1. Introduction

The doctoral work presented herein highlights determination and analysis of foodstuffs and drinks laced with stupefacient compounds by chromatographic technique. These stupefacients are misused for the purpose of cheating with criminal intention. Poisoning is an important health problem in India. Induced poisoning by robbers during travel using public transport is an emerging social and public health emergency in India, which has not been adequately addressed before. Over the last couple of years reports of foodstuff and drink spiking have appeared on a regular basis in the Indian media. Official statistics and literature survey shows that there has been a dramatic increase in the number of reported drug facilitated crime in train, bus, taxi and air travel or local markets [1-4].

Indian railway network especially long distance trains which carry workers from metro cities or industrial cities to the remote places are the main target of such crime. Almost daily such cases are reported in various railway stations/junctions across India. The general information obtained by the victim is that, after having a foodstuff/drink from co-passenger, they fell unconscious and unable to narrate more. The miscreant then decamps with the valuable things and let the victim helpless with no identity and money. Usually police or railway official bring them to the hospital. After regaining conscious the symptoms reported by victims of alleged drug-assisted robbery include confusion, decreased heart rate, dizziness, drowsiness, impaired judgment, impaired memory, lack of muscle control, loss of conscious and nausea [5]. This feeling is subjective and rarely conforms to the reality. In some cases victims under amnesic drug influence are able to actively participate in actions, but state a blank mind on these (anterograde amnesia). This is a very painful experience for the victim, doctors, nurses and the officer concerned. In the absence of toxicological screening facilities specific diagnosis and care of the patients is also limited. Standard guidelines and facilities with
Intensive Care Unit for case management are not adequate. Also the rehabilitation is limited especially for the victims who are confused and certainly embarrassed with the scenario.

2. **Stupefacient drug**

Stupefacient drug (Table-1) is defined as a drug or an agent that possess the property of making a person stuporous [6], a state of unresponsiveness in which a person seems unaware of the surroundings. The condition occurs in neurologic and psychiatric disorders. The person may be totally or almost totally immobile and unresponsive, even to painful stimuli [7]. Stupor is similar to coma, a profound or deep state of unconsciousness [8]. The affected individual is alive but is not able to react or respond to life around him/her.

As per literature survey and version of the accused involved in the misuse of stupefacient drugs related crime / government railway police (GRP) version / forensic science laboratory findings it was concluded that, foodstuff and drink are being spiked with stupefacient drugs like diazepam (tranquilizer), ketamine (general anaesthetic) and lidocaine (local anaesthetic) for the purposes of committing crime. Among these three lidocaine is mostly used in combination, either with diazepam or ketamine.

2.1. **Diazepam**

Diazepam [7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2(3H)-one, C\textsubscript{16}H\textsubscript{13}ClN\textsubscript{2}O] an example of tranquilizer and is one of the popular and frequently prescribed medication for the last 40 years, also having anti-convulsant properties, used in the treatment of anxiety related disorders. Diazepam appears to act on areas of the limbic system, thalamus and hypothalamus, inducing anxiolytic effects. Its actions are due to the enhancement of Gamma-Amino Butyric acid (GABA) activity [9].
2.1.1. Disposition in the body

Rapidly and completely absorbed after oral administration, with peak plasma levels occurring within about 30 to 90 min. The main metabolic reactions are N-demethylation, 3-hydroxylation, and glucuronic acid conjugation. The major active metabolite is desmethyldiazepam [nordazepam] which accumulates during chronic dosing; other metabolites include oxazepam and temazepam, both of which are active. The formation of nordazepam and oxazepam is catalysed by the P450 isoenzymes CYP2C19 and CYP3A. Only small traces of unchanged diazepam are excreted in the urine and the relative amounts of metabolites are variable and appear to be dose-dependent. About 70% of a dose is excreted in the urine, mainly as oxazepam glucuronide and conjugated desmethyldiazepam, together with smaller amounts of conjugated temazepam. Diazepam and its metabolites cross the blood–brain barrier and the placenta and are also found in breast milk \[10\].

