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Case Report

Analysis of Ketamine, a Rave Drug in Pakistan, using Gas Chromatography Coupled with Mass Spectrometer and Flame Ionization Detector: A Case Study

تحليل الكيتامين، عقار الحفلات في باكستان، باستخدام كروماتوغرافيا الغاز المقترنة بمطياف الكتلة وكاشف التأين باللهب: دراسة حالة

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Abstract

Ketamine is an arylcycloalkylamine, classified as cyclidine and chemically related to phencyclidine (PCP). Ketamine can be identified using modified Scott's Test and Alkaline Gold Bromide test. This case study involved the analysis of a Ketamine sample.

The sample was analyzed qualitatively by chemical spot tests, FT-IR and GC-MS without derivatization. Furthermore, a developed and validated method was used for the quantitative analysis of Ketamine using Gas Chromatography with a Flame Ionization Detector (FID).

The certified reference standard of Ketamine in the range of 10-100µg/mL was used for developing linear correlation with regression coefficient ($R^2 = 0.9997$) for the method. The method produced percentage of sample as 90.27%.

The above mentioned techniques and methods provide comparable qualitative and quantitative analytical results helping law enforcement agencies and the forensic community in screening and quantification of ketamine using GCMS coupled with FID.

Keywords: *Forensic Sciences, Ketamine Analysis, GCMS, FTIR, Narcotic/Illicit Drug Abuse, FID*

المستخلص

الكيتامين هو arylcycloalkylamine، يُصنف على أنه cyclidine ويرتبط كيميائياً بـ phencyclidine (PCP). ويمكن تحديد الكيتامين Ketamine باستخدام اختبار Scott واختبار Alkaline Gold Bromide المعدّل.

واشتملت دراسة الحالة هذه على تحليل عينة الكيتامين الواردة إلى المختبر حيث تم تحليل العينة نوعياً عن طريق اختبارات البقع الكيميائية، وطريقة FT-IR وطريقة GC-MS دون اشتقاق. وعلاوة على ذلك، استخدمت طريقة مطورة وتم التحقق من صلاحيتها للتحليل الكمي للكيتامين باستخدام كروماتوغرافيا الغاز المقترنة مع كاشف تأين اللهب. وأجريت الدراسة ضمن المجال المعتمد من الكيتامين في نطاق ١٠-١٠٠ ميكروجرام / مل مع خطية لمنحنى المعايرة بقيمة (R2 = ٠,٩٩٩٧). وكانت نسبة استرداد الاستخلاص ٩٠,٢٧٪.

ونلاحظ من خلال الدراسة أن التقنيات والأساليب المذكورة أعلاه توفر نتائج تحليلية نوعية وكمية مناسبة تساعد وكالات وأجهزة إنفاذ القانون ومجتمع الأدلة الجنائية على الفحص وتحديد الكمي لمادة الكيتامين.

الكلمات المفتاحية: علوم الأدلة الجنائية، تحليل الكيتامين، *GCMS, FTIR, FID*، تعاطي العقاقير/المخدرات غير المشروعة.

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1. Introduction

Ketamine is 2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone [1]. It exerts activity at multiple sites in the brain, primarily acting on the NMDA-receptor by antagonizing it. There are various street names for Ketamine such as K, K-Hole, Kate and Special K etc. Ketamine and other drugs like alcohol, gamma-hydroxy butyric acid (GHB) and flunitrazepam are regarded as date rape drugs causing disorientation and loss of consciousness [2]. The nasal route (such as snorting and inhaling) is the most common route of abuse beside tablets at raves [3]. The effects of Ketamine intoxication range from pleasant dreams to intense visual hallucinations [4]. Ketamine is responsible for hallucination and delirium which is the primary reason for its abuse [5]. At higher doses, it causes elevated blood pressure and psychosis. It is also abused orally and can also be injected [6]. Ketamine can be identified using modified Scott's Test and Alkaline Gold Bromide Test producing a deep purple to blackish purple color. The later one is regarded as a highly specific test for Ketamine [7]. Ketamine is a phencyclidine class of molecules and has been primarily used as a veterinary anesthetic. It is categorized as essential medicine by the World Health Organization [8]. Since 1961 after discovering its role as a drug of abuse, it has not been used in humans, except for a few short term surgical procedures. It is scheduled as a controlled substance in 70 out of 100 countries, due to its increasing use in the illegal drug market. However, it is available over the counter in Pakistan and is used as a dissociative anesthetic for animals. During recent times, it has become one of the uncommon abused recreational drugs in Pakistan and has caused deaths due to overdose throughout the world. Ketamine is categorized as an NPS (new psychoactive substance) according to the UNODC World Drug Report 2017. The NPS includes those drugs which are not controlled by international drug control convention but may cause serious public health issues. Other drugs in this class include tryptamines, synthetic cathinones, synthetic cannabinoids, plant based substances, piperazines, phenethylamines, phencyclidines substances and aminoindanes.

