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"An Experimental Approach to Detect the Presence of the Date Rape Drug Ketamine in Non-Biological Samples"

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I. Introduction

1.1 What Is Ketamine

Ketamine is a drug used in human and veterinary medicine, primarily for the induction and maintenance of general anesthesia, usually in combination with a sedative. Other uses include sedation in intensive care, analgesia (particularly in emergency medicine), and treatment of bronchospasm. Ketamine has a wide range of effects in humans, including analgesia, anesthesia,hallucinations, elevated blood pressure, and bronchodilation (Peck, 2008). Like other drugs of its class, such as tiletamine and phencyclidine (PCP), ketamine induces a state referred to as "dissociative anesthesia (Bergman, 1999) and is used as a recreational drug(Table 1).

Table 1: Ketamine

KETAMINE	
Systematic IUPAC Name	(RS)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone
Chemical formula	$C_{13}H_{16}ClNO$
Molecular mass	237.725 g/mol

1.2 Stucture Of Ketamine

Ketamine is a chiral compound. Most pharmaceutical preparations of ketamine are racemic; however, some brands reportedly have (mostly undocumented) differences in their enantiomeric proportions. The more active enantiomer, (S)-ketamine, is also available for medical use under the brand name Ketanest S(Krüger, 1998) (Figure 1).

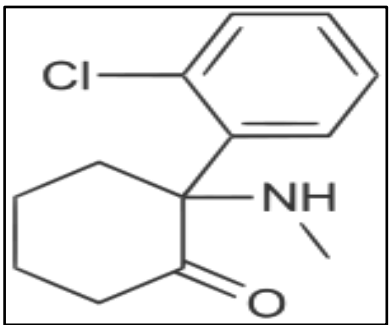

Figure 1: Ketamine structure

Ketamine is a "core" medicine in the World Health Organization's "Essential Drugs List", a list of minimum medical needs for a basic healthcare system. Its hydrochloridesalt is sold as KETANEST, KETASET and KETALAR. Pharmacologically, ketamine is classified as an NMDA receptor antagonist.(Harrison and Simmonds, 1985) At high, fully anesthetic level doses, ketamine has also been found to bind to μ-opioid receptors type 2 in cultured human neuroblastoma cells – however, without agonist activity(Hirotaet al, 1999) – and to sigma receptors in rats. (Narita, 2001) Also, ketamine interacts with muscarinic receptors, descending monoaminergic pain pathways and voltage-gated calcium channels.

II. Literature Review

Ketamine was first synthesized by Calvin Stevens in 1962. The drug was first manufactured in the United States in the 1960s as Ketalar. By the early 1980s a wide range of unauthorized preparations were available in the US including capsules, powder, crystals, tablets and solutions, in addition to the authorized injectable forms. Solutions sold on the street have gone by names such as K, Kay, Jet, Super Acid, vitamin K and Special K (Muetzelfeldtet al, 2008; Degenhardt and Dunn, 2008; Fuhet al, 2006; Drummer, 2001).

Also referred as predator drug, Ketamine drugs are used to assist in the execution of drug facilitated sexual assault (DFSA) as it induces immobility, amnesia and sedation.Ketaminedrugs are typically used by

teenagers and young adults at bars, concerts, parties, and clubs. Ketamine drugs are used to enhance an altered state of consciousness. It has also gradually become popular on the European party scene for its hallucinogenic effects. The use of Ketamines as 'club drugs' has increased significantly over the past 2 decades (Koesters, 2002).

At low doses ketamine induces distortion of time and space, hallucinations and mild dissociative effects. However, at large doses (i.e. over 150 mg) ketamine induces more severe dissociation commonly referred to as a 'K-hole', wherein the user experiences intense detachment to the point that their perceptions appear located deep within their consciousness, thus causing reality to appear far off in the distance (Muetzelfeldtet al, 2008; Siegel, 1978). Since ketamine is odorless and tasteless, it can be added to beverages such as fruit juices, cold drinks, alcohol etc, without being detected, to induce amnesia. Because of such properties, the drug is sometimes misused in a sexual assault, referred as date-rape drug (Hall and Moore, 2008).About 90% of a dose is excreted in the urine in 72h, with about 2% of the dose as unchanged drug, 2% as norketamine, 16% as dehydronorketamine and 80% as conjugates of hydroxylated metabolites.

Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist which interferes with the action of excitatory amino acids including glutamate and aspartate (Anis, 1983). Ketamine has a plasma half-life of 2-4h. It is highly lipid soluble and has a distribution half-life of approximately 7-11 min. Ketamine is metabolized to at least two compounds: first by N-demethylation, to norketamine, which has 1/3-1/2 of the potency of ketamine. Norketamine is further dehydrogenated to produce dehydronorketamine(Huang, 2005; Kim, 2008). The parent compound and both major metabolites are further transformed by hydroxylation and conjugation prior to elimination.

Various instrumental techniques have been used for the determination of ketamines. For example,LC-MS/MS (Koichi, 2011). Kim et al developed a GC-MS method for the qualification and quantification of ketamine (Kim, 2010).

III. Methodology

3.1 Materials And Chemicals Used

1) Borosil glass Beakers
2) Borosil volumetric glass vials
3) Borosil measuring cylinders
4) TLC jar
5) TLC spraying bottle
6) TLC plates
7) Glass capillary
8) Methanol
9) Isopropyl Alcohol
10) 10 Chloroform
11) Iodoplatinate spray reagent
12) Acetone
13) Millipore water
14) Ketamine
15) A clean wood piece
16) A clean white paper
17) A clean white cotton cloth
18) A cleanone inch thick cotton quilt
19) A clean white floor tile piece
20) Surface coating of wall

3.2 Procedure

1) Ketamine standard solution: 5mg ketamine in 5ml methanol was prepared.

2) Six substrates: wood, paper, cloth, quilt, floor tile and surface coatings of wall of the area 10x10=100 cm2 were made ready. 2 ml of ketamine solution was sprayed using a sprayer on each substrate separately and left to dry for 4 hours (Figure 2) (Table 1).

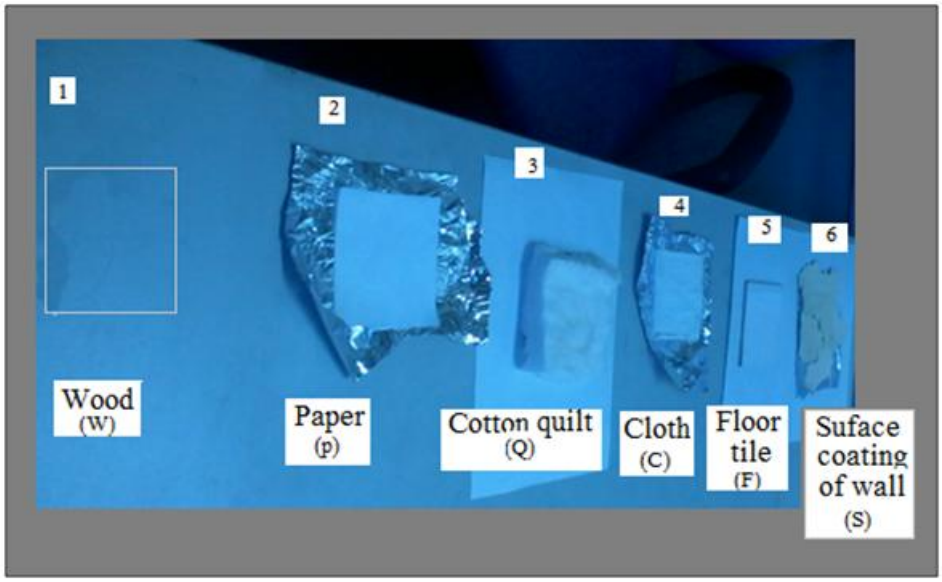

Figure 2: Experimental Set Up For Six Different Substrates.

