

# Forensic Examination of Benzodiazepines: A Case Study



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## Abstract

Benzodiazepines are currently among the most frequently prescribed drugs all over the world. These drugs are misused because of their wide variety of color, have no specific smell, hence easy to mix with any eatable and can be easily available without prescription. Considering their increased potential for addiction and abuse in case of crime, suicide and drug facilitated sexual assault. Benzodiazepines are a class of psychoactive drugs whose core chemical structure is the fusion of benzene ring and a diazepene ring. In this study, sedatives were extracted from cream biscuits using drug extraction procedure and analysed using thin layer chromatography (TLC) and Fourier Transform Infrared Spectrometry (FTIR). In extraction procedure, Diethyl ether and chloroform (3:1) were used. In TLC, two solvent systems Chloroform: Methanol (9:1) and Ethyl acetate: Methanol: Ammonia (15:5:0.5) were used. FTIR spectrum analysis also confirmed the match of suspected sample with the standard Lorazepam. Benzodiazepines are challenging to analyse in FTIR because of their varied color which sometimes interfere to give appropriate results. Therefore, the separation and identification of these compounds is of great interest and it is important to develop an efficient sample preparation procedure as well as method able to determine benzodiazepines in different matrices.

**Keywords:** Benzodiazepines; TLC; FTIR; Drug analysis; GABA receptors

## Introduction

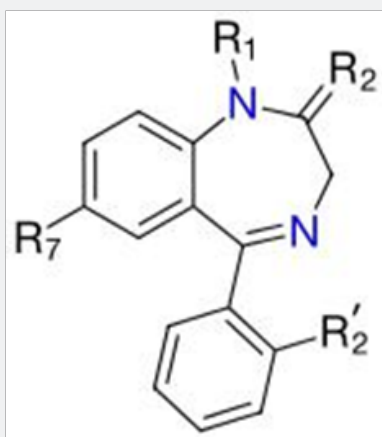


Figure 1A: Structure of Benzodiazepine.

Benzodiazepines are a class of psychoactive drugs whose core chemical structure is the fusion of benzene ring and a diazepene ring. Drug is any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of recipients. Chlordiazepoxide (Librium) was the first of the benzodiazepines discovered by Leo Sternbach (1965) to be marketed as a sedative and an anxiolytic. They enhance the inhibitory actions of the neurotransmitter gamma-amino butyric acid (GABA), located

in the brain [1-4]. These drugs are extensively absorbed when taken orally and achieve peak blood concentrations in about 1 hour. Benzodiazepines become highly protein bound after absorption. Many of them are metabolized and excreted into bile from which they may undergo reabsorption back into the blood (Figure 1A).

Mechanism of action: GABA controls the excitability of neurons by binding to the GABAA receptor. The GABAA receptor is a protein complex located in the synapses of neurons. All GABAA receptors contain an ion channel that conducts chloride ions across neuronal cell membranes and two binding sites for the neurotransmitter gamma-aminobutyric acid (GABA) [5,6]. The GABAA receptor is a heteromer composed of five subunits, the most common ones being two  $\alpha$ , two  $\beta$ , and one  $\gamma$  ( $\alpha 2\beta 2\gamma$ ). Benzodiazepines bind at the interface of  $\alpha$  and  $\gamma$  subunits on the GABAA receptor. Benzodiazepines once bound to the benzodiazepine receptor, the benzodiazepine ligand locks the benzodiazepine receptor into a conformation in which it has a greater affinity for the GABA neurotransmitter. This increases the frequency of the opening of the associated chloride ion channel and hyperpolarizes the membrane of the associated neuron [7].

The inhibitory effect of the available GABA is potentiated, leading to sedatory and anxiolytic effects. For instance, those

ligands with high activity at the  $\alpha 1$  are associated with stronger hypnotic effects, whereas those with higher affinity for GABA<sub>A</sub>

receptors containing  $\alpha 2$  and/or  $\alpha 3$  subunits have good anti-anxiety activity (Figure 1B & 1C).

