. Bromadiolone is an anticoagulant inhalant rodenticide used in rural and urban areas to control rodent. Over the last many decades, it has been observed that the use of rodenticide has been increased in promoting public health in hospitals, outdoor and home, with many generations coming into existence. The easy availability of rodenticide for use in public hygiene and agricultural has caused them to be used for suicidal and homicidal purposes. Bromadiolone, one of the most commonly available rat kill poison acts by inhibiting the synthesis of Vitamin K in blood, thereby causing internal bleeding and hemorrhage. Reported literature on the detection of bromadiolone poisoning mention detection of TLC spot by visualization under UV light only. The present study aimed for developing spray reagent for visualization of the Bromadiolone spots on Thin layer chromatography plates.

INTRODUCTION

Rats are among the most destructive type of pests, damaging crops and the stored food material. A number of rodenticides are available in India for killing these rodents and among them; the most common compounds include zinc phosphide, Bromafuran, Bromethalin and Bromadiolone. There is little doubt that they are effective rodenticides, but now a day their misuse is also increasing. These chemicals are used for the purpose of suicide and even for homicide. Based on their chemical structure, anticoagulant rodenticides may be grouped into two categories: hydroxycoumarins and indandiones main focus here is on the rodenticide Bromadiolone which belongs to hydroxycoumarin group.[1,6]The IUPAC name of Bromadiolone is 3-[3-(4'-bromobiphenyl 4-yl) 3-hydroxy-1-phenylpropyl]-4-hydroxycoumarin (chemical formula: $C_{30}H_{23}BrO_4$). Its molecular weight is 527.41g/mol.

Bromadiolone is a second-generation anticoagulant that has hydroxy-4-coumarin for the control of rodents such as rats and mice, including those resistant to warfarin and first-generation anticoagulants.[2] The second generation anticoagulant rodenticide has longer body retention as compared to first generation and therefore tend to lead to a longer period of bleeding. They are available in local market with the trade names like Mooshkill, Raccumin cake and Dr.Pest. The Bromadiolone is a white to off-white powder. Its water solubility is very low i.e.20 mg/l at 20°C. It is slightly soluble in solvents like Dimethyl Formamide, Ethyl Acetate and Ethanol [3]. It is also soluble in acetonitrile which is used in present study. The acute oral LD in rats is reported to be 1.75mg/kg and doses were 250mg/kg [4]. The ready-to-use baits is a blend of cereals and paraffin- containing 0.005% bromadiolone. In humans, bromadiolone lowers the activity of prothrombin, thereby making the coagulation process slower. It can be absorbed via the gastrointestinal tract, skin, and respiratory system[8]. Oral administration has been reported to have been more toxic. Due to having anticoagulant properties, it acts by disrupting the normal blood clotting mechanisms by inhibiting the recycling of vitamin K_1, a cofactor of primary importance for post ribosomal carboxylation of blood clotting factor II (prothrombin), causing an increased bleeding tendency in rodents [6]. It acts as antagonist to vitamin-K, which is required for blood clotting. It acts by binding to the enzyme vitamin K 2,3epoxide reductase, thereby interrupting the cellular recycling of vitamin K. Liver is the main target organ of this rodenticide, as the synthesis of vitamin K 2,3 epoxide reductase takes place in liver. Rat liver contains 1–2 number mole(s) of enzyme per gram of tissue which is a 4–5-fold overcapacity for maintaining effective vitamin K recycling. The bioavailability is approximately 50% and the minimum plasma concentration is reached approximately 6-9 hr after ingestion. Toxicity to human being was also studied. Many poisoning incidents (both intentional and unintentional) have been reported.[8] A few cases of intoxications from occupational exposure to anticoagulants have also occurred.[13] Symptoms of acute intoxication by anticoagulant rodenticides range from increased bleeding tendency in minor or moderate poisoning to massive haemorrhage in more severe cases. The signs of poisoning develop with a delay of one to several days after absorption.[8] Warfarin is associated in humans with the induction of developmental malformations when taken as a therapeutic agent during pregnancy [7]. No cases of developmental defects following the use of anticoagulants as rodenticides have been reported in case of bromadiolone. Other symptoms include somnolence (sleepiness, drowsiness), weakness, frequent urination, increased thirst [6]. Forensic science laboratories receive a variety of samples for toxicological analysis, sometimes it becomes difficult to identify the exact poison, Bromadiolone being a commonly used poison its identification method is important from forensic point of view.

