Naif Arab University for Security Sciences
Arab Journal of Forensic Sciences and Forensic Medicine
المجلة العربية لعلوم الأدلة الجنائية والطب الشرعي
https://journals.nauss.edu.sa/index.php/AJFSFM

Determination of Tramadol, Tapentadol, Venlafaxine, and Ketamine in Non-Biological Matrices: A GC-MS Study on Drug Stability and Environmental Impact in Forensic Paraphernalia

تحديد الترامادول، التابنتادول، الفينلافاكسين، والكيتامين في المصفوفات غير البيولوجية: دراسة باستخدام GC-MS حول استقرار الأدوية والتأثير البيئي في أدوات الأدلة الجنائية

Diksha Thakura[1], Neeti Prakash Dubey[2]*, Rajvinder Singh[3]
[1]Department of Forensic Science, University Grants Commission, New Delhi, India
[2]Regional Forensic Science Laboratory, Central Range, Mandi, India
[3]Department of Forensic Science, M. D. University, Rohtak, Haryana, India

Received 21 Dec. 2024; accepted 16 Feb. 2025; available online 16 Jun. 2025.

Abstract

An effective, simple, rapid, eco-friendly, and robust method for simultaneous determination of Tramadol, Tapentadol, Ketamine, and Venlafaxine in paraphernalia like metallic bottle cap and syringe using Gas Chromatography-Mass Spectrometry was developed and validated for the examination of case exhibits received in the forensic laboratories. The method was developed by creating variations in the Gas Chromatography operating conditions. The method was validated according to UNODC and ICH guidelines. Linearity using calibration plots, accuracy, precision, recovery, specificity, robustness, ruggedness, LOD, and LOQ were ascertained for all drugs and found in the recommended ranges by standard guidelines. Stability studies for the drugs in different environmental conditions were also presented in this research work. The validated method was successfully applied to real samples. The technique effectively identified all drugs selected in this study; in the presence of Codeine, Morphine, Dextromethorphan, Diacetylmorphine, and, 6-Monoacetylmorphine. The validated method is suggested for routine chemical analysis of exhibits in Forensic laboratories.

Keywords: Forensic sciences, drugs of abuse, method validation, tramadol, ketamine, tapentadol, venlafaxine, paraphernali.

المستخلص

تم تطوير طريقة فعالة، بسيطة، سريعة، صديقة للبيئة، وقوية لتحديد كمي متزامن للترامادول، التابنتادول، الكيتامين، والفينلافاكسين في أدوات تعاطي المخدرات مثل أغطية الزجاجات المعدنية والحقن باستخدام كروماتوغرافيا الغاز-مطياف الكتلة، وتم التحقق من صحة هذه الطريقة لفحص المعروضات المقدمة إلى المختبرات الجنائية. تم تطوير الطريقة عن طريق إحداث تغييرات في ظروف تشغيل كروماتوغرافيا الغاز. تم التحقق من صحة الطريقة وفقًا لإرشادات مكتب الأمم المتحدة المعني بالمخدرات والجريمة (UNODC) والمجلس الدولي لتوحيد المتطلبات الفنية للمستحضرات الصيدلانية للاستخدام البشري (ICH). تم التحقق من الاتساق الخطي للقياسات باستخدام منحنيات المعايرة، والدقة، والضبط، والاسترداد، والنوعية، والمتانة، والصلابة، وحد الكشف (LOD)، وحد الكمية (LOQ) لجميع الأدوية، ووجد أنها ضمن النطاقات الموصى بها في الإرشادات القياسية. تم أيضًا تقديم دراسات الاستقرار للأدوية في ظروف بيئية مختلفة في هذا العمل البحثي. تم تطبيق الطريقة التي تم التحقق من صحتها بنجاح على عينات حقيقية. حددت التقنية بفعالية جميع الأدوية المختارة في هذه الدراسة؛ في وجود الكودين، والمورفين، والديكستروميثورفان، وثنائي أسيتيل مورفين، و6 - أحادي أسيتيل مورفين. في الختام، يُقترح استخدام الطريقة التي تم التحقق من صحتها للتحليل الكيميائي الروتيني للمعروضات في المختبرات الجنائية.

الكلمات المفتاحية: علوم الأدلة الجنائية، إساءة استخدام العقاقير، التحقق من صحة الطريقة، ترامادول، كيتامين، تابنتادول، فينلافاكسين، أدوات تعاطي المخدرات.