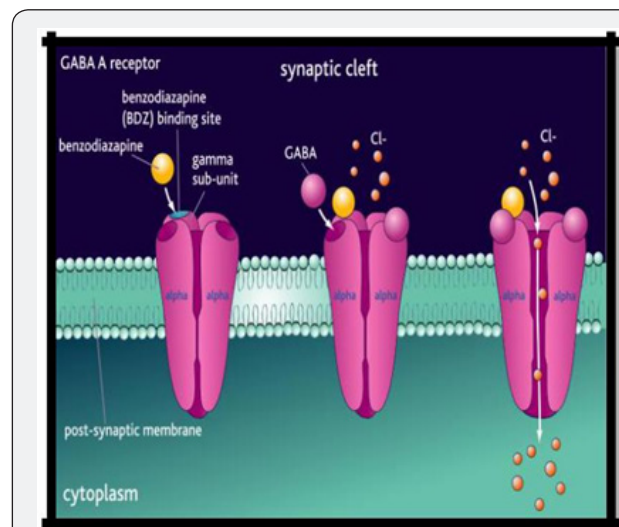


Figure 1B: Mechanism of Benzodiazepines.

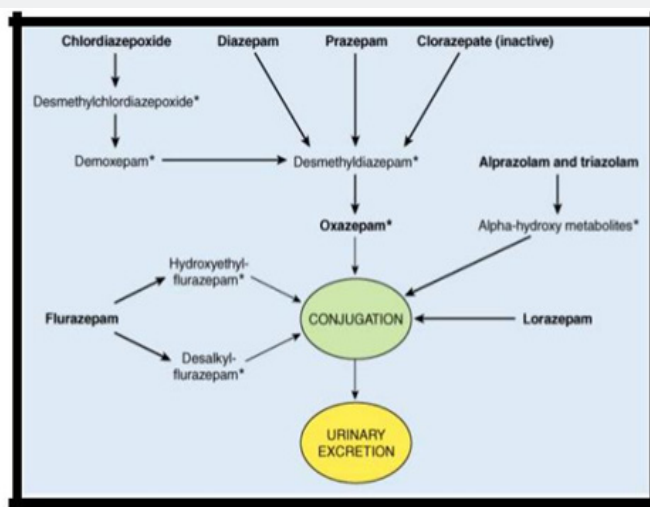


Figure 1C: Metabolism of Benzodiazepines.

Routes of Administration are oral, injection, smoking, rectal administration. Fatal dose of benzodiazepines is 100 to 300 mg/kg body weight. It is used for medical purposes such as seizures, insomnia, general anesthesia, muscle relaxation, alcohol withdrawal, panic attacks etc. Symptoms found after intake of these drugs are vertigo, sleep disturbance, dizziness, drowsiness, loss of orientation, memory impairment, aggression, irritability, slurred speech, nystagmus, diplopia, dysarthria, ataxia, staggering walk, shallow breathing, sedation, somnolence, comma, muscle spasms, convulsions, vomiting etc. The commonly used Benzodiazepines are Diazepam, Flurazepam, Chlordiazepoxide, Nitrazepam, Oxazepam, Alprazolam and Lorazepam [8,9].

### Case Study

Some people have found new tricks to deprive trains and bus passengers of their cash and valuables. These crooks are using any form of eatable including buy offerings (Prasad), laddoo, biscuits or soft drinks as a bait to rob passengers.

On Jan 19. Kunal, resident of Rohini West, New Delhi was drugged through biscuit in a train and then robbed. He was on his way to home from Akshardham Mandir, New Delhi. He told the police that, the stranger seated next to him offered biscuits. Few minutes after eating biscuits he became drowsy and lost consciousness. He does not remember anything. Next day, he found himself in hospital with a glucose drip on. The crook escaped with victims' bag containing Rs. 5000, laptop and some official files. Hospital authorities diagnosed Kunal illness as consumption of sedatives. Police found opened biscuit packet from the place of crime with still two cream biscuits left which they forwarded to FSL in sealed condition for further opinion on whether it contain any sedative or not [10-13].