Various drug detection methods have been developed using various techniques such as UV-Visible Spectrophotometry, GCMS, and GC-FID, etc. Hollow fiber liquid phase micro extraction combined with GC-FID has been used to quantify drugs like amphetamines, caffeine, along with Ketamine in Urine [9]. The method developed in this study was found to be effective and concise for simultaneous determination of a given range of illegal drugs. The proposed method was found to be quite simple for the quantification of Ketamine samples as far as extractions and sample preparation were considered.

2. Materials and Methods

2.1 Chemicals

Methanol and Chloroform were purchased from Sigma Aldrich USA. Ketamine HCl (99.91%) was purchased from Cerilliant Corporation, USA. Distilled water was used for extraction.

2.2 Sample Preparation

2.2.1. Qualitative analysis using GC-MS

For qualitative confirmation of the sample using GC-MS, a solid sample of about 1-2 mg was transferred to a

Agilent GC-MS vial and about 1 mL of methanol was added. The aqueous sample was dried at room temperature and residue was analyzed for confirmation.

2.2.2. Quantification using GC-FID

2.2.2.1. Calibrators

Stock solution of Ketamine (100 µg/mL) was prepared in a 10 mL volumetric flask. Six calibrators of various strengths as 10, 20, 40, 60, 80 and 100 µg/mL were prepared by transferring respective volumes of stock solution into a 10 mL volumetric flask. The flask was filled to 10 mL with methanol to get desired concentrations.

2.2.2.2. Quality control

Two quality control of 30 µg/mL and 70 µg/mL each were prepared from different stock (100 µg/mL) by accurately transferring 300 µL and 700 µL of stock separately in a 10 mL volumetric flask and making volume with the same solvent.

2.2.2.3. Case sample preparation

1 mg of dried sample was transferred in a 10 mL volumetric flask containing 2-3 mL of Methanol. The remaining volume was made up by adding methanol to get a stock solution of 100 µg/mL. Five mL of stock sample was diluted to 10 mL in a volumetric flask to get a 50µg/mL solution.

2.3 Spot Tests

The dried sample was preliminarily tested by spot test according to SWGDRUG guidelines for the presence of any narcotic substance with Mecke, Marquis, Modified Cobalt Thiocyanate, Ehrlich and Lieberman [10]. Preliminary color tests/spot tests are usually performed on a dried sample for the presence or absence of controlled substances as initial detection. The respective reagent was dropped in three wells (labeled as sample, standard and blank) of a clean dry spot testing plate. A small amount of sample was placed in the labeled sample well along with a small amount of Standard Ketamine. Modified cobalt thiocyanate reagent gave a specific dark purple precipitate/color for the presence of ketamine.

2.4 Instrument Conditions

2.4.1. GC-MS analysis

Agilent Gas Chromatograph 7890A in Scan Mode fitted with a split/split-less injector and an ALS (auto-liquid sampler) 7693 and 5975C Triple Axis Detector MSD system was used for GC-MS analysis. An Agilent DB-5MS Ultra Inert column (20m x 180µm x 0.18 µm) was used for the deatection of ketamine using 1µL injection volume, split mode at 250 ^{0}C and 31.8 psi. Oven temperature rampage for GC was set to 150 ^{0}C for 1 min then 25 ^{0}C/min to 300 ^{0}C for 5 min up to 12 minutes. The column flow was set to 01 mL/min. The MS source (230 ^{0}C) and MS quad (150 ^{0}C) were used.

