Short Communication

Thin Layer Chromatography of Benzodiazepines

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ABSTRACT

Many sedative hypnotic drugs are available on prescription. The most commonly used drugs of this type are the benzodiazepines, of which approximately thirty are in common use with different pharmacokinetic characteristics and clinical effects. Most use of benzodiazepines is short-term or confined to induce sleep.

These drugs are increasingly being used by miscreants to stupefy victims prior to robbery or rape. Self-induced or accidental overdoses with these drugs are also quite common.

Detection of intact benzodiazepines, as well as from biological materials of a deceased victim is often necessary in forensic practice. Thin Layer Chromatography (TLC) has always been a better choice for the preliminary examination of various drugs of abuse. Literature has evidenced the use of some TLC solvent systems for the separation of some benzodiazepines but in this paper we have introduced a single TLC solvent system which can be of immense use in the preliminary screening of some commonly encountered benzodiazepines. The authors believe that it is an improved, alternative TLC solvent system for the separation of benzodiazepines that can be utilized by forensic laboratories.

Key Words: Benzodiazepine; Thin layer chromatography (TLC).

Introduction

Barbiturates were introduced in the mid-twentieth century and became the most popular sleeping pills of the previous century. However, combination of alcohol and barbiturates caused many accidental deaths by overdose, besides numerous suicidal ingestions. To address these problems, scientists developed safer sleeping pills in the1970s: the benzodiazepines. However, over a period of time it has become clear that they share a number of similar problems with barbiturates.

Benzodiazepines (minor tranquillizers) are medicines with calming, anxiolytic (anxiety relieving) and hypnotic (sleep inducing) properties. Usually prescribed in tablet or capsule form, benzodiazepines come in a variety of shapes and colours. They are usually taken orally, and once absorbed into the bloodstream, circulate throughout the body, slowing down the central nervous system. Benzodiazepines act on the central nervous system producing sedation, muscle relaxation and lower anxiety levels. The major problem with regard to these drugs is chronic abuse.

Benzodiazepines are generally available only on medical prescription. Illegal use, possession, manufacture or supply of benzodiazepines carries heavy penalty. However, benzodiazepines are often chronically abused, they are intentionally or accidentally taken in overdose. Diazepam is the most commonly prescribed benzodiazepine in many countries.[1]

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Benzodiazepines are commonly abused by alcoholics, and are often obtained illegally. They are taken partly to alleviate the anxiety associated with alcohol use, but also because the mixture of alcohol and benzodiazepines produces a desirable effect. This drug once added to drinks becomes hard to taste but combination of benzodiazepines and alcohol can be fatal. Benzodiazepines have also been used as a "date rape" drug because they can strikingly harm and even abolish functions that normally allow a person to resist or even want to resist sexual aggression or assault. Some street users have moved on to taking large amounts of oral benzodiazepines in combination with injected opiates such as buprenorphine.[2]

Shah et al found some interesting age-standardized benzodiazepine associated mortality rates in England where they found that benzodiazepines caused 3.8% of all death caused by poisoning from a single drug.[3] Similarly, Charlson et al in a review have summarized benzodiazepine-related mortality.[4] Prevalence rates of benzodiazepine usage greater than 20% have been reported in certain population groups.[5] Drummer et al studied 16 deaths associated with toxic concentrations of benzodiazepines during a period of 5 years.[6] Using case-control responsibility analysis, they examined the role of benzodiazepines in driver fatalities in on-road motor vehicle accidents. Benzodiazepines showed a positive association with driver-responsible fatalities.

Of late, cases of stupefaction being indulged in as a way of robbing victims are increasingly being reported from various parts of India.[7]

Various laboratory methods exist for the detection of benzodiazepines in biological samples, e.g., thin layer chromatography, gas chromatography, gas-liquid chromatography, high performance liquid chromatography, etc.[8-10] Thin layer chromatography (TLC) is a simple, rapid and convenient method frequently used for identifying many pharmaceutical substances including benzodiazepines.[11] Hancu et al described TLC separation of eight most frequently used benzodiazepine derivates, i.e., alprazolam, bromazepam, chlorazepate, chlordiazepoxide, diazepam, nitrazepam, oxazepam and their degradation products after acid hydrolysis.[12] Bhoi and Kamat have also described a TLC method for the extraction and separation of oxazepam, diazepam, lorazepam and nitrazedpam from food samples.[7]

This study was aimed to develop a useful, rapid and sensitive TLC solvent system for the separation of various commonly available benzodiazepine derivatives in the market.

Materials and Methods

Samples: Eight samples of pharmaceutical benzodiazepines (*Tranax 0.25, Tranax 0.5, Insonia, Normozin, Tensyn-plus, Libropar, Clonis-md 0.25, Dizeral-m*) were purchased from the local market for this study. The compositions of these benzodiazepine derivates have been mentioned in **Table 1**.

