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Rapid screening of ketamine in confiscated orange juice by thin layer chromatography

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Abstract : Poisoning by unidentified duping agent particularly during journey is an important security and health problem in India. The intention behind the act is to stupefy the person and commit robbery or other crimes. 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 duping drug. In this paper, a new, fast, cost effective and reproducible thin layer chromatographic screening method is discussed for the determination of ketamine in orange juice. The solvent system used for the determination of ketamine is water : butanol (40 : 60) and dragendorff reagent as localizing agent, which shows orange coloured spot at R_f value 0.68. The developed method was applied to real and spiked orange juice sample giving satisfactory result.

Keywords : Ketamine, duping agent, food stuff, beverage, thin layer chromatography.

Introduction

Travelling is not always filled with fun or enlightening, there are some dark side of travelling also and that is the possibility of being targeted by bad elements while travelling. Unfortunately, onboard long distance train and bus drug assisted robberies is a menace for travelers and a challenge for police. The motive behind this may be robbery, sexual assault or both.

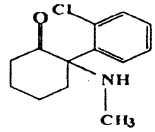
Based on data collected during the research work the modus operandi of gang involved is in these kind of activity was also studied. In general member of the gang use to arrive at the railway station a few hours before the schedule departure time of the train and identify their probable victims and get into friendly terms with them. The victims are normally those people who usually carry cash and valuables with them. The member of gang would board the train along with the unsuspecting passengers and during the journey they would offer them foodstuff or drink laced with duping drugs. Sometimes the member of the gang also 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.

The duping agents which are selected for this purpose do not have colour, smell or taste and are hard to detect once dissolved in a drink or foodstuff, but the preferred drug used for this purpose is ketamine, was described as tasteless, odourless and colourless[1]. The major effects of ketamine are sedation and visual disturbances[2]. It produces vivid hallucinations, paranoia, impulsive behavior, sensory distortions, muscle rigidity and loss of pain perception. In humans, following a single dose (approximately 6–13 mg kg^{-1}), amnesia is observed which may be evident for 1–2 h.

Pharmacologically ketamine is considered a dissociative anesthetic, which means that the drug distorts the user's perception of sight and sound produces feelings of detachment dissociation from the environment and self[4].

The drug also has anesthetic properties that have been

Table 1

Name and trade name	Structure	Chemical formula and molecular weight	pH	pKa	log *P*
Ketamine (ketaject, ketalar, ketaset, ketavet, ketina etc.)		$C_{13}H_{16}ClNO$ Mol. wt. 237.7	5.5	7.5	3.1

used in both human and veterinary medicine. Along with anesthetic benefits, there are certain reactions to ketamine that make it appealing to illicit users. In some circumstances, ketamine has been known to produce illusions or hallucinations that are enhanced by environmental stimuli – this may be one reason that the drug has become increasingly popular in the past few years.

The hallucinatory effects of ketamine last approximately for an hour or less, but the user's sense of judgment and coordination may be affected till 24 h following initial use[5]. Oral ingestion usually produces effects in 15–20 min[2]. Thus making this drug as one of the favourite among lifters.

Various techniques have been developed for the identification of ketamine and its biotransformed products were determined in biological fluids by thin layer chromatography (TLC) and gas chromatography (GC)[6], in biological fluids by gas chromatography-mass spectrometry (GC-MS)[7], in plasma by high performance liquid chromatography with UV-detection (HPLC/UV)[8]. A method has also been reported for the determination of cocaine and ketamine by high performance liquid chromatography (HPLC)[9].

Instrumental techniques are fast, reliable but at the same time expensive and normally not used for screening purpose, needs trained person to handle. A simple analytical method for rapid screening of drugs is needed for use in areas that do not have fully equipped laboratories but the method should fulfill some criteria i.e. must be inexpensive, require limited facilities, minimum training and capable of quickly analyzing the drugs. Among various analytical techniques thin layer chromatography is such a method which satisfies these requirements. Thin layer chromatography is a simple, cost effective, fast, maintenance-free, reliable and uses limited amount of solvents for the screening of drugs.

The present work highlights a simple TLC method for the determination of ketamine and its application in orange juice after a simple solvent extraction technique. Solvent system used is environment friendly, non toxic and safe for human health. The main advantage is the low-volatility of this solvent system and in line with green chemistry which is an eco-friendly and an alternative to conventional chemistry practices.

Results and discussion

Optimization of mobile phase :

In order to establish the mobile phase that gives best result for the separation of ketamine, according to elutropic series of solvents, the used mobile phase was varied from non polar to polar in different combinations.

