Recovery of Ketamine from Ribena using Liquid-Liquid Extraction Followed by Gas Chromatography Techniques

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ABSTRACT: Raves and night clubs are always associated with recreational drugs, which are also referred as 'club drugs'. Dissolving illegal drugs, such as ketamine, into liquid forms to be disguised as bottled soft drinks is one of the current drug concealment methods. This study is aimed to develop a method for detection and quantitation of ketamine contained in soft drink. Recovery efficiency, in term of precision and accuracy, of liquid-liquid extraction (LLE) method used to extract ketamine from the soft drink 'Ribena' was carried out. Gas Chromatography–Mass Spectrometry (GC-MS) analysis was used to confirm the presence of ketamine in the extracts, followed by Gas Chromatography–Flame Ionisation Detector (GC-FID) analysis to quantify the amount of ketamine. Repeated extraction on same sample produced consistent recovery results (RSD = 6.77%). In spiked samples, the average recovery percentage of ketamine was found to be 87.27 ± 5.72 %. In conclusion, the analytical procedures of LLE, coupled with GC-MS and GC-FID in recovery study were found to be useful to extract, detect and quantify ketamine in drug-laced soft drink.

Keywords: Recovery study, recreational drugs, liquid-liquid extraction

Introduction

Drugs remain as the global threat to health and development, including Malaysia. According to the United Nation Office of Drugs and Crime (UNODC), the number of illicit drug users was estimated to be 246 million people, at about 5.2% of the world population in 2013 [1]. It was observed that the prevalence of drugs users remained stable for past three years [1]. However, the increase of world population reflected the continuous increasing trend of the number of drug users [1]. The increased demand of illegal narcotic drugs has triggered the drug trafficking and smuggling businesses to meet continuous demand as evident by frequent news reported on drug trafficking and smuggling activities [2]. This indicates the number drug trafficking and smuggling that went through the security system could also be increased.

The fastest growing classes of recreational drugs, which was referred as 'club drugs', have been associated with the all-night parties, including raves and night clubs [3]. Substances that grouped in the classification of club drugs include 3,4-methylenedioxymethamphetamine (MDMA or ecstasy), gamma-hydroxybutyrate (GHB), ketamine, Rohypnol®, D-lysergic acid diethylamide (LSD) and methamphetamine [4]. These drugs have been used for personal excitement or being spiked in drinks. Among these drugs, ketamine (Fig. 1) are particularly common in night parties, and therefore have been identified for this study.

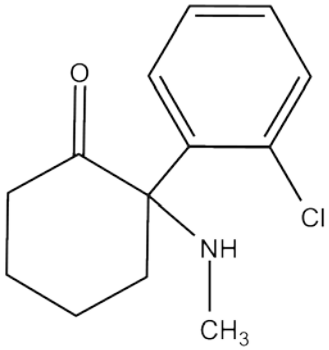

Fig. 1: Chemical Structure of Ketamine [5]

Drugs are often transported into targeted area or country through shipment hidden in food cans, exported coffee bags, prepared food such as pickles, fruits, and frozen food such as fish or shrimp [6]. These food items were used as medium to conceal drugs to avoid detection. For ketamine, dissolving drugs into liquid

forms in bottled alcohol or soft drinks are the upcoming trend [7-9]. The chieftain was hardly detected due to involvement of many people and stages in smuggling [6]. Very often, drug products were seized by authorities but the responsible people for the crime often escape except the packers [6].

It was observed that the bottled soft drinks laced with drugs such as ketamine had become the current trend of drug smuggling in Malaysia [6-9]. Hence a recovery study need to be done to develop a suitable method in order to detect and quantify the amount of specific drugs contained in the soft drinks at the Department of Chemistry Malaysia for the increase of such samples submitted for analysis. The present study is aimed to evaluate the recovery efficiency of liquid-liquid extraction (LLE) of ketamine from 'Ribena'. Chloroform was used in LLE to extract the ketamine from 'Ribena', Gas chromatography-mass spectrometry (GC-MS) was utilised to confirm the presence of ketamine in the extracts, followed by Gas Chromatography-Flame Ionisation Detector (GC-FID), to quantify the amount of exracted ketamine.