2.1.2. Therapeutic Concentration and toxicity

As cited in literature, after a single oral dose of 10 mg, peak blood concentrations of 0.14 to 0.19 mg/L (mean 0.15) was attained in 1 to 1.5 h. and an average peak concentrations of 0.03 mg/L of desmethyldiazepam was attained after 24 h. \[10\]

Toxic effects may be produced if the blood concentrations is > 1.5 mg/L, fatalities caused by diazepam alone are rare, but may occur if blood concentrations is greater than 5 mg/L.

2.2. Ketamine

Ketamine \([(RS)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone, C_{13}H_{16}CINOH]\), an arylcyclohexamine, acts by blocking the receptor for N-methyl-D-aspartate (NMDA)-an excitatory amine found in human brain and is believed to effect opioid, adrenergic, cholinergic and serotonin receptors. The subjective experiences of ketamine intoxication range from pleasant dreams to intensely visual or
polysensual hallucinations or even a brief full-blown delirium may occur. It is still used owing to its ease of administration and its effects: a strong sedative, it is also associated with analgesia and amnesia. Its dissociative effects results in illegal use of the drug [11].

2.2.1. Disposition in the body

Ketamine is rapidly distributed in the tissues including the brain and placenta following parenteral administration. It has a bi- or triexponential elimination pattern. In the first phase, ketamine exerts its anaesthetic effects in the CNS and it is then redistributed into peripheral tissues and metabolised in the liver. About 90% of a dose is excreted through urine in 72 h, with about 2% of the dose as unchanged drug, 2% as norketamine, 16% as dehydronorketamine and 80% as conjugates of hydroxylated metabolites. Norketamine (which has about one-sixth of the potency of ketamine) and dehydronorketamine are found in the serum in concentrations similar to those of ketamine [10].

2.2.2. Therapeutic Concentration and toxicity

After a single intravenous injections of 2.5 mg/kg, serum concentrations of about 1 mg/L was achieved within 15 min.

During continuous intravenous infusion at an average rate of 41 μg/kg/min, a mean steady-state plasma concentration of 2.2 mg/L was reported; norketamine and dehydronorketamine attained mean peak plasma concentrations of 1.1 mg/L and 0.7 mg/L, respectively, in about 3 h. [10].

Ketamine produces hallucinogenic effects similar to those of phencyclidine and is subjected to abuse also.

In a case of fatal homicide caused by an overdose of ketamine, the concentration of ketamine in viscera samples were as follows:
blood 27.4 mg/L, urine 8.51 mg/L, bile 15.2 mg/L, brain 3.24 mg/L, liver 6.6 mg/L, kidney 3.38 mg/L [10].

### 2.3. **Lidocaine**

Lidocaine \([\text{2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide, C}_{14}\text{H}_{22}\text{N}_{2}\text{O}]\) is a local anaesthetic of amide type, inhibits fast sodium channels and depresses automaticity within the His-Purkinje system and the ventricles, but has a variable effect and may shorten the effective refractory period and action potential duration.

#### 2.3.1. Disposition in the body

Readily absorbed from the gastro–intestinal tract, mucous membranes, damaged skin (poor absorption from intact skin), and intramuscular injection. Metabolism occurs in liver rapidly, with about 90% of a dose being dealkylated to form monoethylglycinexylidide which is 60 to 80% as potent as lidocaine and glycinexylidide which is also active. Further metabolism occurs and metabolic reactions also include hydrolysis, and ring hydroxylation. Less than 10% of a dose is excreted in the urine as unchanged drug in 24 h, 40 to 70% as 4-hydroxy-2,6-xylidine, and about 4% as the active monoethylglycinexylidide, excretion of unchanged drug is increased if the urine is acid. Lidocaine crosses the placenta and the blood–brain barrier and it is excreted in breast milk [10].