Sample Treatment: After dissolving in ethanol, the selected samples were spotted on silica gel 60 F_{254} TLC plates of dimensions 20 x 20 cm (Merck, Germany) with the help of fine-bore glass capillaries. Three TLC chambers were saturated with the three mobile phases, i.e., Solvent system 1-ethanol:diethyl ether:acetone (60:20:20); Solvent system 2-ethanol:diethyl ether:acetone (75:15:10), and Solvent system 3-ethanol:diethyl ether (60:40). The plates were made to run for a standard distance of 10 cm and later dried at room temperature. They were examined in daylight, ultraviolet light, iodine fumes, and lastly, after spraying with Dragendorff's reagent [Solution A: 1.7 g basic bismuth nitrate in 100ml water/acetic acid (4:1); Solution B: 40 g potassium iodide in 100 ml of water; 5 ml of solution A and 5 ml of solution B were mixed together in 20 ml acetic acid and 70 ml water]. Photographs of these plates were taken with the help of a digital camera, and hRf values were recorded using templates (**Table 2**).

Table 1 Details of Study Samples

Drug Code	Brand Name	Composition
S1	Tranax 0.25	Alprazolam 0.25 mg
S2	Tranax 0.5	Alprazolam 0.5 mg
S3	Insonia	Zolpidem tartrate 10 mg
S4	Normozin	Chlordiazepoxide 10 mg & Trifluoperazine HCl 1 mg
S5	Tensyn-plus	Propranolol hydrochloride 20 mg & Diazepam 2.5 mg
S6	Libropar	Chlordiazepoxide 10 mg & Trifluoperazine HCl 1 mg
S9	Clonis-md 0.25	Clonazepam 0.25mg
S10	Dizeral-m	Propranolol HCl 10 mg & Diazepam 2 mg

Table 2 Details of Solvent System, TLC Spots and hRf values

Sample Code	Solvent System-1 Ethanol:Diethyl ether:Acetone (60:20:20) Room temp: 34°C	
	No. of spots	hRf value
S1	1	70
S2	1	40
S3	2	84 94
S4	2	25 80
S5	3	34 78 95
S6	3	24 79 94
S9	2	05 71
S10	2	30 95

Results and Discussion

In this study, a single solvent system has been developed for the separation of various benzodiazepines. Relevant details are laid out in **Table 2**. It is evident from the table that the best results were obtained in Solvent system-1 comprising ethanol:diethyl ether:acetone (60:20:20). Th e other two solvent systems also produced satisfactory results but effective separation of all samples could only be obtained in Solvent system-1. UV method can be considered of some value because some components of drugs fluoresced well under UV light. Results obtained with iodine fuming method are also fairly satisfactory. Exposure to Dragendorff's reagent produced some colour spots.

In an earlier study, Hancu et al had separated eight benzodiazepines, i.e., alprazolam, bromazepam, chlorazepate potassium, chlordiazepoxide, diazepam, nitrazepam, and oxazepam using four mobile phases: chloroform:methanol (9:1), chloroform: acetone (4:1), ethyl acetate: methanol: ammonia (17:2:1), hexane: chloroform:methanol (5:5:1), and acetone: chloroform:isopropanol (8:1:1).[12] Each benzodiazepine sample could be separated from the others by using an appropriate mobile phase. Any of the benzodiazepines could be identified by combining the results obtained with different mobile phases. Bhoi and Kamat used food samples for the extraction and separation of four drugs, i.e., oxazepam, diazepam, lorazepam and nitrazepam, prepared by dissolving 10 mg of respective active ingredients in 10 ml of 95% ethyl alcohol separately. TLC plates were developed in a pre-saturated chamber containing solvent system benzene: acetonitrile: methanol (8:1:1). The spots were visualized on TLC by spraying with iodine- potassium iodide reagent, followed by 10% copper sulphate aqueous solution. One spot each for oxazepam, diazepam, lorazepam and nitrazepam was observed at hRf 40, 85, 45 and 65 respectively. The reagents possessed sensitivity to detect oxazepam and diazepam to the level of 1mcg, whereas the more commonly used Dragendorff's reagent did not respond to this limit. A similar observation was made in the present study regarding the capacity of Dragendorff's reagent, though the number of samples in the earlier study was much less.

Conclusion

There are a number of confirmatory tests available for detecting benzodiazepines in biological and other samples. The most favoured is HPLC, although both GC and TLC techniques are also frequently used by many laboratories. TLC has remained one the most desirable methods of investigation in Forensic Science Laboratories because of inexpensive equipment and ease of performing the procedure. This study has concentrated on developing a preliminary screening of some benzodiazepines by using only a single TLC solvent system to facilitate easy and rapid identification of a wide range of benzodiazepines.

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