Material and Methods

Chemical and Reagents

All solvents used were of analytical grade and HPLC grade. Ketamine (95.32%, as hydrochloride salt) and Nortriptyline (≥98%, as hydrochloride salt) were obtained from Sigma Aldrich. Chloroform (HPLC grade, in liquid form), sodium hydroxide (Analytical grade, in crystal form) and sodium sulphate anhydrous (Analytical grade, in powder form) were obtained from Merck (Whitehouse Station).

Ketamine Standard Solution

Ketamine standard solution was prepared by dissolving 52.49 g of ketamine HCl in 50 mL of distilled water (1.0 mg/mL).

Internal Standard Solution

Internal standard solution was prepared by dissolving 1.50 g Nortriptyline HCl in 2.5 litre of chloroform (0.6 mg/mL chloroform).

Sodium Hydroxide Solution

Sodium hydroxide (NaOH) solution used for pH adjustment of soft drink samples was prepared by dissolving 40.0 g NaOH crystals in one litre of distilled water.

Samples

Soft drink composite sample was obtained from Department of Chemistry Malaysia. The process of sampling from the seized evidences, followed by labelling were carried by the chemists. The sample was blackcurrant juice, which was 'Ribena' was clearly labelled and suspected to contain ketamine.

To determine precision of repeated extraction on same samples, the extraction was performed repeatedly on the samples to study the consistency of the amount of desired drugs extracted from the soft drink sample. For every extraction, 25 mL of case sample was used.

For the recovery study using spiked samples, three solutions were prepared from the case sample in 25 mL volumetric flask: sample blank solution, low-level spiked solution (adding 0.1 mg/mL of ketamine into the sample blank solution), and high-level spiked solution (adding 0.3 mg/mL of ketamine into the sample blank solution).

Liquid-Liquid Extraction

All the samples prepared in the previous section were subjected to LLE. The prepared aliquotes were diluted in 1:1 ratio using distilled water. The pH value of all mixture (average pH 2-3) was adjusted to alkaline (~pH 11) using 1.0 M NaOH solution. It was then poured into separating funnel together with 25 mL of chloroform. After shaken vigorously for 2 to 3 mins, the bottom layer (chloroform layer) was eluted and filtered through sodium sulphate anhydrous into an evaporating dish. The extract was then heated to dryness and reconstituted using internal standard solution and poured into 10 mL volumetric flask.

GC-MS Analysis

GC-MS analysis was performed with Agilent GCMS 6890N GC equipped with an Agilent HP-5 (5% Phenyl Methyl Siloxane) capillary column (30 m length, 250 µm i.d., 0.25 µm film thickness). The injector temperature and

the interface was set at 250°C and 280°C, respectively. Oven temperature was held at 80°C for 1 min and then rose to 310°C at a rate of 10°C/min, and held for 5 min. Helium was used as the carrier gas. The 5975B MS detector operates in ionisation mode at ionisation energy of 70 eV, with scan range of 40 to 450 m/z. Around 1 µL of extract was injected into a GC vial for analysis.

GC-FID Analysis

GC-FID analysis was performed by an Agilent 5890N GC system equipped with a HP-5 (5% Phenyl Methyl Siloxane) capillary column (30 m length, 250 µm i.d., 0.25 µm film thickness). The carrier gas used was helium. The flow rate of carrier gas was set at 1.0 mL/min. The injection and detector temperature were kept at 250°C and 280°C, respectively. The column temperature was initially programmed at 200°C for 6.5 min and followed by a ramp of 90°C/min to 270°C and hold for 4.5 min. The volume injected for each extract was 1 µL. The concentration of ketamine in the extracts were quantified based on the standard calibration curve of ketamine with the equation:

PeakArea = 278.3 (ketamine concentration) +2.3328

Precision of Repeated Extraction on Same Sample

To study the precision of analyte recovery, the mean, standard deviation (SD), and relative standard deviation (RSD) were calculated. The GC-FID reading for each sample was considered as precise if its RSD value was below 10%.

Recovery Study on Spiked Sample

To study the accuracy of extraction method, the recovery percentage of ketamine was calculated for both low- and high-spiked sample solutions using mean values of ten injections for each sample.