#### 2.3.2. Therapeutic Concentration and toxicity

In plasma the range is about 2 to 5 mg/L and the toxic effects are associated with plasma concentrations greater than 6 mg/L, and fatalities are reported when blood concentration crosses 14 mg/L [10].
Table-1. Common stupefacient drugs, Group (Class), Physical appearance, taste/odour and site of action

<table>
<thead>
<tr>
<th>Drug (proprietary names)</th>
<th>Group (class)</th>
<th>Physical appearance</th>
<th>Taste/odour</th>
<th>Site of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>Benzodiazepines</td>
<td>Tablet-A white or yellow crystalline powder, Liquid-usually clear</td>
<td>Bitter/odourless</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>(Diapam, Dialar, calmose, Umbrium, Valium etc.)</td>
<td>(Tranquilizer)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>Anaesthetics</td>
<td>Pill-usually white crystalline powder, Liquid-usually clear</td>
<td>None</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>(Ketalar, Ketalin, Ketaset, Ketavet, Ketina etc.)</td>
<td>(General Anaesthetic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lidocaine</td>
<td>Anaesthetics</td>
<td>Pill- a white to slightly yellow crystalline powder, Liquid-usually clear</td>
<td>None</td>
<td>Peripheral nerves</td>
</tr>
<tr>
<td>(Xylocaine, Xylocard, Xyloox, Lidocard, Ultracaine etc.)</td>
<td>(Local Anaesthetic)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.4. Ideal stupefying drug characteristics

Some of the characteristics of an ideal stupefacient drug are as follows:

- The drug should be odourless, tasteless, and colourless. This allows administration to the intended victim without any warning signs that the victim can detect by the normal bodily senses of smell, taste and sight.

- The drug should be readily soluble, preferably in water. This allows miscibility in normal foodstuffs and drinks.

- The drug should have a fast onset of action. This allows a time period in which the culprit can attempt to create an alibi.

- The drug should have a low-dose lethality, which means low amount of toxic material for administration.

- The drug should be easily available, but not traceable, so that it will leave no investigative trail that would lead to the culprit.
• The drug should be chemically stable. This makes it easy to store without loss of potency.

3. Foodstuff and drink spiking

Spiking is deliberate adding of a chemical or drug into food and drink (alcoholic or non-alcoholic) so as to intoxicate or immobilize an unsuspecting person [12-13].

For stupefaction, the drug is often mixed either in bakery products such as cream biscuits [14], fruits or in liquid foods, like juice [15], milk [4], coffee, Indian sweet [16], tea or coffee [17] etc., for the purposes of cheating or deceiving a person. The effects from foodstuff and drink spiking depend on the type and quantity of the additive used and can include vomiting, loss of consciousness, poor coordination and balance, slurred speech, muscle spasms, respiratory difficulties and loss of control.

4. Motive and modus operandi of culprit

One way to look at the motive of a culprit is to study how the victim is selected: some culprits choose a specific individual, whereas others choose someone at random. The motives of these two types of culprits are same. During the research work the modus operandi of gang involved is in these kind of activity was also studied and are as follows:

(I) Member of the gang use to arrive at the railway station a few hours before the schedule departure time of the train and identify their probable victims and get friendly with them. The victims are such person who usually carries cash and valuables with them. This is a common trend with people who are in return journey back to their home. They would board the train along with the unsuspecting passengers and during the journey they interact with the passenger and would offer them foodstuff or drink laced with stupefying drugs.
(II) The gang passes on the information collected from the fellow passenger to their counterpart so that they can plan the crime and supply the required foodstuff to them in the course of travel. The innocent victims were thus rendered unconsciousness after consuming the stupefacient laced foodstuff and drink and had their belongings stolen by the accused suspects.

In the past, there have been instances of crime reported by law enforcement agencies in which diazepam lidocaine and ketamine was intentionally added to food items with a motive to commit a crime (4,14,18-26).