2.4.2. GC-FID quantitative analysis

An Agilent 7890B GC/5973 MSD system was used in this study applying validated GC method. A DB-5ms Capillary Column (30 m x 0.25 mm x 0.25 µm); Agilent 122-5532DB-5MS was used. GC oven temperature was programmed at 150 °C for 1 min then 25 °C/min to 300 °C for 3.5 min with a total run time of 10.5 minutes. A 2-µL aliquot was injected in split ratio of 50:1 with split flow 50

mL/min mode. The front injection port was set at 240 °C. The FID was operated with heater at 250 °C with Helium (99.999% pure) as carrier a gas. The data was evaluated by Chem Station using responses of samples and calculating regression coefficient with auto integration parameters.

2.5 FTIR Analysis

Thermo Scientific Nicolet iS10 smart iTR™ was used for initial confirmation of sample using FTIR-Spectroscopy (11). The sample was analyzed as such on FT-IR to obtain IR spectrum and confirmed the presence of Ketamine using representative wave numbers 1696, 747, 1142, 1120, 712, and 1027 cm-1 (Figure-1).

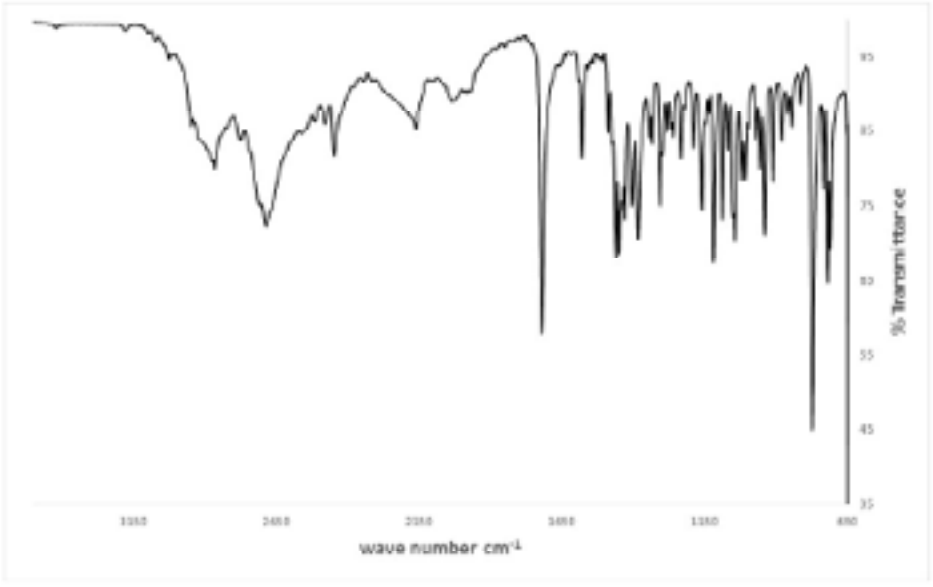

Figure 1- *FT-IR spectrum of ketamine sample.*

3. Case History

Custom officials apprehended a suspect who was travelling by international flight from Islamabad airport to Bangkok. The accused was suspected of carrying an unknown liquid in plastic bottles. He claimed it was Aab-e-Zam-Zam (The Holy Water) upon questioning. The officials used Field Testing Kits for presumptive testing and found it to contain Ketamine dissolved in water. The recovered liquid was submitted to the Narcotics Unit, Punjab Forensic Science Agency, Lahore for further confirmation and analysis.

The scope of this study includes identification, confirmation and quantitation of suspected controlled substances seized during trafficking by law enforcement agencies.

4. Results and Discussion

Modified Cobalt Thiocyanate reagent produced a particular dark purple precipitate/color of tested dried sample (dried at 60 ^{0}C). For confirmation, GC-MS in Scan mode along with Chem Station was used and the results showed a peak at the retention time 4.361 minutes and principle MS ions 180, 209, 182, 152, 181, 211, 138 (m/z), using AAFS reference library for Ketamine sample (Figure-2, 3) and by comparing with literature [12]. The GC-MS analysis of reference standard was also done to match the RT with the

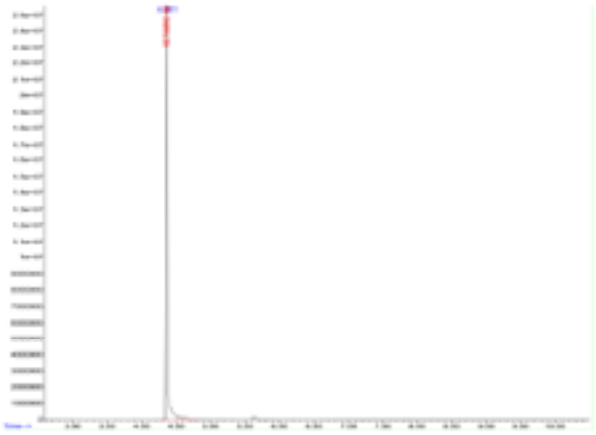

Figure 2- *Gas chromatogram of ketamine sample using scan mode (the peak at retention time 4.361 for ketamine). Note: retention time at X-axis and abundance at Y-axis.*