Result and Discussion

GC-MS Analysis

From GC-MS analysis, the extract from the sample was found to contain ketamine and nortriptyline (the internal standard) (Fig. 2). Ketamine was eluted at 4.754 min. Fig. 3 shows the mass spectrum of ketamine. Fragment at m/z 180 was the base peak, followed by 209. The molecular ion was observed at m/z 237.

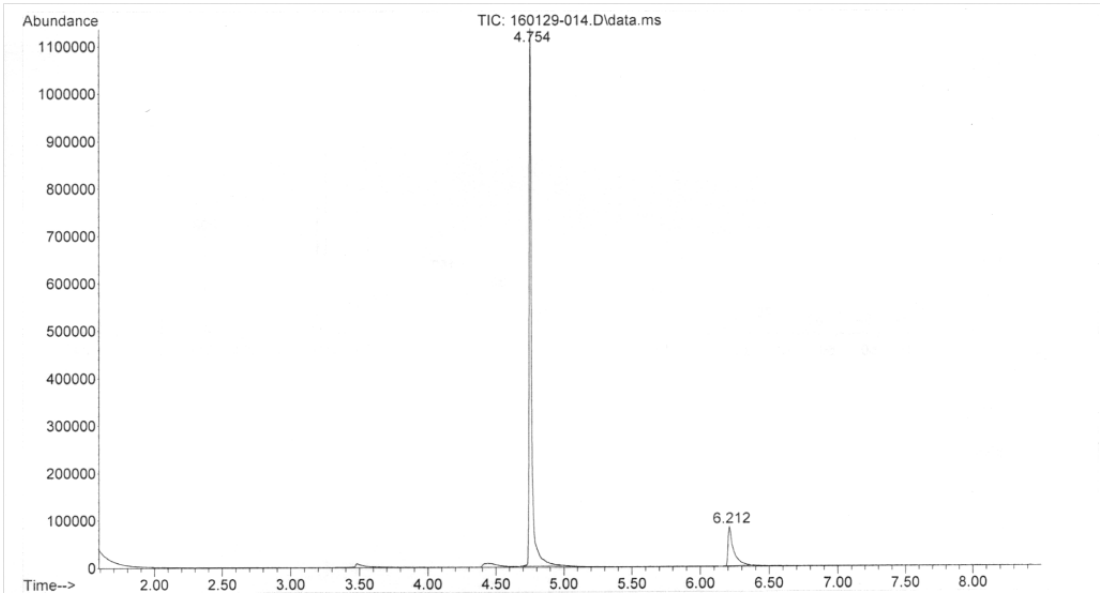

Fig. 2: GC-MS Chromatogram of sample A1

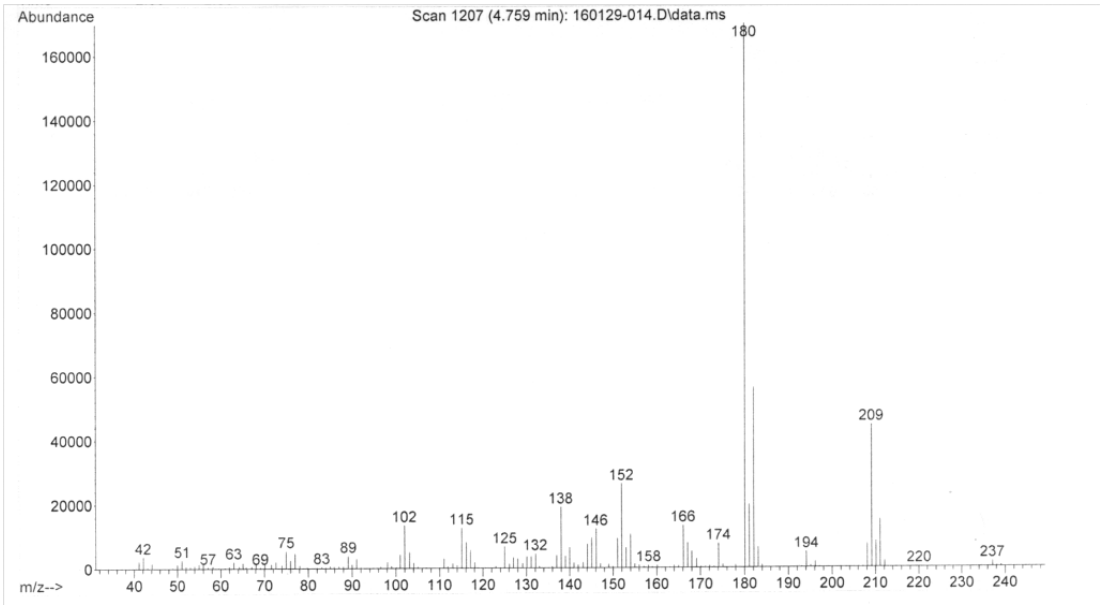

Fig. 3: Mass Spectrum of ketamine in sample A1

Fig. 4 shows the proposed fragmentation of ketamine. The initial ionization at nitrogen results in α-cleavage of the fragile bond and stabilisation of the positive charge on nitrogen [10]. Loss of carbon monoxide to produce m/z 209 can occur with two ways [10]. Firstly, with relative ease, the ring closure occurs to form a radical ion with similar stability as the molecular ion, which the radical site is moved nearer to nitrogen (pathway a). Secondly, the generation of a primary radical site, which lead to further fragmentation (pathway b). Subsequent losses of methyl (pathway c) and ethyl radicals (pathway d) lead to the ions at m/z 194 and 180, respectively. Loss of larger radical, which is ethyl radical is more preferred due to the higher stability of both radical and resultant ion products [10].

GC-FID Analysis

Precision of Repeated Extraction on Same Sample

For GC-FID analysis, the precision results of repeated extractions were tabulated in Table 1, showing the values of mean, SD and RSD. Hence, the repeated extraction of ketamine from Ribena sample was found to be precise, with RSD = 6.77%.

Table 1: GC-FID readings for repeated extractions of all samples

Sample	Mean (mg/mL)	SD (mg/mL)	%SD
A1	1.8518	0.1253	6.77%

Accuracy in Extraction on Spiked Samples

For recovery study using spiked samples, the calculations of recovery percentage for each level of spiked concentration (low and high) with respect to the unspiked sample concentration was calculated (Table 2). The average recovery percentage was reported at 87.27 ± 5.72 %.

The unspiked sample concentration (**A**) was the ketamine concentration that extracted from original case sample. The calculated spiked concentration (**B**) was the resultant ketamine concentration that will be spiked into the unspiked samples, which expected concentration (**C**) can be calculated by simple addition of **A** and **B**. Spiked concentration (**D**) was the ketamine concentration extracted from the spiked samples. The recovery percentage (**E**) was calculated using the following formula:

$$\text{Recovery (\%)} = \frac{\text{Spike concentration, D}}{\text{Expected Concentration, C}} \times 100\%$$