Table 2: Legend Table

COMPOUNDS	LEGENDS
Ketamine	K
Methanol	M
Isopropyl alcohol	I
Wood piece	W
White paper	P
White cotton cloth	C
Cotton quilt	Q
Floor tile piece	F
Surface coating of wall	S

3)Sample lifting: the samples from all 6 substrate were swabbedby using 6 inch wooden cotton sticks soaked in two different solvents – methanol and isopropyl alcohol separately for each substrate.
Samples lifted by using methanol andisopropyl alcohol swabbing from the substrates were kept in glass beakers and were marked as mentioned in the Table 2.

Table 3: Samples Used For Analysis after Methanol and Isopropyl Alcohol Swabbing

S.No.	Samples Lifted By Methanol Swabbing	Samples Lifted By Isopropyl alcohol Swabbing
1	KWM	KWI
2	KPM	KPI
3	KCM	KCI
4	KQM	KQI
5	KFM	KFI
6	KSM	KSI

4) Extraction: The basic extraction (liquid: liquid extraction) using Chloroform:Ether (1:3 ratio) was done with all samples separately.
5) TLC (Thin Layer Chromatography) wasrun for all the collected samples intwo different solvent systems: **i)** Ethyl acetate: Methanol: Ammonia (8.5:1) and **ii)**Methanol: Ammonia (9:1).AcidifiedIodoplatinatesolution was sprayed which was used as the visualizing agent.

IV. Resuls And Discussion

4.1 Results

I) **TLC Results**

1) Samples swabbed with Isopropyl alcoholTLC solvent system: Ethyl acetate: Methanol: Ammonia (8.5:1:)
Spray reagent: Acidified Iodoplatinate solution

Table 4: TLC Result 1

S.No.	SAMPLES	Rf VALUE
1	Standard	6.1/7.0 = 0.87
2	KWI	5.9/7.0 = 0.84
3	KPI	5.9/7.0 = 0.84
4	KCI	5.9/7.0 = 0.84
5	KFI	6.0/7.0 = 0.85
6	KQI	6.0/7.0 = 0.85
7	KSI	6.1/7.0 = 0.87

2) 1) Samples swabbed with Methanol

TLC solvent system: Methanol: Ammonia (10:1)

Spray reagent: Acidified Iodoplatinate solution

Table 5: TLC Result2

S.No.	SAMPLES	Rf VALUE
1	Standard	6.0/7.0 = 0.85
2	KWM	5.9/7.0 =0.84
3	KPM	5.8/7.0 =0.82
4	KFM	5.7/7.0 =0.81
5	KSM	5.8/7.0 =0.82
6	KCM	5.7/7.0 =0.81
7	KQM	5.9/7.0 =0.84

V. Discussion

In the current experimental setup the extraction ofKetamine drug was done successfully from all the substrates swabbed with Methanol and Isopropyl solvents.All the 14 samples showed positive result in TLC visualized with AcidifiedIodoplatinate visualizing agent.

It is pertinent to mention that extraction of ketamine was challenging and difficult from the surface coating of wall (S) from the samples "KSM" and "KSI" as the matrix was complex because of the presence of pigments and coating constituents which apart from ketamine also bleeded into the solvents while swabbing and created difficulty in the application of TLC technique for identification.

The samples "KSM" and "KSI" needed further cleanup procedure which we were able to do by subjecting it to extraction (basic extraction) with Chloroform: Ether (1:3) solvent mixture with a drop of ammonia in it. The extraction was repeated three times in order to get maximum recovery.It was then filtered using anashlessWhatmanfilter paper No. 42 containing Anhydrous AmmoniumSulphate and was washed three times with Methanol.

The solvent was dried at room temperature (RT) and the residues of both the samples "KSM" and "KSI" were used for spotting on the TLC plates. After the mentioned cleanup procedure the samples responded to TLC technique.

It suggests that extraction of ketamine drug was difficult in the samples "KSM" and "KSI" but, our extraction procedure was well enough for other samples ("KWM", "KCM", "KPM", "KQM", "KFM"; "KWI", "KCI", "KPI", "KQI", "KFI") where no such problems was faced in extracting the ketamine drug from them.

In future course of action, we may use advanced cleanup procedures and techniques for the rapid extraction of drugs from the physical evidences at scene of crime. Further, some more polar and non-polar solvents including water may also be tried as swabbing agents for different class of drugs which have been reported to be used in drug facilitated sexual assaults.

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