International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue A15, March 2021
ISSN:2320-7817(p) 2320-964X(0)

Original Article Open Access

TLC analysis of plant alkaloid (Strychnine) in toxic baits using novel solvent system

Ingole Nirwan[1] and Dubey Mishra Swati[2*]

[1]Assistant Professor & Research Scholar, [2]Assistant Professor, Shri Vaishnav Institute of Forensic Science Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore.
*Corresponding author Email: swati.dubeymishra@gmail.com

Manuscript details:

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Cite this article as:
Ingole Nirwan and Dubey Mishra Swati (2021) TLC analysis of plant alkaloid (Strychnine) in toxic baits using novel solvent system, *Int. J. of. Life Sciences*, Special Issue, A15: 42-44.

Article published in Special issue of International e-Conference on "Forensic Biology" (ICFB-2021) organized by Department of Forensic Biology & IQAC, Government Institute of Forensic Science, Nagpur, Maharashtra, India date, February 28th & 29th, January, 2021.

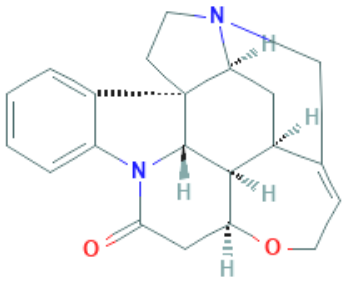
ABSTRACT

Strychnine is obtained from the seeds of Strychnosnux vomica. It is an Indian origin plant and the seeds of nux-vomica are readily available in villages and often used for malicious purposes. Strychnine is highly toxic to the human as well as animals. It is used as indoor and outdoor pesticide specifically for the control of rats, gophers and moles. It is recommended for underground use. A complete and systematic detection of poisons is carried out by Forensic toxicologist. Nowadays sensitive instrumental techniques are available for detection of poisons but at the same time these techniques are more expensive and need sophisticated laboratory infrastructure. Therefore simple, cost effective and rapid thin layer chromatography method is the choice of most analysts for the screening of poisons. In this study, solvent system was developed for analysis of strychnine using Thin Layer Chromatography. This method was also applied to identify the strychnine in toxic baits using novel solvent system which will be useful for detection of strychnine in animal poisoning cases.

Keyword: Strychnine; Toxic baits; Pesticide; Thin Layer Chromatography

INTRODUCTION

Human are killing animal for various purposes like food purposes, medicine purpose, animal population control etc. Illegal killing of animal's especially rare species animal is becoming the serious threat to the nation. Many poisons are reported which are used in killing the animal. The choice of poison depends on many factors like availability of poison, effectiveness of poison, cost and administration procedure. All this contributes to the selection of poisons by the animal killergenetic etiology remains largely unknown (Reddy *et al.*, 2005). Strychnine is one of the plant based poison which are reported in many animal poisoning cases in different countries (Brown *et al.*, 1996, De Saqui *et al.*, 1996, Mali *et al.*, 2017). Strychnos nuxvomica plant is easily available in In- dia (Rathi *et al.*, 2008). The seeds of this plant contain strychnine and can be easily extracted from the seeds (Amir *et al.*, 2013).

Structure (National Center for Biotechnology Information 2021)	**Molecular Formula & Molecular Weight**	***pKa***
	$C_{21}H_{22}N_2O_2$ 334.41 g/mol	8.26

As these seeds are easily available and strychnine can be easily extracted from the seeds. Strychnine poisoning often reported in many intentional, accidental animal poisoning cases in India and it is also reported for the poisoning of wild animal. Secondary poisoning also reported due to consumption of bait by the non-targeted animals (Mali *et al.*, 2017). Strychnine is easily available and more effective than other poisons.

Many analytical techniques are available for the identification of strychnine in various samples. (De Saqui *et al.*, 1996, Patel *et al.*, 2012), but thin layer chromatography is still the method of choice because it is cost effective, sensitive and handy method. Very few work has been reported for the analysis of strychnine in animal bait samples. In this study Dichloromethane, Methanol and Petroleum Ether were used for the screening of strychnine in animal bait sample using Thin Layer Chromatography.

MATERIAL AND METHODS

Materials and reagents:

Standard of Strychnine was purchased from Sigma aldrich, analytical grade chemicals i.e. Dichloromethane, Methanol, Chloroform and petroleum ether were purchased from *Loba Chemie.* Seeds of Strychnos Nux Vomica were purchased from market. Apple was used to prepare poisoned bait.

Apparatus: Pre coated Silica Gel Plate (Silica gel 60 F254, Merck KGaA, Germany), Digital Balance, UV Cabinet were used for analysis. Glass tanks were used to develop TLC plate.

Sample preparation:

Standard Sample:

1 mg of strychnine standard was dissolved in 10 ml of methanol and filtered through 0.45 µm nylon membrane filters and stored in amber glass volumetric flask under refrigeration.