* Corresponding Author: Neeti Prakash Dubey
Email: neetiprakash999@gmail.com

doi: 10.26735/XNLE5002

1. Introduction

In recent years, India has witnessed a concerning rise in the misuse of prescription drugs, particularly opioid analgesics and stimulants, which pose a significant public health risk. These drugs exhibit pharmacological effects akin to conventional narcotics or emerging psychotropic substances, making them a preferred choice for abuse. Their affordability, easy accessibility, and potent effects have led to widespread misuse [1].

Tramadol and Tapentadol are synthetic narcotic analgesics that act on the central nervous system to relieve mild to severe pain. They are among the most commonly prescribed drugs and are known for their potent pain-relieving properties. Both drugs carry a significant risk of addiction and abuse [2,3]. Venlafaxine, an antidepressant with recreational misuse potential, is another prescription drug of concern. It functions as a Serotonin and Norepinephrine Reuptake Inhibitor (SNRI), enhancing neurotransmitter levels in the brain and prolonging mood elevation in abusers [4]. Ketamine abuse is another global concern, with varying rates of misuse reported across different regions. As a phencyclidine derivative, Ketamine has dissociative, analgesic, and hallucinogenic effects, making it popular as a club drug and for date rape. Its misuse has been documented in academic journals and widely discussed on social media platforms. When abused, Ketamine can lead to physical and psychological dependence, with long-term use resulting in tolerance, increased addiction risk, and severe health issues [5].

The illicit trafficking and abuse of these drugs have become a global issue, exacerbated by the COVID-19 pandemic, which led to stricter surveillance on conventional drugs like Heroine, Morphine, Cocaine, etc., and a greater reliance on prescription drugs due to the reduced availability of other substances. In 2019, Tramadol was the second-most seized pharmaceutical opioid globally [6].

Forensic science laboratories have noted a trend of pharmaceutical opioids, such as Tramadol and Tapentadol, being frequently involved in cases of poly-drug abuse. These substances are commonly encountered in the form of tablets, capsules, and powders commonly detected in seized drug samples suspected to contain heroin and in paraphernalia recovered from crime scenes. Drug traffickers often intentionally add these drugs as adulterants to heroin to increase its volume and potency for greater financial gain. Identifying these drugs, especially when present in low concentrations or mixed with other substances, presents a significant challenge for forensic analysts during routine examinations.

Limited instruments have been studied for detecting the chemical constituents of illicit drugs. Nowadays, the most widely used techniques for estimating Tramadol alone or in combination forms are spectroscopic methods, reversed-phase high-performance liquid chromatography, and liquid chromatography-mass spectrometry [7-13]. Gas chromatography-mass spectrometry is another explored technique for detecting illicit drugs of abuse [14-16].

This study aimed to develop and validate a sensitive GC-MS-based analytical method for the simultaneous analysis of Tapentadol, Ketamine, Tramadol, and Venlafaxine in the presence of other drugs like Morphine, Codeine, Diacetylmorphine, 6-MAM (Mono acetyl morphine), and Dextromethorphan in non-biological matrices found at crime scenes. Additionally, the study aimed to evaluate the impact of environmental conditions on simulated paraphernalia. The combination of drugs investigated in this research, within the most commonly encountered paraphernalia under

different environmental conditions, has not been previously documented and is of significant forensic importance. The method was validated according to ICH and UNODC guidelines and tested on real samples of seized paraphernalia. The new method was flexible, fast, and cost-effective, making it suitable for forensic analysis.

2. Material and Methods

2.1. Drugs and chemicals

Certified Reference Material (CRM) for Tramadol Hydrochloride (w/w 0.997 mg/mg), Tapentadol Hydrochloride (w/w 0.998 mg/mg), Venlafaxine Hydrochloride, Trihexyphenidyl Hydrochloride (Internal Standard-IS), and Ketamine has been purchased from the Indian Pharmacopeia Commission, Sector-23 Raj Nagar, Ghaziabad, Uttar Pradesh. The chemical structure for these compounds is depicted in Fig. 1. Methanol (HPLC grade-Merk) was used for preparing standard stock solutions and was procured from MS RAM traders, India.

2.2. Preparation of Stock standard solution and working solution

Stock solutions of 100 μg/ml were prepared in methanol for all the drugs using CRM. Two working standard solutions 10 μg/ml, and 1 μg/ml were prepared by diluting the appropriate volume of the standard stock solution (100 μg/ml) of drugs with methanol.