Table 2 show that most satisfactory result was obtained using solvent system I water : butanol (40 : 60 v/v) (Fig. 1). When the combination of water : methanol (40 : 60 v/v) and water : propanol (40 : 60 v/v) were used the spots of ketamine was highly retained on stationary phase. Using solvent system IV propanol : butanol (50 : 50 v/v), V propano : methanol (50 : 50 v/v) and VI butanol : methanol (50 : 50 v/v) the spots were totally solvated in the mobile phase and moves with solvent front. In solvent system VIII water : butanol (80 : 20 v/v) tailing was observed. Using solvent system VII water : bu-

Table 2

Sl. no.	Solvent system	Ratio of solvent used	R_f
I	Water : butanol	40 : 60	0.68
II	Water : methanol	40 : 60	0.35
III	Water : propanol	40 : 60	0.20
IV	Propanol : butanol	50 : 50	0.88
V	Propanol : methanol	50 : 50	0.91
VI	Butanol : methanol	50 : 50	0.84
VII	Water : butanol	20 : 80	0.31
VIII	Water : butanol	80 : 20	0.92
IX	Water : butanol	45 : 55	0.78
X	Water : butanol	35 : 65	0.54

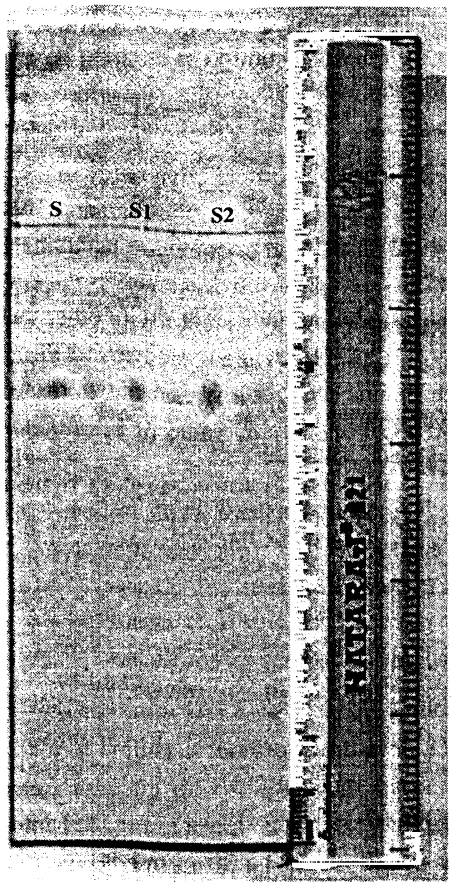


Fig. 1. Thin layer chromatography of ketamine : S = pure ketamine, S1 = real orange juice sample containing ketamine and S2 = spiked orange juice sample containing ketamine.

tanol (20 : 80 v/v), LX water : butanol (45 : 55 v/v) and X water : butanol (35 : 65 v/v) did not give any satisfactory result.

Finally water : butanol (40 : 60) was selected as the optimum mobile phase for the identification of the compound. Using the above mobile phase the R_f for the ketamine was 0.68.

Selection of localization reagent :

Three localizing reagent was used for the development of the spot i.e. mandelin's reagent, marquis reagent and dragendorff reagent. Mandelin's reagent and marquis reagent did not give any result. Finally dragendorff reagent was used. Using dragendorff reagent orange spot was obtained, where as with the same reagent blank spot (orange juice without ketamine) did not give any result.

Limit of detection, repeatability and reproducibility :

For Limit of detection (L.O.D), different concentration from 2 μg/ml to 500 μg/ml of ketamine were prepared and sprayed with dragendorff reagent. The concentration which could be visualized either under UV visualization chamber or using dragendorff reagent was 30 μg/ml.

For repeatability and reproducibility study the spots of three different concentrations of ketamine i.e. 50 μg/ml, 100 μg/ml, 150 μg/ml were developed using the optimum mobile phase and dragendorff as localizing reagent. The experiment were carried out on inter and intraday and inter laboratory basis spread over a period of two month.

For all the experiment carried out the result were almost similar. Hence it could be concluded the method developed is stable.