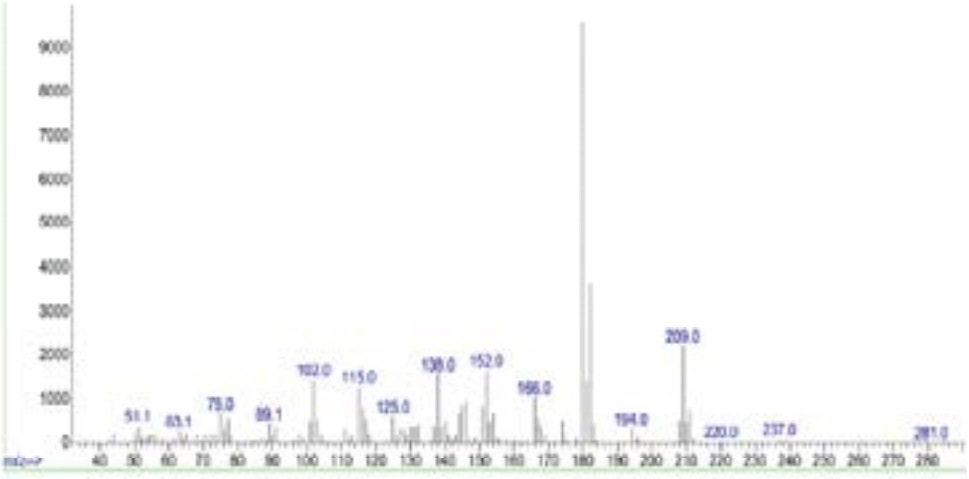

Figure 3- *Mass spectrum of ketamine sample using GC-MS using scan mode. Note: m/z at X-axis and abundance at Y-axis.*

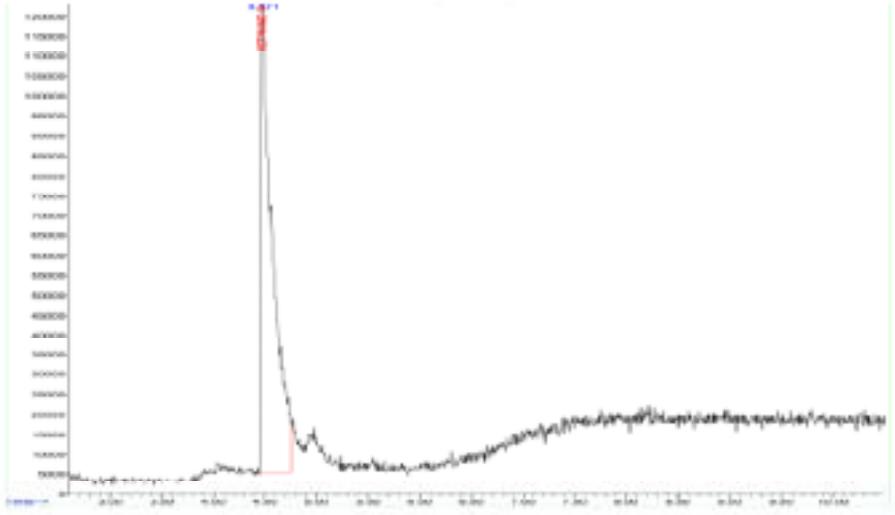

Figure 4- *Gas chromatogram of ketamine standard using scan mode (The Peak at Retention Time 4.47 for Ketamine). Note: Retention time at X-axis and Abundance at Y-axis.*