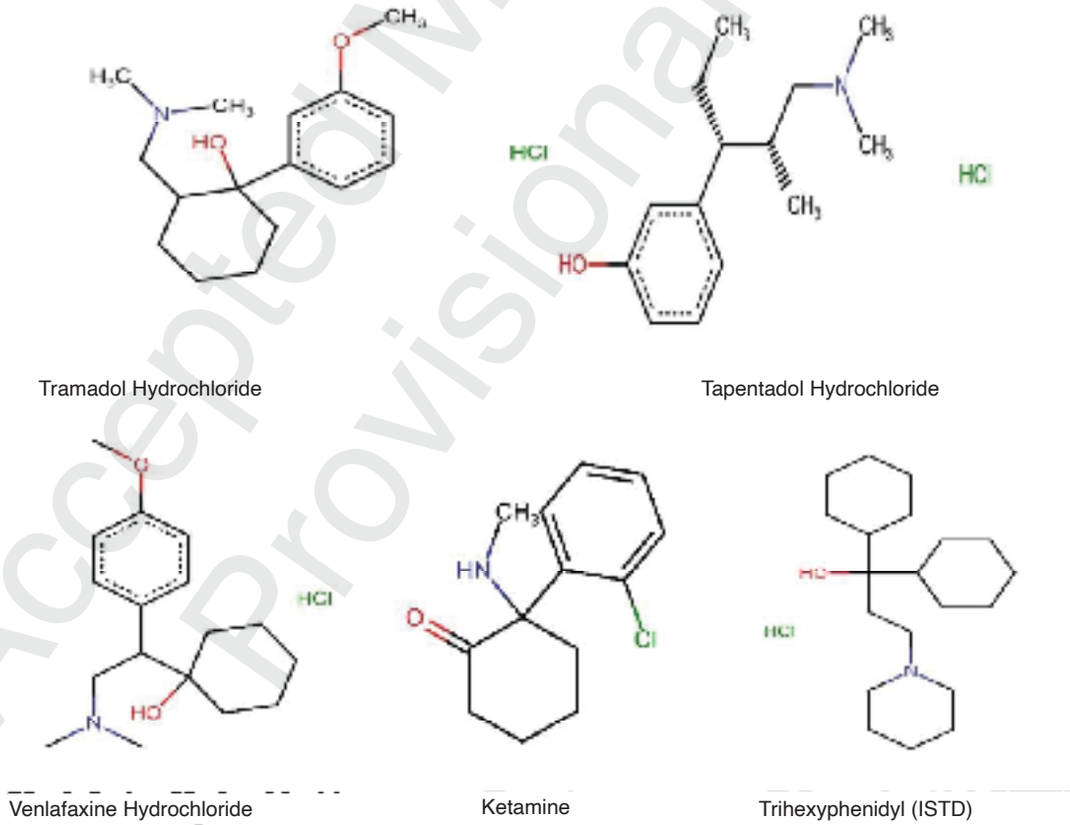

Figure 1- *Chemical structures of the selected drugs in this research study. Source: Marvin was used to draw, display, and characterize chemical structures, substructures, and reactions. Marvin JS 24.3.0 (re389bc4da912), 2024, ChemAxon (http://www.chemaxon.com).*

Figure 2- *Metallic bottle cap used by abusers to administer drugs of abuse.*

Figure 3- *Aluminium foil- common drug paraphernalia used for inhaling drugs.*

Figure 4- *Syringes used to inject drugs into the body.*

2.3. Selection of Paraphernalia as Non-biological Samples

The most frequent ways to inoculate drugs are intravenous, inhalation, snorting, sniffing, ingesting, etc. Among these, intravenous injection and inhalation are the most commonly used methods of drug ingestion due to their rapid effect. So, the syringe, metallic foil, and metallic bottle cap; presented in Fig. 2, 3, and 4 are selected based on the pattern of the case exhibits received in the Forensic Science Laboratory, Mandi.

2.4. Sample Preparation

From the working solutions (10 μg/ml), concentrations of 80 ng/ml, 600 ng/ml, and 1200 ng/ml of each drug were spiked onto simulated paraphernalia (controlled test samples prepared to mimic real forensic evidence), including cap surfaces, aluminum foil surfaces, and disposable syringe. For cap and aluminum,

Table 1- *Analytical Instrumental Parameters*

GC Operational Parameters	Mass-spectrometer Operational Parameters
Oven: starting at 100.0 °C	Ion Source Temp: 200^{0}C
Ramp 1: 25.0 °C per minute until 290 °C hold for 10minutes	Interface Temp: 220^{0}C
Column: Restek Rtx-5Sil MS, 30m x 0.32mm ID, .25µmdf	Solvent Cut Time: 3.0 Minute
Injection volume: 1 µl	Full scan and SIM mode
Injector Temp: 250 ^{0}C	
Carrier Gas: Helium	
Carrier Gas flow: 2.0 mL/min	
Mode – Run in split less mode.	