The main focus of this study is the development of spray reagent for detection of bromadiolone spot on TLC plates. Techniques such LC-ESI-MS with liquid extraction, reverse phase Ion- pair HPLC with SPE, UPLC MS/MS has been used for its detection [8]. These techniques are costly, thin layer chromatography, being inexpensive and simple method for analysis of poisons has been used for this study. After TLC, spot visualization is the next step for comparing the sample material along with standard sample. So far, the reported literature suggests detection of bromadiolone spot on TLC by visualization under UV light only [14]. By studying the detailed structure of the compound, possible spray reagents have been tried for visualization of spots in the present study.

MATERIALS AND METHOD

Reagents: Acetonitrile, Methanol, Toluene, Ethyl Acetate, Glacial Acetic Acid, Laboratory Grade Water. LR grade EP solvents have been used in the present study.

Preparation of standard solution: Standard solution of Bromadiolone was prepared by dissolving accurate quantities of powdered samples in acetonitrile.

Extraction of bromadiolone from samples purchased from market- Samples of Bromadiolone (Brand name Mortin® and Rogan® containing 0.005% Bromadialone) were purchased from the local market. These are available in solid cake form (25g), encased in plastic wrapping. Before analysing them, the samples were finely minced and dissolved in acetonitrile and methanol and kept overnight to facilitate proper extraction.

Color test: Preliminary examination was done through different color test targeting functional group moiety present in the bromadiolone structure i.e. Bromine, -OH bond and coumarin group.

Chromatographic procedure - Thin-layer chromatography was performed on precoated Silica Gel 60 F_{254} plates (Merck). TLCs were run in the following different solvent systems:

Detection - Following the development, the chromato graphic plates were dried and observed under U.V. light (254 and 365nm range). The bromadiolone spots were detected by spraying the plates first by (1) Diazotized sulphanilic acid reagent followed by (2) 1N Sodium Hydroxide solution. The result of colour observed on TLC plates has been tabulated in table II.

RESULTS AND DISCUSSION

Table 1 represents solvent systems used for the separation and identification of bromadiolone standard and samples. Table II represents color test targeting different functional groups and results obtained. Table III summarizes the color results obtained for each solvent system under U.V. light exposure prior to spray, after spraying Chromatographic plates with spraying reagent (1) & (2) in visible light and under UV light after spraying with reagent 1 &2. It has been observed that the excellent one-dimensional chromatographic separation obtained with majority of solvents, however separation with solvent system 1,4 and 5 are showing prominent results of bromadiolone. Moreover, the time of development with Chloroform: Acetic acid: water was observed to be less as compared to benzene containing solvent systems. As both market samples have dyes and other ingredients, during chromatographic procedure various other band representing dyes and other ingredients of sample were observed. The color impurities were removed by filtering the sample solution through silica powder and charcoal.

CONCLUSION

Rodenticides are most commonly used for killing rats and mice and because of its effectiveness they are increasingly employed for committing suicide and even homicide. This work may be useful as preliminary examination including color test for the rapid identification and confirmation of bromadiolone on TLC plates using sulphanilic acid spraying reagent followed by NaOH in the absence of instrumental facility like LC-ESI-MS with liquid extraction, reverse phase Ion- pair HPLC with SPE, UPLC MS/MS.

Table 1 : Solvent systems for TLC of Bromadiolone.

Solvent system	Composition
1	Hexane: Benzene: Rectified Spirit: Acetic Acid (60:20:10:10)
2	Hexane: Benzene: Ethyl Alcohol: Acetic Acid: Ammonia (5:2:2:0.5:0.5)
3	Ether: Chloroform: Acetone (60:30:10)
4	Toluene: Ethyl Acetate: Acetic Acid (40:50:10)
5	Chloroform: Acetic Acid: water (4:1:1)
6	Ether: Chloroform: Acetone (12:6:2)

Table 2 : Color test targeting different functional groups.

Target group	Test	Observation
Br^- OH^-	Reaction with Silver Nitrate R-Br + AgNO3 ➞ R-NO3 + AgX	Pale yellow color further treating with dil. ammonia solution color disappears
-OH	Reaction with hydrochloric acid R-OH+ HCL ➞ R-CL+ H2O	Cloudy appearance
Coumarin group	1ml sample solution is mixed with Diazotized sulphanilic acid solution. Then NaOH is added and shake vigorously.	Dark orange yellow color.