### Materials and Methods

Cream biscuit which was the suspected sample found at the crime scene at New Delhi was used as the sample and sedatives were extracted using drug extraction procedure and analysed using thin layer chromatography (TLC) and Fourier Transform Infrared Spectrometry (FTIR).

- a) Extraction Procedure: Crushed cream biscuits were taken in an evaporating dish. 50 ml of distilled water was added and shaken properly. This mixture was filtered and transferred into the separating funnel. 90 ml of Diethylether and 30 ml of Chloroform (3:1) was added into the filtrate in a separating funnel and shaken properly. Lower aqueous layer was taken out. Upper organic layer was passed through sodium sulphate anhydrous in an evaporating dish. Air dried the evaporating dish.
- b) Thin Layer Chromatography: TLC plate was cut (10cmx20cm) and activated by keeping it in the oven for 20 minutes. Solvent systems were prepared using Chloroform: Methanol (9:1). The TLC chamber was filled to a depth of about one cm from the bottom and allowed to saturate. A vertical line of 1.5cm was drawn apart from the bottom of the TLC plate. Purified extract dissolved in chloroform were serially spotted on the vertical line of the TLC plate along with the standards (phenargan, DAM, diazepam (basic), lorazepam (basic), nitrazepam (basic)). Allowed the TLC plate to run. The plates were removed from the chamber and mark the solvent front immediately with the pencil and let the solvent dried off the plate. TLC plates were visualized under UV light (254 nm) for characteristics fluorescence or absorbance. Sprayed the spraying reagent Dragendroff and the R<sub>f</sub> values for each spot was calculated. The whole process was revised for second solvent system using Ethyl

acetate: Methanol: Ammonia (15:5:0.5) on the basis of initial results.

c) FTIR: KBr pellets were prepared using KBr powder mixed with extracted sample. Scan the KBr pellets in FTIR.

sample was done using Thin Layer Chromatography and Fourier Transform. In TLC by calculating Rf value comparison was made and in FTIR principle peak values of sample and standard were compared.

**Results and Discussions**

Considering that benzodiazepines can harm the people if misused, on the basis of the case study, analysis of suspected

**Thin layer chromatography**

**Solvent system 1:** Chloroform: Methanol (9:1) (Figure 2 & 3, Table 1)

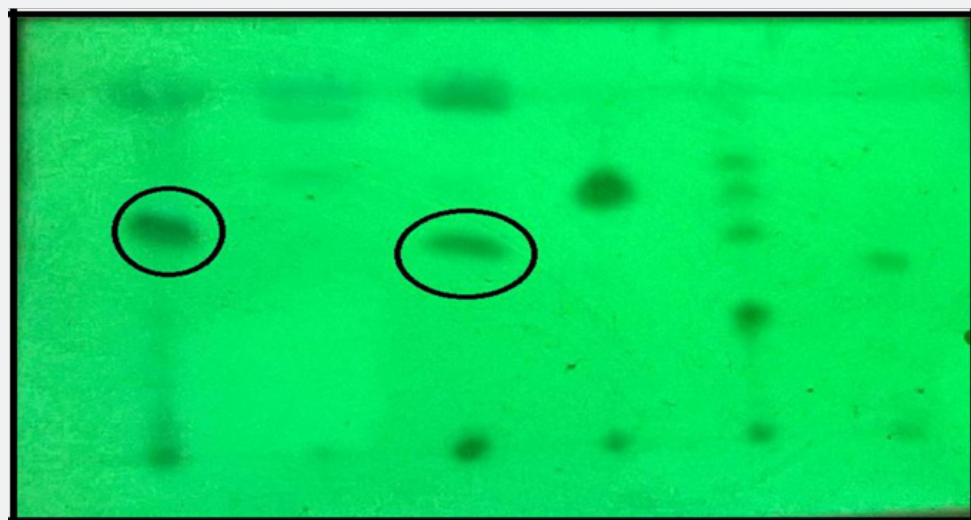


Figure 2: Showing TLC plate under UV Light: Chloroform: Methanol (9:1).