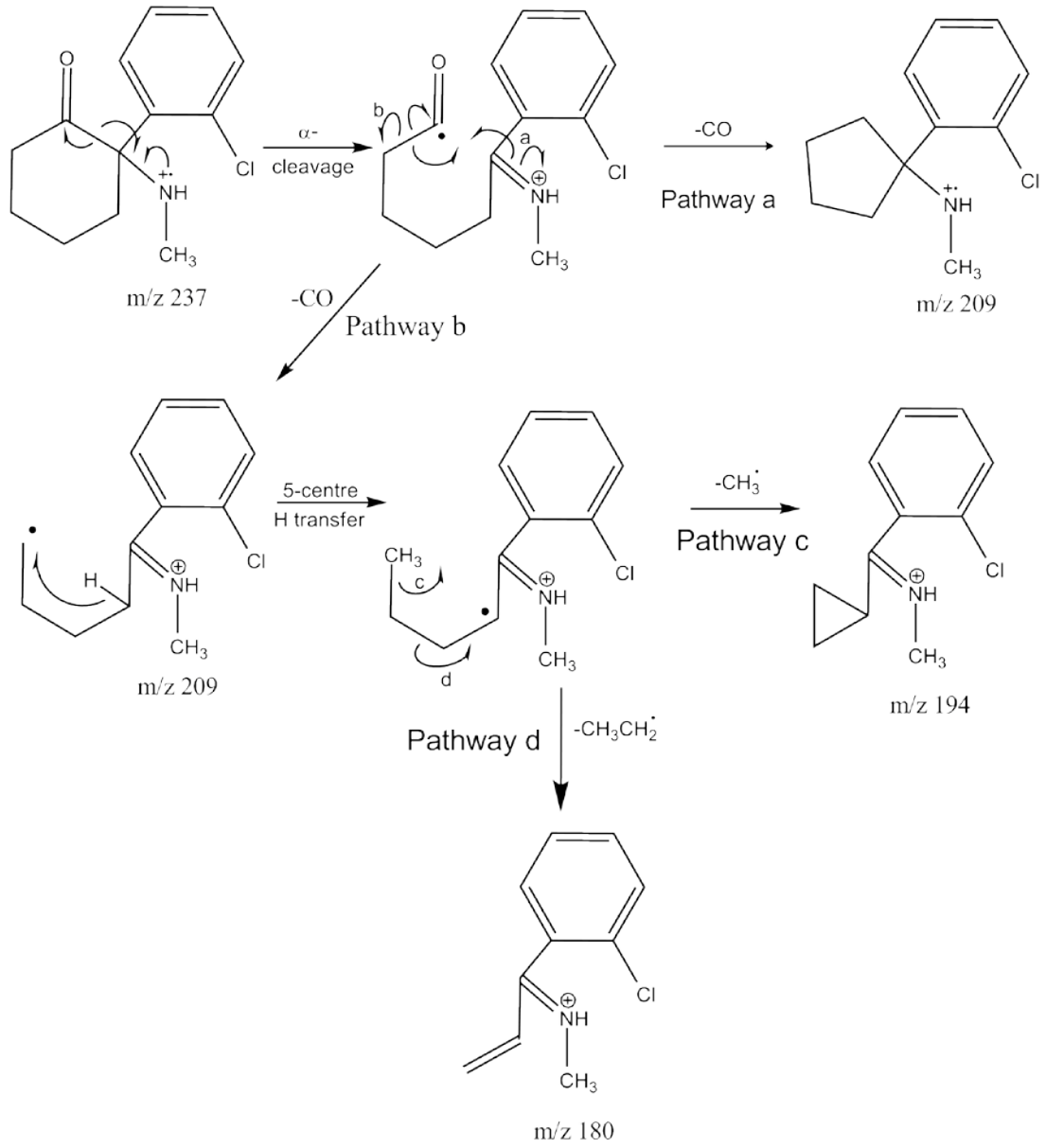

Figure 4: Proposed fragmentation of ketamine [10].

Table 2: Calculations of recovery percentage for low-level and high-level spiked sample

Level	Unspiked Concentration (mg/mL)	Calculated Spiked Concentration (mg/mL)	Expected Concentration (mg/mL)	Spiked Concentration (mg/mL)	Recovery (%)
	A	B	C=A+B	D	E=(D/C)*100
Low	0.039062	0.100067	0.139129	0.115785	83.22
High	0.039062	0.300201	0.339263	0.309789	91.31
				Mean	**87.27**
				SD	**±5.72**

The ketamine concentrations of unspiked and spiked samples were the average GC-FID readings of 10 repeated injections for each sample.

The method, which is called matrix spiking [11], is used to evaluate the performance of an analytical procedure when testing a specific sample (matrix) type. In the study, the performance of LLE on 'Ribena' as matrix was evaluated. The good recovery percentages for both low- and high-spiked samples increased the confidence in the accuracy and validity of the LLE technique.

Conclusion

LLE was useful for extracting drugs such as ketamine from soft drink matrices. GC-FID analysis showed that the extraction method was found to be precise and accurate for ketamine. Together with other possible factors, such as solvents, matrix effects, pH *etc*, more studies need to be done to ensure a more efficient LLE method, and to make quantitation more precise and accurate.

The present study provides useful information on the recovery efficiencies of LLE performed on 'Ribena'as matrix. This gives the direction of future studies to develop more reliable techniques in quantitation of dangerous drugs in the soft drinks. In forensic settings, the accurate quantitation can assist in estimating the actual amount of drugs in the seized evidences, particularly in concealed soft drinks, which can assist in the prosecution of the guilt under the Dangerous Drugs Act 1952 in Malaysia.

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