5. The problem and proposed work

Foodstuff and drink spiking is a complicated phenomenon which may take place in any locations and anyone could be its victims with a variety of different spiking additives, but with a common motive of commits crime either against body or property. During literature survey, it was observed that only a few research works has been carried out using the proposed compound i.e. diazepam, ketamine and lidocaine in foodstuff and drink.

A reversed-phase high performance liquid chromatography technique was developed for the simultaneous determination of both bupivacaine and ketamine in plasma [27]. Method has also been reported for the determination of cocaine and ketamine by high performance liquid chromatography (HPLC) [28]. A sensitive method developed for the determination of ketamine and nor-ketamine by micro-liquid chromatography/mass spectrometry was developed as part of a clinical trial in a pediatric population [29]. Quantitative detection of ketamine, norketamine, and dehydronorketamine in urine often chemical derivatization was detected by gas chromatography-mass spectrometry [30] and by liquid chromatography-tandem mass spectrometry was also reported [31].
Quantitative determination of diazepam, nitrazepam and flunitrazepam in tablets using thin-layer chromatography-densitometry technique [32], diazepam in cream biscuits by high performance liquid chromatography [14] and in soft drinks by high performance thin layer chromatography (HPTLC) was also reported [33]. Determination of flunitrazepam and its metabolites in blood by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry [34], and micellar liquid chromatography (MLC) procedure was reported for the determination of several benzodiazepines in serum [35].

Toxicology results from 1014 cases of claimed drug-facilitated sexual assault (DFSA) was analysed at the Forensic Science Service, London Laboratory between January 2000 and December 2002, where, either a whole blood sample and/or a urine sample was analysed for alcohol, common drugs of abuse and potentially stupefying drugs using various analytical techniques [36].

A simple and rapid reversed phase high performance liquid chromatography method, for the simultaneous determination of lidocaine and cetrimonium bromide was also reported [37]. A case on the use of lidocaine anaesthetic drug in train robbery was also reported in the literature [4]. High performance liquid chromatography method with ultraviolet detection coupled with a solid-phase extraction sample clean up technique was developed for the analysis of five local anaesthetics and four antihistaminics in cosmetic products. The accuracy of the method was verified by spiking experiments on home-made cosmetic samples [38], for the determination of lidocaine and monoethylglycine xylidide in human serum containing various concentrations of bilirubin for the assessment of liver function [39], for the estimation of oxycodone and
lidocaine in rectal gel [40] and separation of tolperisone and lidocaine in commercial products available in Thailand [41].

Although many analytical methods are available for the detection of these drugs but only a few references were available for the determination of lidocaine, diazepam or ketamine in foodstuff and drink and it is noteworthy to mention here that in Indian scenario these are the most common drugs used to dupe the passengers in trains and buses. With reference to above the main aim of the present doctoral work was to identify the most frequently used stupefiant drug and to developed a simple and sensitive procedure for the determination of these drugs either individually or simultaneously in foodstuff using liquid chromatographic method i.e. thin layer chromatography, high performance liquid chromatography and micellar liquid chromatography which simplifies the determination of these compounds. Moreover, there is also a need for using such methodology which is cost effective as well as sensitive without ruling out the importance of fast analysis time.

Chromatography is a method in which the components of a mixture are separated on an adsorbant column in a flowing system [42]. The adsorbant material or stationary phase, first described by Tswett [42] in 1906, has taken many forms over the years, including paper [43], thin layer of solids spread over glass plates [44], immobilized liquids [45], gels [46] and solid particles packed in columns [47]. The flowing component of the system or mobile phase is either a liquid or a gas.

Liquid chromatography (LC) which is one of the forms of chromatography, is an analytical technique which is used to separate a mixture in solution into its individual components. Liquid chromatography is the generic name used to describe any chromatographic procedure in which the mobile phase is liquid.
Thin layer chromatography (TLC) is a versatile method of analysis, the equipment required is relatively, compact and easy to use. A large number of samples can be analyzed at the same time and the analysis can be sensitive and selective. However, a limitation regarding the accuracy of TLC can be expected, when a mixture containing large number of compounds is to be separated. At this point there may exist a chance of false positive test. Still for screening purpose TLC is most successful and recommended technique.