Figure 3: Showing TIC plate after spraying with Dragendorff: Chloroform: Methanol (9:1).

Table 1: Rf value of different Benzodiazepines in solvent system Chloroform: Methanol (9:1).

Benzodiazepines	Distance Travelled By Solvent (cm)	Distance Travelled By Solute (cm)	Rf Value
Case Sample	8	4.4	0.55
Diazepam	8	6.5	0.81
Lorazepam	8	4.3	0.53
Nitrazepam	8	5	0.62
Dam	8	1.8	0.22
Phenergan	8	3.4	0.42

On the basis of above table Rf value of Case sample is almost similar to the Rf value of standard Lorazepam.

Solvent system 2: Ethylacetate: Methanol: Ammonia (15:5:0.5) (Figure 4 & 5, Table 2)

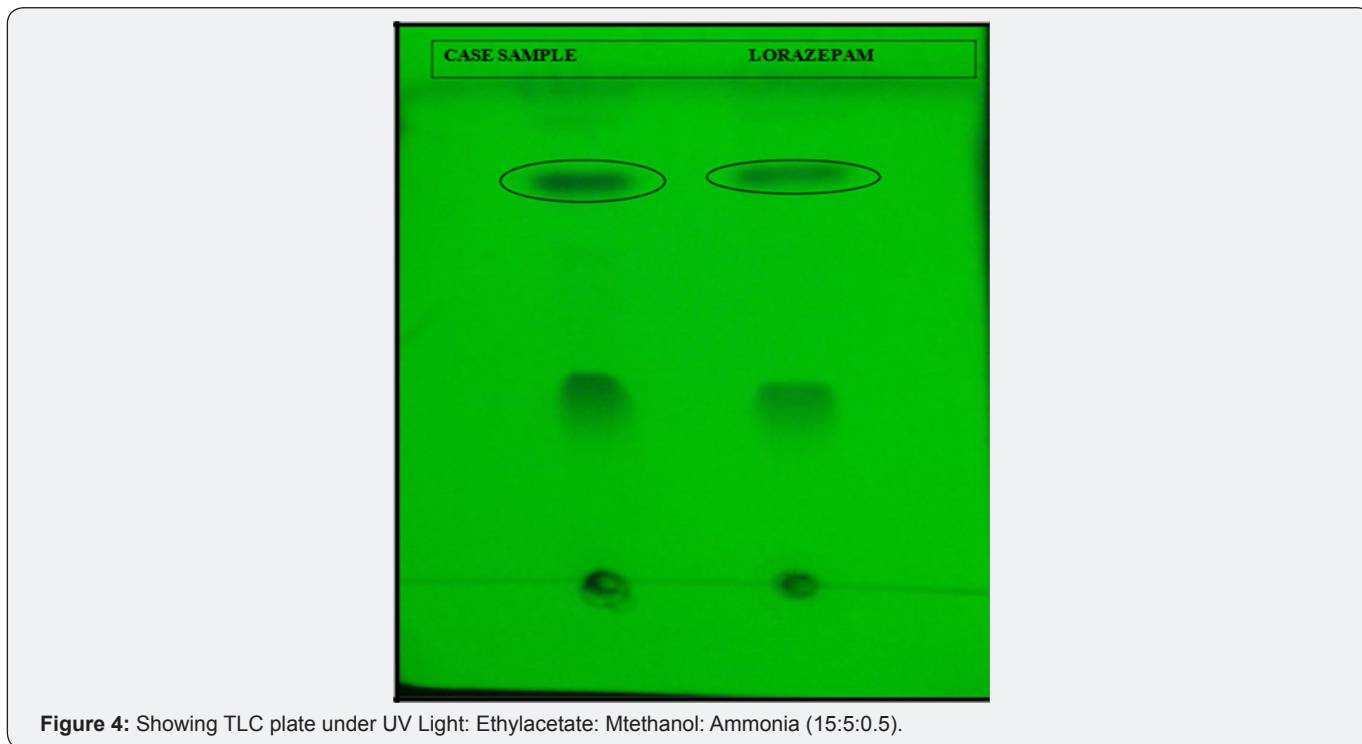


Figure 4: Showing TLC plate under UV Light: Ethylacetate: Methanol: Ammonia (15:5:0.5).

