

## DETECTION OF ALPRAZOLAM IN BROWN SUGAR ADDICT'S URINE BY HPTLC

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### ABSTRACT

Thin-layer chromatography is a quick, simple and economic analytical method for separation of different analytes. A simple, sensitive high performance thin layer chromatographic method has been developed for determination of alprazolam in brown sugar addict's urine. Alprazolam possesses anxiolytic, sedative, hypnotic, anticonvulsant, and muscle relaxant properties.<sup>1</sup> In India nowadays street samples of brown sugar are adulterated by the drug alprazolam. The brown sugar samples are adulterated at every stage of drug trafficking. When those samples reach the end users they contain very small amount of heroin as compared to adulterants used. The present work deals with the detection of unchanged alprazolam in urine samples of brown sugar addict. The acid hydrolysis<sup>2</sup> of urine sample at 15 psi pressure was carried out and extracted at pH 9.2 with solvent chloroform and isopropyl alcohol IPA in the ratio 8:2. The solvent layer evaporated to dryness and residue dissolved in methanol and used for HPTLC. The drug alprazolam was separated on a HPTLC plate. The detection of alprazolam is confirmed by GC-MS analysis. The detection limit of alprazolam by this method found was 1µg.

### KEY WORDS

Alprazolam, adulterant, brown sugar addict, urine, HPTLC

### INTRODUCTION

The term controlled drug is any substance, the possession or supply of which is restricted by law because of its potential harmful effect on the user. Such drugs are known as scheduled drugs /substances. They comprise licit materials (i.e. those manufactured under license), the illicit products of clandestine factories & some natural products. Many of the drugs currently abused were once not only on open sale but often promoted as beneficial substances by the food & pharmaceutical industries. Consumption of illegal drugs is widespread globally. Among all benzodiazepines alprazolam has the highest misuse potential. Alprazolam is the most commonly misused benzodiazepine. It can be

used to enhance the effects of other CNS depressant drugs eg alcohol or heroin

In India the clandestinely prepared brown sugar samples contain heroin along with other opium alkaloids or crude heroin (diacetyl morphine) is adulterated at every stage of drug trafficking for monetary gain. Previously the major adulterants used were paracetamol, diazepam, caffeine, sugar etc. But since from last few years the trend has been changed. The tranquiliser drug alprazolam has been introduced as an adulterant in street brown sugar samples. Brown sugar samples are so adulterated that when they reach the end users the samples contain very low percentage of heroin and higher percentage of alprazolam. Therefore an attempt to detect alprazolam in brown sugar addict's urine is

made. So far HPTLC, GC-MS, LC-MS methods for determination of alprazolam along with other benzodiazepines have been reported.

Several analytical methods for the detection of multiple benzodiazepine drugs in human blood and urine samples have been reported using GC<sup>3</sup>, HPLC<sup>4</sup>, GC/MS<sup>5-10</sup>, LC/MS<sup>11-14</sup> and LC/MS/MS<sup>15-16</sup>.

## MATERIALS & METHODS

All reagents used were of analytical grade, obtained from E.Merck India Ltd. Dragendorff's reagent used was freshly prepared. Reference standard drug alprazolam was obtained from open market. Reference standard morphine was obtained from Govt. opium and alkaloids factory, Neemach.

Sample preparation – All reagents used were of analytical grade.

- i) Reference standard solution –
  - a) Alprazolam – 1 mg/ ml in methanol was prepared.
  - b) Morphin-1mg/ml in methanol was prepared.
- ii) Test samples-Brown sugar addict's urine samples.
- iii) Test sample preparation – 10ml of brown sugar addict's urine was taken in a test tube. 2ml of concentrated hydrochloric acid was added to it and acid hydrolysed in an autoclave at 15 psi pressure for 30 minutes. The solution cooled and made alkaline with 3ml of concentrated ammonium hydroxide solution. The pH of the solution was adjusted to 9.2. The solution was then extracted with mixture of solvents chloroform and isopropyl alcohol in the ratio 8:2. The organic layer separated, evaporated to dryness, residue dissolved in methanol, filtered, concentrated and used for HPTLC analysis.
- iv) High Performance Thin Layer Chromatography (HPTLC).

## Equipment

TLC plates of 10 x 20 cm size having layer thickness 0.25 mm and precoated with <sup>60</sup>F<sub>254</sub> silica gel E. Merck were used.

The plates were activated in oven at 110 °C for 10 minutes, then removed, cooled to room temperature. Standard drug solutions and samples under examination were applied on the HPTLC plate with an applicator. The plate was then developed in solvent system Benzene: Acetonitrile: Methanol in the ratio 8: 1: 1. The TLC chamber was pre saturated with solvent system for about 10 minutes. The plate was developed until the solvent front travels to 10 cms from the spotting. Then the plate was removed from chamber, air dried and sprayed with Dragendorff's reagent. Orange spots were obtained. The Rf values were noted.