the outer surface was spiked. In contrast, the syringe was spiked from the inner surface of the barrel by introducing the needle into the drug solution and retracting the plunger in the backward direction. After spiking, the drugs were allowed to dry. The spiked samples were categorized into four groups for this study:

a. Spiked simulated samples analyzed immediately.
b. Spiked simulated paraphernalia samples left for 24 hours at 25°C with 35-40% humidity, then extracted and analyzed.
c. The spiked cap and aluminum foil surfaces were placed in an incubator at 50°C for 30 minutes, then extracted and analyzed.
d. The spiked cap and aluminum foil surfaces were placed in an incubator at 70°C for 30 minutes, then extracted and analyzed.

The syringe was not subjected to categories c) and d) due to the possibility of deformation of the syringe surface. Samples were prepared in triplicate for each group. To extract the spiked surfaces of the cap, aluminium foil, and disposable syringe, 1.5 ml of methanol was used to wash the surfaces. The wash was collected and filtered through a 0.45 μm nylon syringe filter. Trihexyphenidyl (25 μl of 250 ng/ml) was added as an internal standard to each extract, and the volume was adjusted to 2 ml.

2.5. Instrumental Conditions

The samples were analyzed using a GCMS-QP 2020, Shimadzu coupled with a mass spectrometer. The mass spectrometer was operated at 70 eV in the electron impact (EI) mode. The analytes were determined by injection of standard solutions and internal standard solutions. The data output was achieved using scanning (SCAN) mode for identification and selected ion monitoring (SIM) mode for quantification using characteristic ions. The GC and mass spectrometer optimized operational parameters are mentioned in Table 1.

2.6. Validation

The proposed analytical method for the identification of Tramadol, Tapentadol, Ketamine, and Venlafaxine on the surface of selected paraphernalia was validated as a quantitative method. The following parameters were considered for method validation: Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, specificity, selectivity, carryover, matrix effect, and recoveries.

The calibration curve was performed with standards of the final concentrations of 10, 25, 50, 100, 250, 500, 1000, and 1500 ng/ml in methanol. A calibration curve was generated to confirm the linear relationship between the analyte peak areas versus the analyte concentration.25 μl of Trihexylphenidyl

(derived from a working standard of 10 μg/ml) was added as ISTD to each calibrant solution. The slope, intercept, and corelation coefficient (R2) were calculated as regression parameters. The calibration curve with a correlation coefficient (R2) equal to or greater than 0.99 was considered linear.

The precision was evaluated using measurements of the repeatability (intraday) and intermediate precision (interday). The repeatability was investigated using working standard solutions prepared at three different concentration levels. The samples were injected in triplicate and the results were expressed as the Relative Standard Deviation of measurements (RSD%). Intermediate precision was determined by a second analyst at the same concentration levels of repeatability after two consecutive days.

The accuracy of the method was measured through a recovery assay. To perform this assay, working standard solutions were prepared in triplicate at three different concentration levels. The accuracy was expressed as the percentage of the amount recovered in the sample compared to the nominal standard concentrations. In addition, RSD% and accuracy were required to be within ±20%.

The LOD and LOQ of Tramadol, Tapentadol Venlafaxine, and Ketamine were ascertained using the linear calibration model. The LOD was estimated using the formula:

LOD = (3.3Sy)/Avgm

where Sy is the standard deviation of the y-intercept and Avgm is the average slope.

The robustness of the method was tested by introducing small changes in the flow rate of the mobile phase namely Helium (±0.1 ml/min), and column ramp temperature (±2 °C). The robustness tests were carried out using working standard solutions containing 600 ng/ml of each analyte. The stability assay was performed using six replicate injections under a variety of storage and handling conditions.

3. Results and discussions

3.1. Method validation

The validation of the analytical procedures was carried out under ICH guidelines and and UNODC [17,18]. According to these guidelines, the key parameters to ensure the acceptability of the performance of an analytical method are selectivity, linearity, limits of detection and quantification, precision, accuracy, matrix effects, recovery, and robustness.