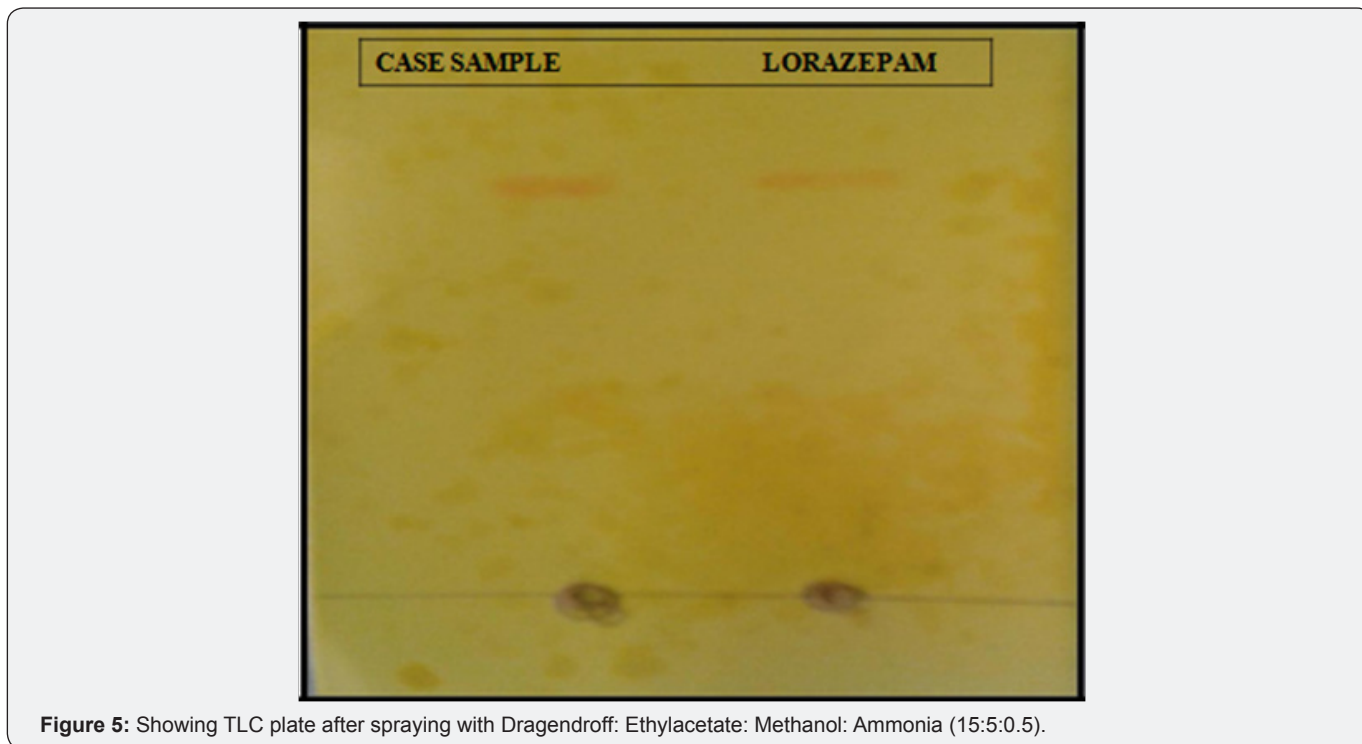


Figure 5: Showing TLC plate after spraying with Dragendorff: Ethylacetate: Methanol: Ammonia (15:5:0.5).