Fig. 1 : Solvent system-Hexane: Benzene: Rectified Spirit: Acetic Acid (60:20:10:10)

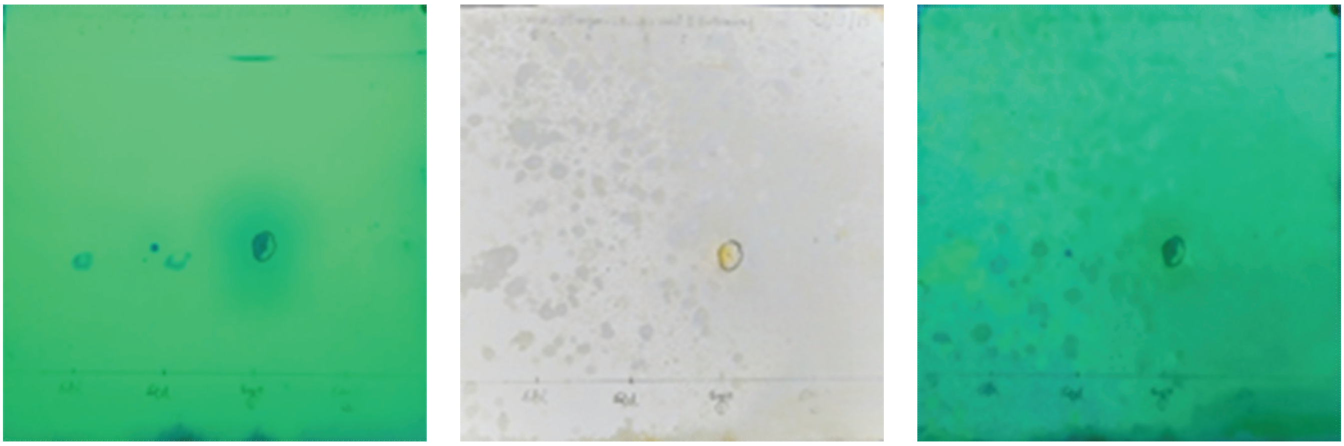

Fig.2 : solvent system- Toluene: Ethyl Acetate: Acetic Acid (40:50:10)

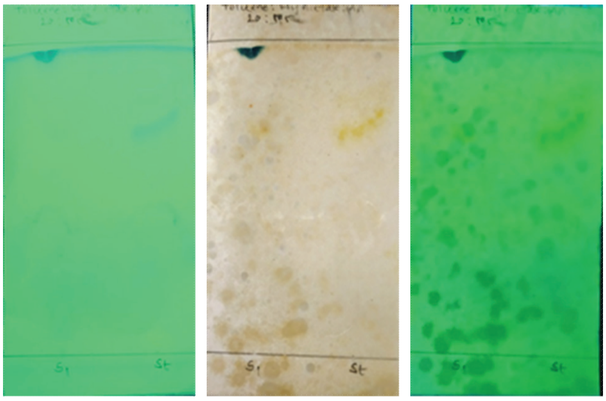

Fig. 3 : Solvent system Chloroform: Acetic Acid: water (4:1:1)

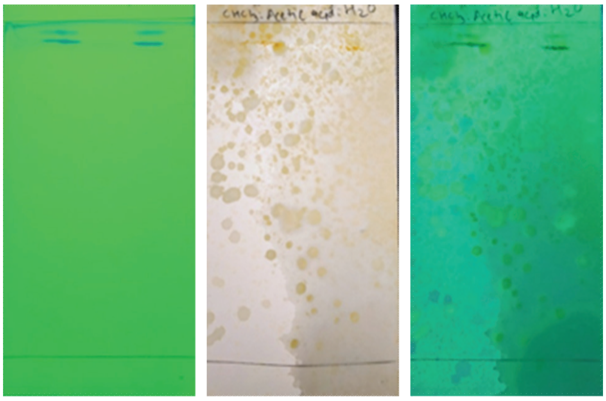

Table 3 : The color results obtained for each solvent system under U.V. light exposure before spray, after spraying Chromatographic plates in visible light and under U.V light.

Sr.No	Solvent system	U.V light before spray	Visible light After spraying with (1) & (2)	U.V light (after spraying)
1	Hexane: Benzene: Rectified Spirit: Acetic Acid (60:20:10:10) (Fig.ii)	2 spots	Brown Orange	Dark brown
2	Hexane: Benzene: Ethyl Alcohol: Acetic Acid: Ammonia (5:2:2:0.5:0.5)	No prominent color observed		
3	Ether: Chloroform: Acetone (60:30:10)	No prominent color observed		
4	Toluene: Ethyl Acetate: Acetic Acid (40:50:10) (Fig.ii)	1 Blue	Yellowish Orange	Orange
5	Chloroform: Acetic Acid: water (4:1:1) (Fig.ii)	2 bands bluish purple And dark blue	Yellow Orange	Orange and brown
6	Ether: Chloroform: Acetone (12:6:2)	No prominent color observed		

REFERENCES

1. Murphy, M. J. (2018). Anticoagulant Rodenticides. *Veterinary Toxicology: Basic and Clinical Principles: Third Edition, 24*(4), 583–612. https://doi.org/10.1016/B978-0-12-811410-0.00046-5

2. Garg, N., & Singla, N. (2014). Toxicity of second generation anticoagulant bromadiolone against Rattus rattus: individual and sex specific variations. CIB Tech. J. Zool, 3(2), 43-48.