## RESULTS AND DISCUSSIONS

The Rf values by high performance thin layer chromatography (HPTLC) analysis<sup>17</sup>, of alprazolam was 0.62 and that of morphine was 0.25. The spots of case samples are observed at Rf values 0.62 and 0.25. The HPTLC analysis showed presence of the drugs morphine and alprazolam. The HPTLC results are confirmed by GC-MS technique.

GC-MS techniques is the unique reliable and full proof tool for identification purpose. In Gas chromatography coupled with mass spectrometric detector (GC-MS), chromatography obtained followed by mass spectrometric detection. In mass spectrometric electron ionization fragment the molecules and with the determination of the mass to charge ratio, determines the relative abundance of the ions which are produced. The GC-MS results indicated presence of morphine and alprazolam without any doubt.

The Rf values obtained by TLC analysis are listed in Table -1. The Rf value of alprazolam was 0.62

and that of morphine was 0.25. The R<sub>f</sub> values of case samples were 0.62 and 0.25 matching with that of alprazolam and morphine. The thin layer chromatogram is shown in figure-1. A typical TIC of the alprazolam is shown in figure-2. The mass spectrum of alprazolam is shown in figure-3 and that of morphine is shown in figure-4. The mass spectrum of case sample exactly tallied with that in library of alprazolam.

When brown sugar is consumed, rapidly hydrolysed to 6-monoacetyl morphine in blood and then more slowly metabolized<sup>18</sup> to the major active metabolite, morphine, normorphine to a very minor extent. Up to 80% of a dose is excreted in the urine in 24hrs mainly as morphine-3-glucuronide. Urine samples are acid hydrolysed mainly to break the linkage between morphine and protein glucuronide, thus made morphine free. The free morphine further extracted in organic solvents chloroform and IPA in the ratio 8:2.

When alprazolam is consumed, it is readily get absorbed and undergoes metabolism. The major metabolites are  $\alpha$ -hydroxy alprazolam and a benzophenone derivative of alprazolam. About 80% of a dose is excreted in the urine in 72hrs of which 11% is unchanged drug. In present work the unchanged drug alprazolam is detected

#### Reproducibility of analysis

Five aliquots of 10 ml each of the same urine sample was taken for analysis and the R<sub>F</sub> values were noted for each aliquot. The values are tabulated in Table-2.

#### Detection Limit

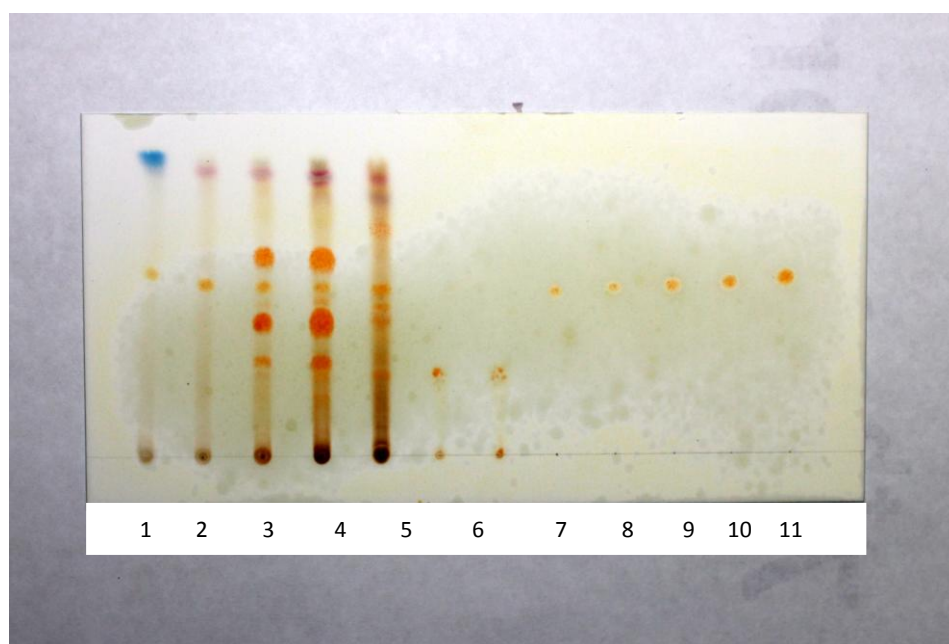
The detection limits of alprazolam were obtained applying 0.5g, 1 $\mu$ g, 2 $\mu$ g, 3 $\mu$ g, 4 $\mu$ g and 5 $\mu$ g alprazolam on HPTLC plate. It was found that the detection limits were 1  $\mu$ g.

Thus the method proposed is very simple, reliable and cheaper for detection of alprazolam in brown sugar addict's urine by HPTLC technique.