Poisoned Bait Sample:

10 ml extract of nux vomica seeds was injected in an apple and kept for 24 hour at the room temperature and then strychnine extracted from apple using methanol. After extraction sample was filtered through 0.45 µm nylon membrane filters and stored in amber glass volumetric flask under refrigeration.

TLC Procedure:

Samples A (Standard) , B(Poisoned Bait) were applied at starting line, about 1 cm away from one edge of the pre-coated TLC plate. Solvent was allowed to run 10 cm from starting line. After the application of sample spot, plate was placed almost vertical in a saturated glass chamber containing the optimum developing solvent. After the development plate was removed from the chamber. The plate was allowed to dry and subjected to detection/identification. TLC plate was viewed under UV light in UV cabinet. After visualization of the spot Rf value was calculated.

RESULTS AND DISCUSSION

Optimization of mobile phase

In order to establish the mobile phase that gives best result for the screening of strychnine, according to elutropic series of solvents, the tried mobile phases were varied from non polar to polar solvents and their various combinations. Finally the optimum solvent system found out to be 70:20:10 (v/v) proportions of Dichloromethane, Methanol and Petroleum Ether respectively and used for the present study. Using the above combination of mobile phase the Rf value of strychnine was found 0.42.

Table 1: Method validation through Repeatability

Day	Number of Trial	R_f Value
Day-1	**1**	0.43
	2	0.42
Day-2	**3**	0.43
	4	0.43
Day-3	**5**	0.41
	6	0.42
Day-4	**7**	0.43
	8	0.43
Day-5	**9**	0.41
	10	0.43
Mean		0.42
± Deviation		0.01

Selection of visualization mode:

Developed plate was visualized under UV light.

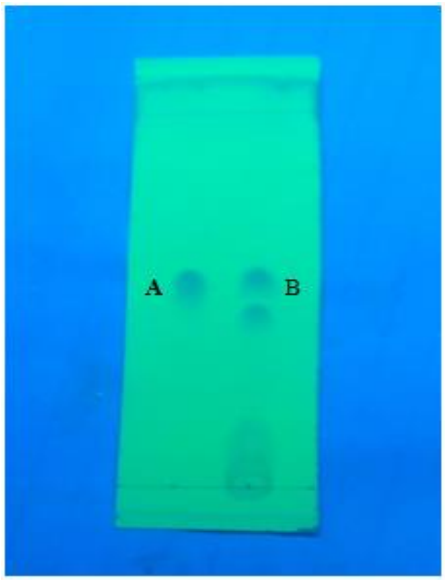


Figure 1 A= Strychnine Standard, B=Strychnine in Bait Sample

Inter-day analysis:
The inter-day repeatability of the method was determined by examine strychnine for five different days using the optimum mobile phase and the changes in the Rf values of strychnine was calculated. The results shown in Table-2 indicate good repeatability of the developed TLC method. The developed method was validated for repeatability and deviation was found ±0.01.

CONCLUSION

Strychnine was identified by Thin Layer Chromatography using an optimum mobile phase Dichloromethane, Methanol and Petroleum Ether (70:20:10) and visualized under UV light. The Rf value of strychnine was 0.42. Method found to be rapid and suitable for routine laboratory analysis of Strychnine. The solvent used is comparatively less toxic moreover no significant interference was observed in the bait sample.

Conflicts of interest: The authors stated that no conflicts of interest.

References

Amir MA, Mujeeb M, Usmani S, Ahmad A, Ahmad S, Siddique WA and Mohammad Aqilda (2013), A Validated Quantitative High Performance Thin – Layer Chromatographic Method for Estimation of Strychnine in StrychnosNux Vomica Seed Extract and Marketed Unani Formulation. *Journal of Applied Science and Engineering,* 149-158.

Brown P, Charlton A, Cuthbert M, Barnett L, Ross L, Green M, Gillies L, Shaw K and Fletcher M (1996), Identification of pesticide poisoning in wildlife. *Journal of Chromatography*, 463-478.

De Saqui P, Nups, P. te Bars, Burgat V (1996) Evaluation of an HPTLC Method for the Determination of Strychnine and Crimidine in Biological Samples. *Journal of Analytical Toxicology*, 185-188.

Mali BD, Akuskar DS and Jivane DM. (2017) Separation and Detection of NuxVomika Alkaloids by Thin-Layer Chromatography. *Journal of Forensic Chemistry and Toxicology*, 95-97.

Patel DK, Patel KP, Duraiswamy B, Dhanabal SP. (2012) Phytochemical analysis and standardization of Strychnos nux-vomica extract through HPTLC techniques. *Asian Pac J Trop Med Dis.,* 56–60

Rathi A, Srivastava N, Khatoon S, Ajay KSR (2008) TLC Determination of Strychnine and Brucine of Strychnosnux vomica in Ayurveda and Homeopathy Drugs. *Chromatographia* , 607–613.

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 441071, Strychnine. Retrieved February 17, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Strychnine.