Table 2: Rf value of different Benzodiazepines in solvent system Ethylacetate: Methanol: Ammonia (15:5:0.5).

Benzodiazepines	Distance Travelled By Solvent (cm)	Distance Travelled By Solute (cm)	Rf Value
Case Sample	8.3	7.7	0.92
Lorazepam	8.3	7.7	0.92

On the basis of above table Rf value of Case sample is exactly similar to the Rf value of standard Lorazepam i.e. 0.92. Hence the suspected sample is confirmed to be Lorazepam.



Fourier Transform Infrared Spectrometry (Figure 6 & 7, Table 3)

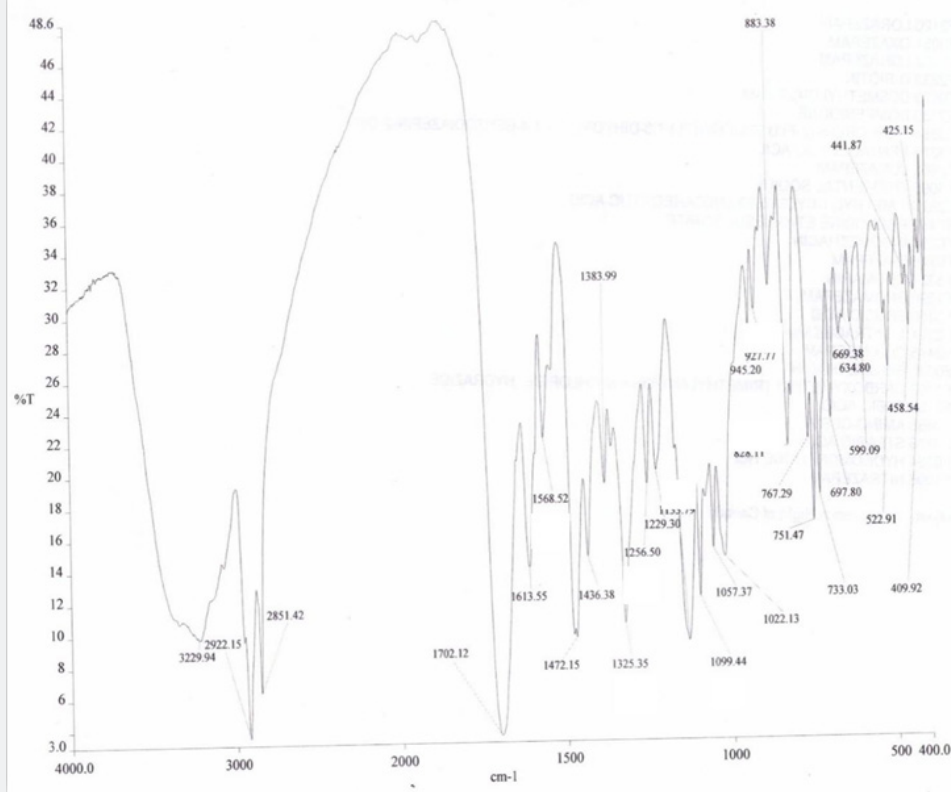


Figure 6: Showing interferogram for the extracted case sample.