**Table 1:**

Name of the Drug	RF values
Alprazolam	0.62
Morphine	0.25
Test urine samples of drug addicts( 1 to 5)	0.62,0.25

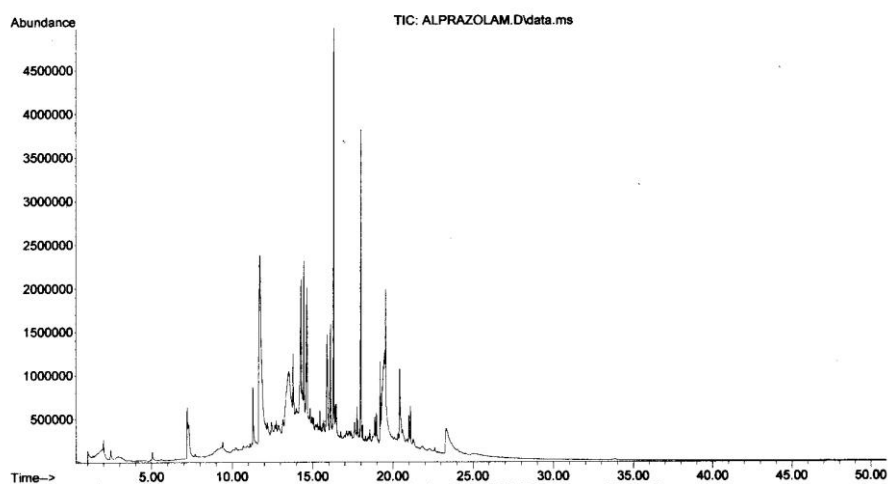
**Fig.-1:**



1 to 5: Test Urine samples of brown sugar addicts.

6 and 7: Ref. std. morphine.

8 to 12: Ref. std. alprazolam 1µg, 2µg, 3µg, 4µg, 5µg respectively.



**Fig.-2: TIC of alprazolam**

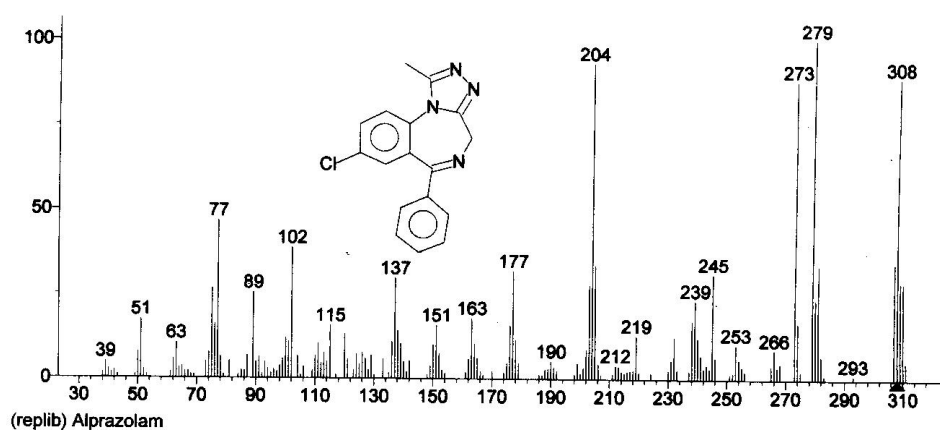


Fig 3: Mass spectrum of alprazolam

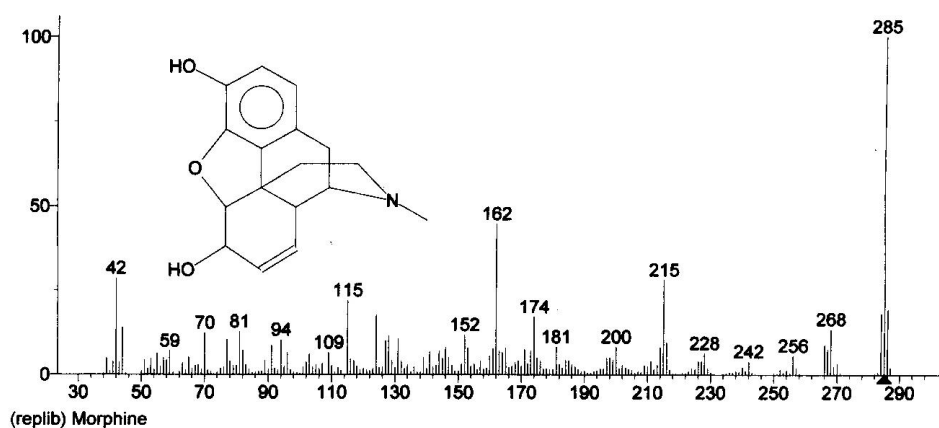


Fig.-4: Mass spectrum of morphine

#### CONCLUSION:

The Indian street samples of brown sugar were adulterated with the tranquilizer drug alprazolam.

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