Experimental

Materials and methods :

Pre-coated 0.25 μm silica gel-G 20 × 20 cm TLC plate was purchased from E. Merck (India) Limited, Mumbai, India. The plates were developed in horizontal chamber ASGI Industries, Agra, India. Sprayer was of ASGI Industries, Agra, India. UV Chamber was of Ideal Scientific Concern, Kolkata, India. Pure sample of ketamine was kindly gifted by Themis Medicare Ltd., Mumbai, India. Orange juice sample containing unknown drug send for analysis to State Forensic Science Laboratory was kindly gifted by them for research purpose and also prepared spiked orange juice sample was based on the methodology adopted by culprit. Analytical grade chemicals and reagents i.e. acetone, ethyl acetate, formaldehyde, potassium iodide, ammonium vanadate, sulphuric acid, bismuth sub nitrate, ammonia, phosphoric acid and water (HPLC grade), were from Qualigens Fine Chemicals, Mumbai, India. Methanol and chloroform were from Merck, Mumbai, India. Propanol and butanol were from Rankem (Ranbaxy), New Delhi, India. Digital balance was procured from A&D Co. Limited, Japan.

Preparation of spray reagent :

Dragendorff reagent : The dragendorff spraying reagent was prepared as mentioned below[10] :

Solution (A) : 2 g of basic bismuth sub nitrate and 25 ml of glacial acetic acid in 100 ml water. Solution (B) : 40 g of potassium iodide in 100 ml of water. Mix. 10 ml of solution (A) and 10 ml of solution (B) and 20 ml of glacial acetic acid followed by 100 ml of water.

Mandelin's reagent[10] is prepared by dissolve 0.5 g of ammonium vanadate in 1.5 mL of water and by adding 100 mL with 98% sulfuric acid.

Marquis reagent[10] is prepared by mixing 1 part of 38% formaldehyde solution with 9 part of 98% sulfuric acid (v/v).

Sample preparation[10] :

10 ml of orange juice sample (real and spiked) was taken and the pH was adjusted to 3 by adding phosphoric acid, followed by extraction with 30 ml of ether twice. Then the 60 ml of ether extract was washed with 5 ml of water and the washing was added to the aqueous solution which was retained for further extraction. To the aqueous solution dilute ammonia was added to adjusting the pH to 8 and was extracted twice with 10 ml of chloroform. The chloroform extract was washed with water. The extract was filtered and evaporates to dryness.

TLC procedure :

Standard solution, extracted, real and spiked orange juice of ketamine was spotted on TLC plate using a micropipette. The spot was plotted 1.5 cm from the edge of the plate. Eleven solvent systems were taken in to consideration based on the elutropic series of solvent and property of analyte (Table 2). The chromatographic chamber containing solvent systems was saturated for 30 min. Solvent front was marked at 10 cm from the spot and the spotted plate was developed in ascending way. After the migration of solvent up to the solvent front the TLC plates were taken out from chromatographic chamber and were air dried for about 15 min. The TLC plate was first viewed in an ultraviolet chamber and then the spots were developed by spraying chemical visualizing reagent. For the development of ketamine mandelin's reagent, marquis reagent and dragendorff reagent were used.

Application :

The developed method was successfully applied, for the analysis of ketamine in different foodstuff and drink like milk, tea, Indian sweet (ladoo), fruits etc. as the method is reliable, involves minimum solvent extraction and less expertise is required so it could be a method of choice for screening purpose.

Conclusion :

A rapid sensitive cost effective ready to use screening method was developed for the determination of most frequently used stupefacient compound ketamine using thin layer chromatography with dragendorff reagent as localizing agent, shows no interference with other dupingdrugs like lidocaine, diazepam and phenobarbital (Table 3). The optimum mobile phase was found out to be water : butanol (40 : 60) and the R_f value of ketamine was 0.68.

Table 3. Visualization of ketamine and other related stupefying drug with dragendorff's reagent

Name of drug	Colour of the spot	R_f
Ketamine (General anesthetic)	Orange	0.68
Lidocaine (Local anesthetic)	Orange	0.93
Diazepam (Tranquillizer)	Orange	0.93
Phenobarbitone (Depressant)	No response	No response

Using the developed method, ketamine in juice resolved and identified successfully with good repeatability and reproducibility. Result is shown in Fig. 1. Limit of detection was found out to be 30 μg/ml for ketamine. This method can be useful for monitoring ketamine in the various fields of investigation : first and foremost the method will be very useful for hospital toxicology management unit, which generally treats victim of this drug. It will be useful for sanitation department of railways which test foodstuff supplied at railway station. It could also be helpful in the quality control of various beverages (alcoholic and non-alcoholic), foodstuffs, and milk products etc. The developed method could be used as a screening technique before proceeding for sophisticated instrumental technique for confirmation.

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