3. https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/bromadiolone (last viewed 1 Aug, 2020)

4. Vandenbroucke.V., Bousquet-Melou.A., De Backer.P., & Croubels.S (2008). Pharmacokinetics of eight anticoagulant rodenticides in mice after single oral administration. *Journal of Veterinary Pharmacology and Therapeutics,* 31(5), 437–445. https://doi.org/10.1111/j.1365-2885.2008.00979.x

5. Berny, P. J., de Oliveira, L. A., Videmann, B., & Rossi, S. (2006). Assessment of ruminal degradation, oral bioavailability, and toxic effects of anticoagulant rodenticides in sheep. *American Journal of Veterinary Research,* 67(2), 363–371. https://doi.org/10.2460/ajvr.67.2.363

6. Valchev, I., Binev, R., Yordanova, V., & Nikolov, Y. (2008). Anticoagulant rodenticide intoxication in animals–a review. *Turkish journal of veterinary and animal Sciences,* 32(4), 237-243.

7. Alshawabkeh, L., Economy, K. E., & Valente, A. M. (2016). Anticoagulation During Pregnancy: Evolving Strategies with a Focus on Mechanical Valves.Journal of the American College of Cardiology, 68(16), 1804–1813. https://doi.org/10.1016/j.jacc.2016.06.076

8. Grobosch, T., Angelow, B., Schönberg, L., & Lampe, D. (2006). Acute bromadiolone intoxication. Journal of analytical toxicology, 30(4), 281-286. https://doi.org/10.1093/jat/30.4.281.

9. Routh, C. R., Triplett, D. A., Murphy, M. J., Felice, L. J., Sadowski, J. A., & Bovill, E. G. T. (1991). Superwarfarin ingestion and detection. American Journal of Hematology, 36(1), 50–54. https://doi.org/10.1002/ajh.2830360111

10. Meehan, A. P. (1978). Rodenticidal activity of bromadiolone--a new anticoagulant. In *Proceedings of the Vertebrate Pest Conference* (Vol. 8, No. 8).

11. Robben, J. H., Kuijpers, E. A., & Mout, H. C. (1998). Plasma superwarfarin levels and vitamin K1 treatment in dogs with anticoagulant rodenticide poisoning. The veterinary quarterly, 20(1), 24–27. https://doi.org/10.1080/01652176.1998.9694831

12. Smith, S.A., Kraft, S.L., Lewis, D.C., & Freeman, L.C. (2000). Plasma pharmacokinetics of warfarin enantiomers in cats. *Journal of veterinary pharmacology and therapeutics,* 23(6), 329-337. https://doi.org/10.1111/j.1365-2885.2000.00269.

13. Narlı Özdemir, Z., Şahin, U., Merter, M., Gündüz, M., Ateşağaoğlu, B., & Beksaç, M. (2016). A Case of Superwarfarin Poisoning Due to Repetitive Occupational Dermal Rodenticide Exposure in a Worker. Turkish journal of haematology : official journal of Turkish Society of Haematology, 33(3), 251–253. https://doi.org/10.4274/tjh.2015.0433

14. Singh, R., & Pamarthi, V.K. (2008). Thin layer chromatographic analysis of Bromadiolone. *Journal of Indian Society of Toxicology,* 4(002), 26-28.

15. Lau-Cam, C. A., & Chu-Fong I. TLC of Coumarin Anticoagulants. Journal of Pharmaceutical Sciences. 1972;61(8):1303–1306. https://doi.org/10.1002/jps.2600610828

16. Rengel, I., Friedrich, A. Detection of anticoagulant rodenticides (4-hydroxycoumarins) by thin - layer chromatography and reversed -phase high-performance liquid chromatography with fluorescence detection. Vet Res Commun 17, 421–427 (1993). https://doi.org/10.1007/BF01839210.

17. Gunja N, Coggins A, Bidny S. Management of intentional superwarfarin poisoning with long-term vitamin K and brodifacoum levels. *Clin Toxicol (Phila).* 2011;49(5):385-390. doi:10.3109/15563650.2011.587126