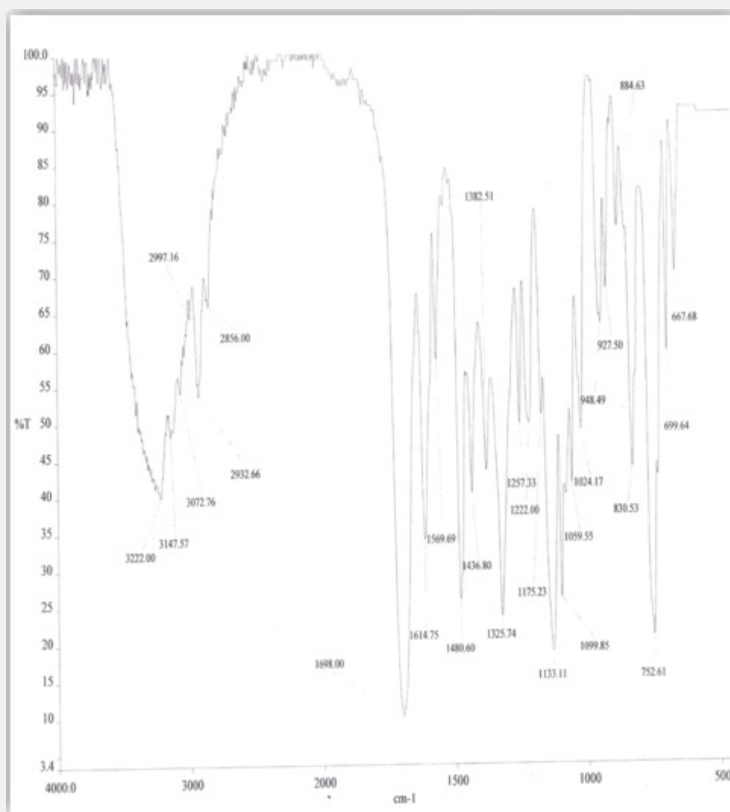


Figure 7: Showing interferogram for the standard Lorazepam.

**Table 3:** Match of principle peaks in FTIR spectrum of extracted sample and standard Lorazepam (cm<sup>-1</sup>) (KBr disk).

No.	Principle Peaks	
	Extracted Sample (cm <sup>-1</sup> )	Standard Lorazepam (cm <sup>-1</sup> )
1	1702.12	1698
2	1613.55	1614.75
3	1568.52	1569.69
4	1436.38	1436.8
5	1325.35	1325.74
6	1256.5	1257.33
7	1133.79	1133.11
8	1099.44	1099.85
9	927.77	927.5
10	828.11	830.53

### Conclusion

On the basis of the case study given, sedatives were extracted from the cream biscuits using drug extraction procedure and analyzed using thin layer Chromatography (TLC) and Fourier Transform Infrared Spectrometry (FTIR). In extraction procedure, Diethyl ether and chloroform (3:1) were used because of the solubility of drugs in them. In TLC two solvent systems Chloroform: Methanol (9:1) and Ethyl acetate: Methanol: Ammonia (15:5:0.5) were used for the confirmation of the sedative found. By analyzing Rf value comparison was made. Rf value of Lorazepam was similar to that of extracted sample i.e. 0.53±0.2 and 0.55±0.2 respectively in the solvent system Chloroform: Methanol (9:1). Rf value of extracted sample was exactly similar to the standard Lorazepam i.e. 0.92±0.2 in the solvent system Ethyl acetate: Methanol: Ammonia (15:5:0.5). KBr pellets (inert in Infra red Spectrum) were for confirmation of Lorazepam using FTIR technique. In FTIR spectrum nearly 10 principal peaks of suspected sample were matched with the standard Lorazepam like 1133.79 cm<sup>-1</sup> (Sample), 1133.11 cm<sup>-1</sup> (Standard), 828.11 cm<sup>-1</sup> (Sample) and 830.53 cm<sup>-1</sup> (Standard) (as shown in Table 3). On the basis of experimental results, it is therefore concluded that the sedative used to rob the victim Kunal was Lorazepam, a Benzodiazepine. Benzodiazepines are challenging to analyze in FTIR because of their varied color which sometimes interfere to give appropriate results. The success of benzodiazepines could be attributed to the fact that they were considered safer and less habit-forming than barbiturates. The widespread use of this class of drugs has occasionally raised concern about recreational benzodiazepine abuse and has led to the erroneous impression that benzodiazepines have a relatively high abuse liability among recreational drug users. These drugs are misused because of their wide variety of color, have no specific smell, hence easy to mix with any eatable and can be easily available without prescription. Considering their increased potential for addiction and abuse, the separation and identification of these compounds is of great interest and it is important to develop an efficient sample preparation procedure

as well as method able to determine benzodiazepines in different matrices.

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