Rapid screening of alcohol deterrent drug “Disulfiram” using Thin Layer Chromatography

S Mourya, S Dubey, D Bose, A Durgabanshi, P Monsanto

Abstract

Disulfiram is presently the only available drug used in the aversion therapy of recovering alcoholics. It acts by inhibiting aldehyde dehydrogenase, leading to high blood levels of acetaldehyde. A thin layer chromatographic methodology to detect and identify disulfiram has been proposed. The detection and identification of disulfiram was done on the basis of Rf values firstly by locating them under UV light followed by chemical treatment involving development of color substances. The efforts were for the development of reliable method for screening of disulfiram in various formulations.

INTRODUCTION

Alcoholism is always a major important problem in many part of the world because alcohol is one of man's oldest and most commonly used psychotropic agent. Prolonged alcohol use can cause intolerance as well as physical and psychological dependence. Indian government agencies are also facing problem of consumption of alcohol beverages mostly in public and work places. In order to overcome this problem most governments have imposed heavy duty on alcohol products. For the de-addiction of alcohol, addicts depend on pharmaceutical cure. Cynamide is one of them but only with modest success. Treatment with disulfiram helps to overcome from drinking habit. It is not a cure for alcoholism, but its use discourages a person from drinking.

Disulfiram, is a synonym of a carbamate derivative tetraethylthiuram disulfide or bis (diethylthiocarbamoyl) disulfide, also known as antabuse. Disulfiram is a relatively nontoxic substance when administered alone, but with alcohol it alters the intermediary metabolism of alcohol. When alcohol is ingested after administration of disulfiram blood acetaldehyde concentration is increased by blocking oxidation of acetaldehyde. Accumulation of acetaldehyde in the blood produces a complex of highly unpleasant symptoms referred to as disulfiram-alcohol reaction, characterized by flushing, palpitation, dyspnea, hypertension, increased pulse rate nausea, vomiting, cyanosis, decreased blood pressure etc. These symptoms usually are followed by drowsiness and sleep, after which the patient recovers fully.

Certain adverse effect observed due to disulfiram-alcohol reactions are diabetes mellitus, hyperthyroidism, epilepsy, cerebral damage, chronic and acute nephritis, hepatic insufficiency. Therefore disulfiram should be used with extreme care in patients having above medical history. Disulfiram should be given to the patient only after the patient has been made fully aware of risks associated with the treatment.

Disulfiram itself can cause adverse effects such as drowsiness, fatigue, headache and skin reactions.

Most of the analytical techniques employed for the determination of disulfiram are based on chromatography. High performance liquid chromatography-Ultraviolet detection (HPLC-UV) of disulfiram and its metabolites have been reported. Advanced analytical techniques like liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS), HPLC-electrospray ionization mass spectrometry (HPLC-MS), HPLC-MS/MS (tandem mass spectrometry) for the analysis of disulfiram and its metabolites in various biological matrices have also been reported. There are several other methods for the determination and estimation of disulfiram in various matrices reported in the literature, including diffusion layer titration at an interdigitated microelectrode array, adsorptive stripping voltammetry at gold disk.
Among the above analytical techniques thin layer
chromatography is a simple, cost effective, fast and reliable
technique for the screening and identification of disulfiram.
Effort has been made using thin layer chromatographic
technique for the rapid determination of disulfiram.

In the Sagar division of state of Madhya Pradesh (India)
some unregistered medical practitioners with the superficial
knowledge of alcohol aversion treatment are selling
disulfiram in altered forms. They also claim that the
preparation is safe and does not have any side effects. The
dose of used drug in preparations is not defined. These
unregistered medical practitioners prescribe the home made
preparations to alcoholic and they consume it without
knowledge of potential hazardous. The present method is
developed to detect the presence of disulfiram in various
formulation provide by unregistered medical practitioners in
Sagar and other part of country side. It is also claimed that
these medications do not contain any pharmaceutical
compound and the de-addiction is completely due to
Ayurvedic medication.

Our team has collected various such formulations and all of
them show the presence of disulfiram. Thus the main aim of
the effort is to develop a rapid, sensitive, cost effective
screening technique for the determination of disulfiram in
various non pharmaceutical formulations.

**Figure 1**

Table 1: Structure, Chemical formula, Molecular weight and
Physical Properties of Disulfiram.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Chemical Formula and Molecular weight</th>
<th>Physical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>C_{10}H_{16}N_{2}S_{4} M.W. 206.55</td>
<td>Disulfiram is white to off white, odorless, crystalline powder, mpF about 709 C, practically insoluble in water, soluble 1 in 66 of ethanol, 1 in 2 of chloroform and 1 in 20 of ether, soluble in acetone, benzene and carbon disulfide.</td>
</tr>
</tbody>
</table>

**EXPERIMENTAL/METHODS**

**MATERIALS**

Standard sample of disulfiram (tetraethylthiuram disulfide)
was obtained from Acros organics-Rankem (New Delhi,
India). Analytical grade chemicals and reagents i.e. acetone,
n-hexane, cyclo-hexane, ethyl acetate, acetonitrile were of
Qualigens Fine Chemicals, (Mumbai, India). Methanol,
chloroform, petroleum ether, tetrahydrofuran, n-heptane,
acetic acid and ammonia were of BDH-E-Merck (Mumbai,
India). Copper chloride, palladium chloride, cobalt acetate,
nickel chloride, ferric chloride, cadmium chloride, zinc
chloride and lead acetate were form Himedia (Mumbai,
India). Digital balance was used of A&D Co.Ltd. (Japan).