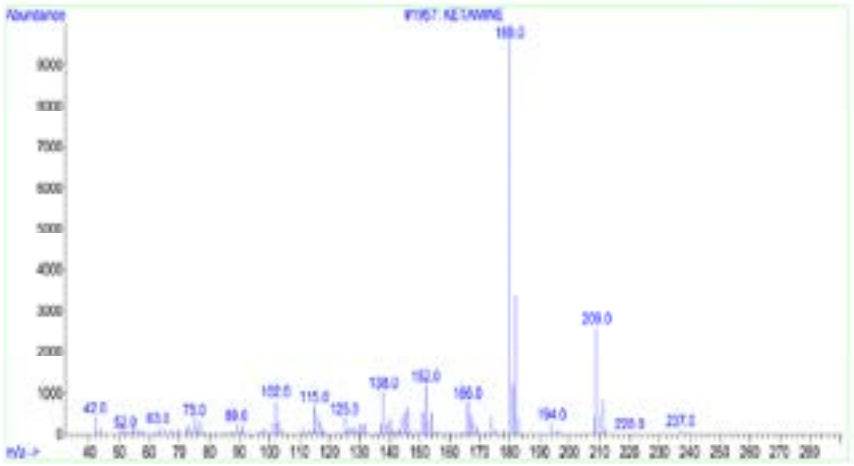

Figure 5- *Mass spectrum of ketamine standard using GC-MS using scan mode. Note: m/z at X-axis and Abundance at Y-axis.*

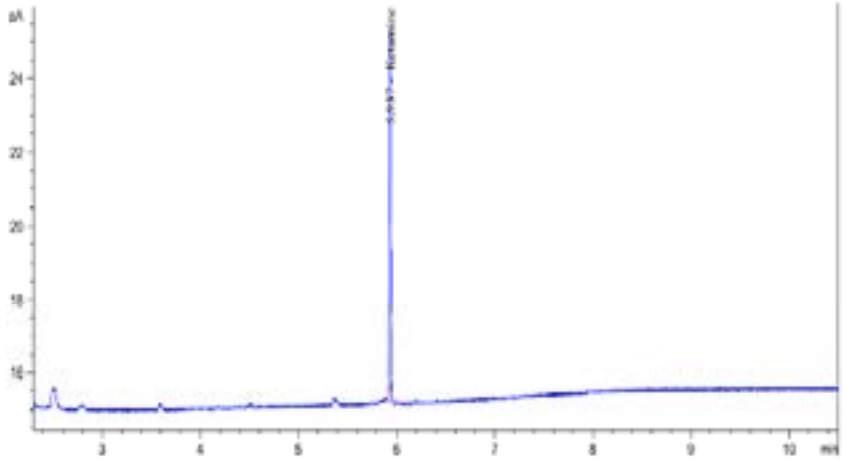

Figure 6- *Gas chromatogram of ketamine quality controls (30µg/mL) using FID (The peak at retention time 5.937 for ketamine). Note: Retention time at X-axis and Abundance at Y-axis.*

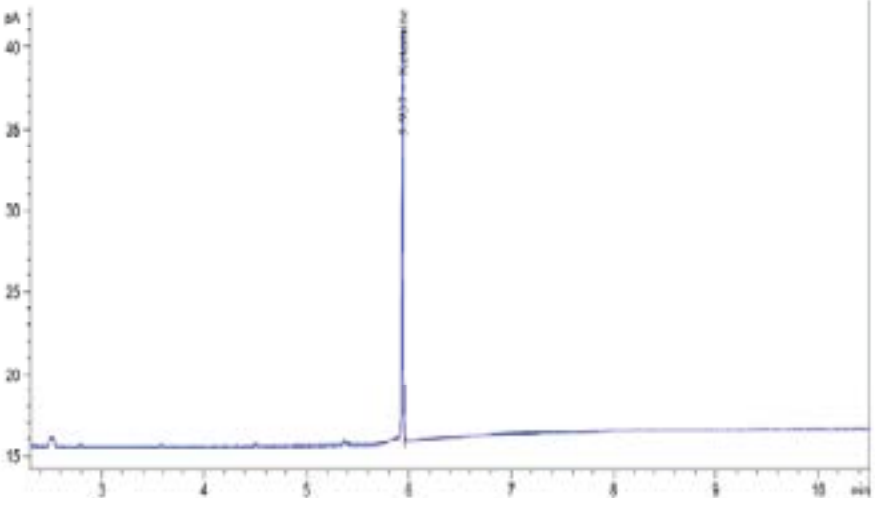

Figure 7- *Gas chromatogram of ketamine quality controls (70 ug/mL) using FID (The Peak at Retention Time 5.937 for Ketamine). Note: Retention time at X-axis and Abundance at Y-axis.*