High performance liquid chromatography (HPLC) is one of the versatile chromatographic techniques, which can satisfy many of the required criteria for the analysis of drugs. HPLC is more flexible than GC as there is an infinite number of mobile phase combinations and stationary phase that can be selected depending on the property of the analyte. There is also a choice while selecting the right detector as there are a variety of detectors, which can be coupled with HPLC starting from simple ultraviolet to the complicated mass spectrometer.

Micellar liquid chromatography (MLC) is a mode of reversed-phase liquid chromatography (RPLC), in which the mobile phase is an aqueous solution of surfactant at a concentration above its critical micellar concentration (CMC). Anionic sodium dodecyl sulphate (SDS) is the most widely used surfactant in MLC, but neutral Brij-35 or cationic N-cetyltrimethyl-ammonium chloride is also used. In these media, the great variety of interactions between the solutes, micelles and stationary phase makes MLC a highly versatile technique, which is appropriate for a wide range of solutes (hydrophobic and hydrophilic compounds) that can be separated in the same run. Furthermore, MLC offers some advantages in simplifying sample preparation also.
7. **Organization of thesis**

   Based on the research work carried out during this tenure, the doctoral work comprises of 5 chapters.

   The introductory chapter (chapter-1) demonstrates about stupefacient drug and its characteristics, foodstuff and drink spiking, motive and modus operandi opted by the culprit. It also explains the nature of problem and the justification behind its selection. Brief description of proposed work is also given in the chapter.

   Chapter-2 discusses about thin layer chromatographic technique and some modification i.e. preparative thin layer chromatography, thin layer rods and high performance thin layer chromatography.

   **Section-2A** deals with the separation of two stupefying drug lidocaine and diazepam by thin layer chromatography. The work highlights the seriousness when these drugs are taken in combination and an effort has been made to screen combination of lidocaine and diazepam in various liquid foodstuffs like milk and soft drink.

   **Section-2B** shows the determination of ketamine in juice by thin layer chromatography. After a general survey based on government railway police version, hospital findings, version of accused and reports from forensic science laboratory it was concluded that ketamine was frequently used as stupefying drugs.

   Chapter-3 discusses the technique high performance liquid chromatography with its chromatographic modes based on polarity of the solvent and the stationary phase, its instrumentation and theoretical consideration.

   **Section-3A** focuses on the simultaneous determination of lidocaine, diazepam and ketamine in food sample by high performance liquid chromatography. This method is simple, precise, and sensitive and successfully applied for the determination of foodstuff laced with these stupefacient drugs.
Chapter-4 discusses the technique micellar liquid chromatography, exploring the capabilities of micellar mobile phases with organic solvents as modifiers to enhance chromatographic parameters and a general idea on retention modeling of MLC.

Section-4A describes the simultaneous determination of three stupefying drug lidocaine, diazepam and ketamine in food samples by micellar liquid chromatography (MLC), allow the direct injection of all these samples.

The final chapter (chapter-5) of the thesis is the conclusion of the research work and incorporates all the future prospective and application of the doctoral work in various fields.
Reference:

[1] The Telegraph, Calcutta-India, Train thugs elude cuffs-Drugged RPF constable rescued from station, hospitalised (22.01.11).

[2] Tribune News Service online edition, Chandigarh, India, Seven arrested for robberies on trains (Chandigarh Index) (03.10.10).

[3] Tribune News Service online edition, Chandigarh, India, Robbery motive of birthday party incident (Ludhiana Index) (05.05.11).


[15] Times of India, Banglore, Robbery on the Udyan Express (27.03.10).

[16] Tribune News Service online edition- Chandigarh, India, Three of family taken ill after consuming prasad (Chandigarh Index) (03.01.11).