Pre-coated 0.25 μm silica gel-G 20×20 cm TLC plate was
purchased from E Merck (India) Limited, Mumbai, India. A
glass tank was used to develop the TLC plates. Used UV
Chamber was of Ideal Scientific Concern, (Kolkata, India).

HPLC grade water (Qualigens fine chemicals, Mumbai,
India) was used throughout the experiments. All the
experiments were performed at 25°C.

Samples sold by unregistered medical practitioners as
alcohol deterrent drug were in form of tablets (S1) and of
green powder (S2). Suspected samples were purchased from
fake unregistered doctors.

**METHODS**

**SAMPLE PREPARATION**

Both sample S1 and S2 were extracted with 10 ml of acetone
and filtered. These samples were stored in amber color glass
containers under refrigeration.

Standard sample of disulfiram was prepared by dissolving
25mg of disulfiram in 10 ml of acetone.

**THE TECHNIQUE**

Samples S1, S2 and standard sample were applied at starting
line, about 1 cm away from one edge of the pre-coated TLC
plate. Precision glass capillaries were used for sample
application. Solvent front was kept at 10 cm from starting
line. After the application of sample spot, the plate was
placed almost vertical in a saturated glass chamber
containing the developing solvent. When the development
was completed the plate was removed from the chamber.
The plate was then allowed to dry and subjected to
detection/identification. First the TLC plate was viewed
under UV light in UV chamber then detection was made by
spraying visualizing agents. After visualization, the spot on
the plate were marked with the pencil. Distance between the
centre of the spots and the base line were measured manually
and the Rf value of resolved spots and the standard was
calculated using the following formula:
RESULT AND DISCUSSION

OPTIMIZATION OF MOBILE PHASE

In order to establish the mobile phase that gives best result for the screening of disulfiram, according to elutropic series of solvents, the tried mobile phases were varied from non polar to polar solvents and their various combinations.

When the combination of n-hexane: water (50:50 v/v), water: petroleum ether (70:30 v/v), water: ethyl acetate (80:20 v/v) was applied as a mobile phase, in all combinations tailing was found. In the combination of water: ethyl acetate (40:60 v/v) spot moved with the solvent and shows tailing, it means in water containing mobile phases compound is retained by the stationary phase. In the combination of hexane: acetone: chloroform (65:35:5 v/v) spot was totally retained by stationary phase. In combination of ethyl acetate: methanol (80:20) and ethyl acetate: ethanol: water: acetic acid (60:15:20:5) sample did not show any retention in stationary phase.

As the above combinations could not lead to proper determination. Finally a combination of two organic solvent i.e. cyclo-hexane (dielectric point \( \varepsilon = 1.890 \)) and ethyl acetate (dielectric point \( \varepsilon = 6.020 \)) were chosen for the optimization in different proportions.

When 100% cyclo-hexane as a mobile phase was selected the compound fully retained on stationary phase. When 100% ethyl acetate was used the sample moves with the solvent front. Following the above trend various combination of cyclo-hexane and ethyl acetate in the ratio of 90:10, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 v/v respectively tried.

Finally the optimum solvent system found out to be 80:20 (v/v) proportions of cyclo-hexane and ethyl acetate respectively. Using the above combination the \( R_f \) value of disulfiram was found out to be 0.82.

DETECTION AND IDENTIFICATION

As the dithiocarbamates have chelating action with metals \(^{16}\), different metallic solutions i.e. copper chloride, cobalt acetate, nickel chloride, lead chloride, zinc chloride, ferric chloride and cadmium chloride were tried for visualization of the spots. Copper chloride was found most sensitive among the above metallic solutions. The 5% solution of copper chloride produces dirty green color with disulfiram. Thus it was used for the localizing disulfiram on TLC plate.

To visualize the spot palladium chloride was also used which is a reported reagent for screening the disulfiram by chemical color test. With palladium chloride spots produces orange color.\(^4\)

While using zinc chloride, nickel chloride, lead acetate, ferric chloride and cadmium chloride no color change were found on the thin layer chromatographic plate.

**Figure 3**

Figure 1: Thin Layer Chromatography of Disulfiram
CONCLUSION

Disulfiram was detected using a mobile phase 80:20 v/v of cyclo-hexane and ethyl acetate respectively and visualizing it first under U.V. light and finally with chemical agent copper chloride. The method is fast and reliable for the screening of disulfiram. The solvent used is non carcinogenic, comparatively less toxic and non water polluting substances. The developed method was used to detect the sample obtained from a pharmacy shop and another obtained from an unregistered medical practitioner as an ayurvedic alcohol deterrent drug. The result was satisfactory for the screening of the drug. No significant interference from other related compounds were observed.

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