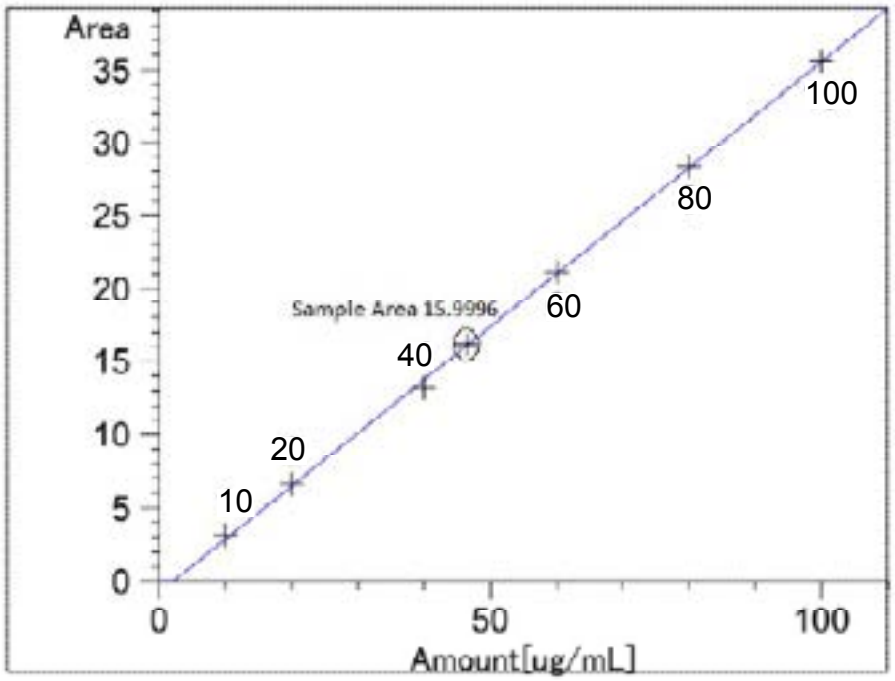

Figure 8- *Calibration curve for ketamine standard (Range 10 µg/mL-100 µg/mL) using GC-FID. .*

Table 1- *Peak areas of calibrators and quality control samples of ketamine standard at RT (5.937 min) using GC-FID.*

Sr #	Concentration (µg/mL)	Peak Area
1	10 (Calibrator-1)	3.2349
2	20 (Calibrator-2)	6.5987
3	40 (Calibrator-3)	13.1771
4	60 (Calibrator-4)	21.0632
5	80 (Calibrator-5)	28.3713
6	100 (Calibrator-6)	35.5545
7	30 (QC Level 1)	10.3453
8	70 (QC Level 2)	24.9328

sample (Figure-4, 5). The quantification of the sample was done on GC-FID using standard calibration in the range from 10 µg/mL to 100 µg/ml standard of Ketamine (Figure 8). Two quality controls (30 µg/mL and 70µg/mL) were prepared for the verification of the method that produced the acceptance criteria of <20% (Figure-6, 7). The calibrators and two quality controls were run in triplicate. The linearity of calibrators showed correlation R^2=0.9997 with equation of straight line as =3.63044e-1 (x)+(-7.65640e-1). The sample (prepared at a theoretical concentration of 50 µg/mL) showed Ketamine concentration of 45 µg/mL (90.27% of the theoretical value) by using regression coefficient and extrapolating the sample amount through it (Figure-8, Table-1). based upon our results and evaluation,

the proposed method proved to be quite simple, reliable and time saving using GC-FID for determining percentage of Ketamine in suspected illicit samples usually submitted in the form of powder, crystalline material or aqueous solution. This method can also be helpful for the Ketamine assay in veterinary context as the drug is commonly used in veterinary practice.

5. Conclusion

The results in this study showed that the proposed qualitative and quantitative method helps forensic drug analysts to determine the nature and percentage of suspected seized material for better understanding of drug profiling and impurity determination. Ketamine has not been a commonly abused drug around the world in the past, but its legal status and effects in recent times render it being a major component of an abusive drug. Its use in various parts of the world, including Pakistan, has markedly increased as a component of ecstasy type tablets in recent times. The analytical results in this study showed that GC-MS and GC-FID using the above method are quite effective and reliable for determining the qualitative and quantitative composition of suspected samples. Method validation, impurity profiling and individual excipient determination are also important future prospects of this study.

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Nill.

Conflict of Interest

None declared.

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