

EXTRACTION, ISOLATION AND DETECTION OF STRYCHNINE FROM BLOOD USING THIN LAYER CHROMATOGRAPHY

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ABSTRACT: Strychnine is a bitter and highly poisonous compound obtained from nux vomica. This alkaloid has occasionally been used as a stimulant. Strychnine transported in body by plasma and erythrocytes. Actually it has slight protein binding site. Strychnine spread in bloodstream quickly and finally distributes to the tissue. 50% of the ingested dose of Strychnine can enter in the tissue within 5 minutes. Its analysis is very important from biological samples such as blood which are easily available for its management and medico legal cases. Many techniques such as HPLC, GLC, GC-MS, LC-MS etc have been use for analysis of Strychnine but these techniques are not only very costly but also time consuming and require more and more sophisticated instruments. An attempt has been made to develop the method for the analysis of Strychnine from blood using Thin Layer Chromatography (TLC).

Keywords : Strychnine, Alkaloid, Analysis, Blood TLC, Nux Vomica etc.

INTRODUCTION

An alkaloid is a naturally occurring nitrogenous molecule that has a pharmacological effect on humans and other animals. Well known alkaloids include Morphine, Strychnine, Quinine, Brucine, etc. Strychnine is an alkaloid which is easily available for suicide and homicide purpose. Many times these are also present in accidental cases (mostly in children).^{1-2,9}

Strychnine was first discovered by French chemist Joseph Bienaime Caventove and Pirre Joseph in 1818 in the saint-Ignatius bean.

Strychnine is a white odorless, bitter, crystalline powder. It is insoluble in water and soluble only in organic solvent. Plant *Strychnus nux vomica* is generally the natural source of Strychnine. Plant *nux vomica* mostly found in Australia and some other countries like India, Sri Lanka etc. it used like a chemically pill, but today it is used for kill rats. Some old historic records told us that preparation counting Strychnine for kill dogs, cats, and birds in Europe. The structure of Strychnine was first determined in 1946 by Sir Robert Robinson and this alkaloid was firstly synthesized in a laboratory by Robert B. Woodward. This was a most famous tragedy in organic chemistry history.

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Both Chemists (Robinson in 1947 and Woodward in 1965) won the Noble prize for his unforgettable research.³⁻⁵



Fig 1 : Strychnine plant and seeds.

Plant nux vomica poisoning was also mentioned in the 1972 movie “The Mechanic” starring Charles Bronson in which young hitman Steve McKenna (Jan Michael Vincent) betrays his mother, aging hitman Arthur Bishop (Bronson) using a celebratory glass of wine spiked with alkaloidal poison (Brucine) leaving Bishop to die of an apparent heart attack. Queen Cleopatra famously forced her servant to commit suicide by means of a Strychnine

trees fruit seeds, which contain lethal level of Strychnine.⁶

Strychnine transported in body by plasma and erythrocytes. Actually it has slight protein binding site. Strychnine spread in bloodstream quickly and finally distributes to the tissue. 50% of the ingested dose of Strychnine can enter in the tissue within 5 minutes. Very rare difference was noted between oral and intra muscular administration of this highly toxic poison in patient killed by Strychnine. Mostly concentration of Strychnine present in blood, kidney, stomach wall and liver, generally 60-100 mg of Strychnine show fatal dose.^{7,8}

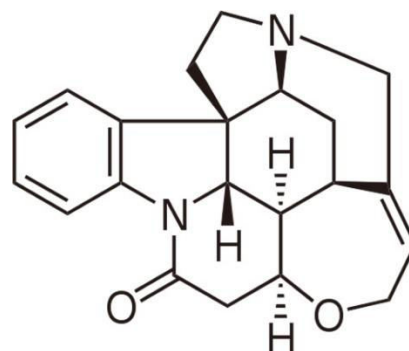


Fig 2: Structure of Strychnine

Many techniques such as HPLC, GLC, GC-MS, LC-MS etc have been used for analysis of Strychnine but these techniques are not only very costly but also time consuming and require more and more sophisticated instruments. Therefore an attempt has been to develop the method for the analysis of Strychnine from blood by using Thin Layer Chromatography (TLC).

EXPERIMENTAL AND DISCUSSION

All chemicals used were of analytical reagent grade. Standard solution (1 mg/ml) of Strychnine was prepared in ethanol.

Preparation of Spraying Reagent

Dragendorff's Reagent

Solution A : 2 gram of Bismuth sub nitrate was dissolved in 20 ml of glacial acetic acid and made up to 100 ml with ultrapure water

Solution B : 40 gram of potassium iodide was dissolved in 100 ml of ultrapure water.

10 ml of solution A and 10 ml of solution B were mixed with 25 ml of glacial acetic acid and volume is made up to 100 ml with ultrapure water.

Extraction From Blood

5 ml blood, 1gm sodium tungstate and 1ml sulphuric acid were mixed in a conical flask. This mixture was then heated for 2-3 minute at 60°C. Contents were then cooled and filtered using filter paper. The Filtrate was made alkaline by adding ammonium hydroxide (pH 8-10) in a separating funnel and extracted with diethyl ether: chloroform (1:3) mixture. The organic layer was separated and marked BE1. The aqueous layer was again extracted with 15 ml and 10 ml ether: chloroform (3:1) mixture respectively. The ether layers were separated and marked BE2 & BE3. All three organic extract BE1, BE2 and BE3 were combined and passed through a pad of anhydrous sodium sulphate and then evaporated up to dryness and residue was dissolve in 0.5 ml ethanol.^{1,4,7}

Chromatography Analysis

Chromatography was performed on TLC plates. Standard solution of Strychnine and extract of blood were spotted onto the TLC plate. The plate was developed in previously saturated TLC chambers using methanol : ammonia (9.5 : 0.5) as mobile phase. The plate was removed from the chamber, Air dry and sprayed Dragendorff's reagent, brown spots were appeared for standard as well as sample on the TLC plate. Strychnine is a organic compound which react with Dragendorff's reagent to give an intense brown spot with white background. The color of the spot was stable for several days. The Rf of sample and standard were 0.40 and 0.41 respectively.



Fig 3 : TLC Chamber

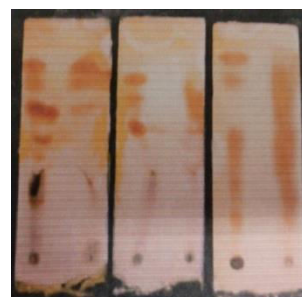


Fig 4 : Developed TLC Plates

CONCLUSION

Developed TLC method is a very simple, cheap, rapid method for analysis of

Strychnine from blood using Thin Layer Chromatography and can be performed in a very less time as compared to sophisticated instruments.

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CONFLICT OF INTEREST:

Authors declared no conflict of interest
