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Original Article

Separation and Detection of Nux Vomika Alkaloids by Thin-Layer Chromatography

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Abstract

Strychnine and brucine are the poisonous alkaloids, derived from seeds of strychnos nux vomica. These seeds are readily available in villages and often misused for poisoning. A systematic and complete detection and quantitation of different poisons including plant poison is carried out by forensic toxicologists. Their method involves screening of poisons and their instrumental assay. Although instrumental methods are sensitive, they are costly and involve elaborate instrumental assay. Therefore, a simple, rapid and cheap thin-layer chromatographic method for separation and detection of bioactive compounds (strychnine and brucine) is described. These alkaloids have -N-C=O group in their structure. This reacts with active chlorine to give corresponding chloramines, which oxidises o-tolidine to give blue coloured compound on thin-layer chromatographic plate. The detection limit was found to be 5µg and 8µg per spot for strychnine and brucine respectively.

Keywords: Strychnine; Brucine; Thin-Layer Chromatography; Chlorine-O-Tolidine.

Introduction

Strychnine and brucine are the main alkaloids obtained from seeds of strychnos nux vomica and other species of strychnos plants, which grow in India [1]. The bark, wood and leaves of the plant contain brucine but not strychnine [2]. Due to ready availability of seeds they are sometimes misused in villages for homicidal or suicidal purposes. Some accidental poisoning cases from villages are reported as the villagers are unaware of their toxicity [3]. Such poisoning cases are received in toxicology division of forensic science laboratory for analysis. Several instrumental methods such as high performance liquid chromatography [4], capillary zone electrophoresis [5], spectrophotometry [6,7] etc., are reported in the literature. But these methods are costly and requires elaborate instrumental assay, hence thin-layer chromatography [TLC] is method of choice for screening of poisons, where large number of biological samples are to be analysed.

Chromogenic reagents such as Dragendorff [8, 9], iodiplatinate followed by UV detection [10] are reported in the literature. TLC [11] and HPTLC [12, 13, and 14] methods are reported in literature for the detection of strychnine and brucine in ayurvedic and homeopathic drugs, nux vomica seeds and biological samples. The objective of this work is to search an alternative and sensitive reagent. We report chlorine-o-tolidine reagent for selective detection of strychnine and brucine.

Materials and Methods

All chemicals used were of analytical reagent grade. Seeds of strychnos nux vomica were obtained from konkan region of Maharashtra. Seeds were dried

and crushed in grinding machine to form powder. It was stored in a glass container. Reference standard strychnine and brucine were purchased from Natural Remidies Pvt. Ltd. Karnataka. Standard solutions of strychnine 1mg/ml and brucine 1mg/ml each in methanol were prepared and were suitably diluted for spotting.

Dried 2 gm powder of nux vomica was extracted in methanol (2x50ml) under reflux for an hour on water bath. The extract was cooled and filtered and concentrated. The volume was made up to 50 ml in a volumetric flask with methanol.

Spray reagents: (I) A 0.05% solution of o-tolidine (S.D. Fine Chemicals, Mumbai) was prepared by dissolving 50 mg o-tolidine in 100 ml 10% acetic acid. (II) Chlorine gas was prepared from a 1:1 mixture of 1.5 % (w/v) potassium permanganate solution and 10% (v/v) hydrochloric acid.

Thin-Layer Chromatography

Standard glass plates (10x20cm) were coated with 0.25 mm layer of silica gel G (Sisco Chemicals , Mumbai) in water (1:2) , allowed to dry at room temperature and then activated at 110°C for about one hour. Aliquots of 10µl each of nux vomica seed extract together with reference solutions of strychnine and brucine were spotted on TLC plate 1.5 cm from the bottom of the plate by means of a micropipette and spots were left to dry in air. The plate was developed by ascending technique, in presaturated TLC chamber, using two solvent systems, chloroform:methanol (9+1) and chloroform:diethylamine(9+1) at 25°C temperature. The mobile phase was allowed to run to a distance of about 10 cm. Approximately 20 ml of solvent was required for run (development time ca 20 min). The plate was removed from the chamber dried in air, was placed for ca 5 min in a chamber containing chlorine gas (prepared 10 min earlier). Excess of chlorine gas was removed from the plate by leaving it to stand in the air; complete removal was tested by spraying the corner of the plate with o-tolidine reagent. When a faint blue colour appeared the plate was uniformly sprayed with o-tolidine reagent then 1 %(w/v) aqueous phosphomolybdic acid.

Results and Discussion

After detection; seed extract, strychnine and brucine standard showed blue spots on a white back ground. A representative reaction for strychnine (I) is represented in Figure 1. The structure of strychnine and brucine reveals that they contain –N-C=O- group. On chlorination they give chloramines (II) which further oxidize with o-tolidine (III) to an intensely blue quinonoid compound (IV). The blue coloured spot began to fade after an hour; it was stabilized for a day by spraying the plate with 1% phosphomolybdic acid solution. Both the mobile phases used gives compact spots. The R_F values and detection limit are listed in Table 1.

Table 1: R_F values and detection limits of nux vomica alkaloids

Alkaloids	Solvent System		Detection limit
	I	II	µg
Strychnine	0.82	0.78	5
Brucine	0.31	0.60	8
Seeds			
Strychnine	0.78	0.74	--
Brucine	0.28	0.56	--

Solvent system: I Chloroform:methanol (9+1), II Chloroform:Diethyamine (9+1)

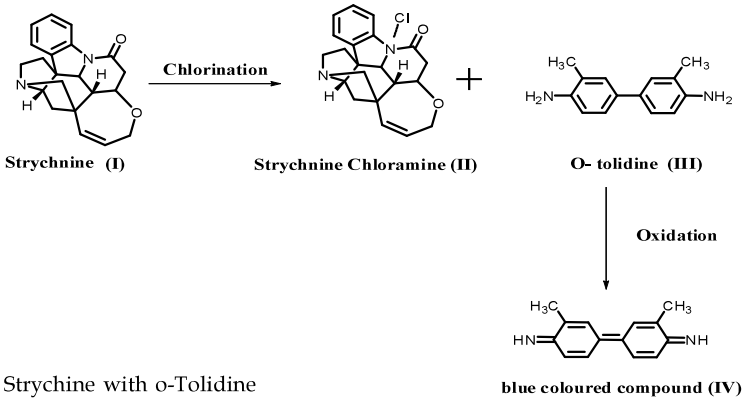

Fig. 1: Reaction of Strychine with o-Tolidine

It is essential to ensure the plate is free from chlorine before spraying with o-tolidine, otherwise the nux vomica alkaloids gives yellow spots, rather than blue. The reagent does not give a colour reaction with other constituents of nux vomica seeds. Dragendorff's and iodiplatinate reagent is reported in literature for detection of nux vomica alkaloids,but also gives coloured spots with several basic nitrogen containing compounds. The method described in this paper involves one-step extraction of alkaloids and less expertise is required than for use of instrumental methods. Chlorination then use of o-tolidine reagent can be used routinely in toxicological analysis for screening of strychnine and brucine in human poisoning cases in single run. This reagent with some modification has also been reported for detection of amino acids [15], benzodiazepines [16] and antidepressants [17].

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