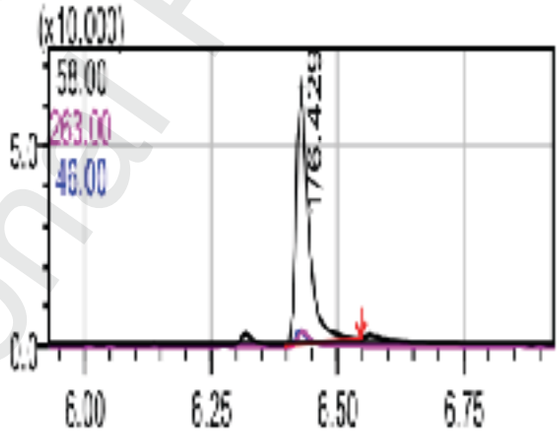

Figure 5- *RT, m/z ratio, and reference ions for Tramadol.*

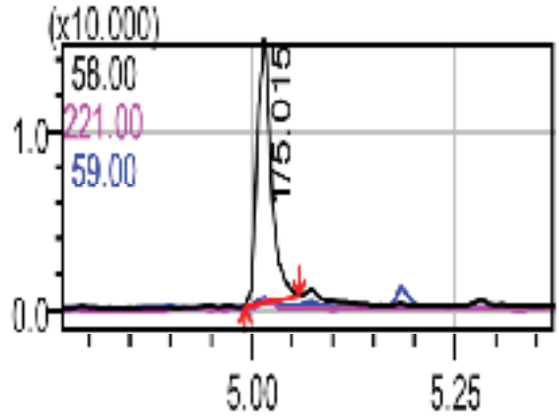

Figure 6- *RT, m/z ratio, and reference ions for Tapentadol.*

3.1.1. Selectivity

The separation achieved using the experimental conditions of the present assay for Tramadol, Tapentadol, Venlafaxine, Ketamine, and their major metabolites are presented in Fig. 5,6,7, 8 and, 9 respectively. Selectivity was indicated by the absence of any internal interference at retention times of peak of interest as evaluated by chromatograms. GC-MS has further confirmed the drugs based on the mass-to-charge (m/z) ratio and the presence of reference ions. The selection of reference ion was performed after scanning all analytes to obtain mass spectrum. The target ions and reference ions were the ones for identification with stable and high intensity. The RT, m/z ratio, and occurrence of reference ions for all the drugs detected in real samples through the proposed method are mentioned in Table 2.

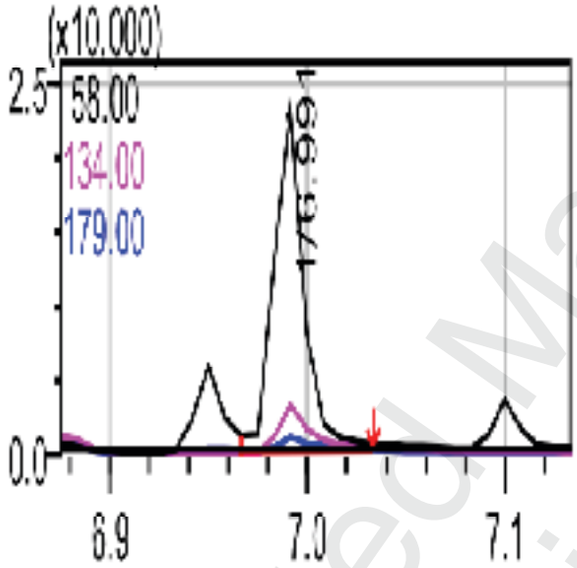

Figure 7- *RT, m/z ratio, and reference ions for Venlafaxine.*

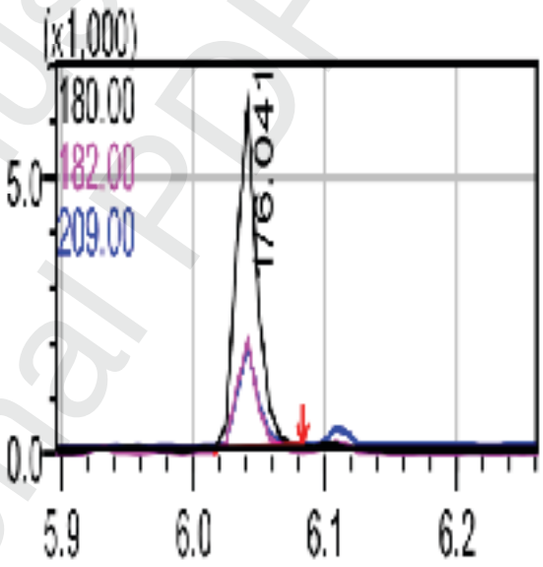

Figure 8- *RT, m/z ratio, and reference ions for Ketamine.*

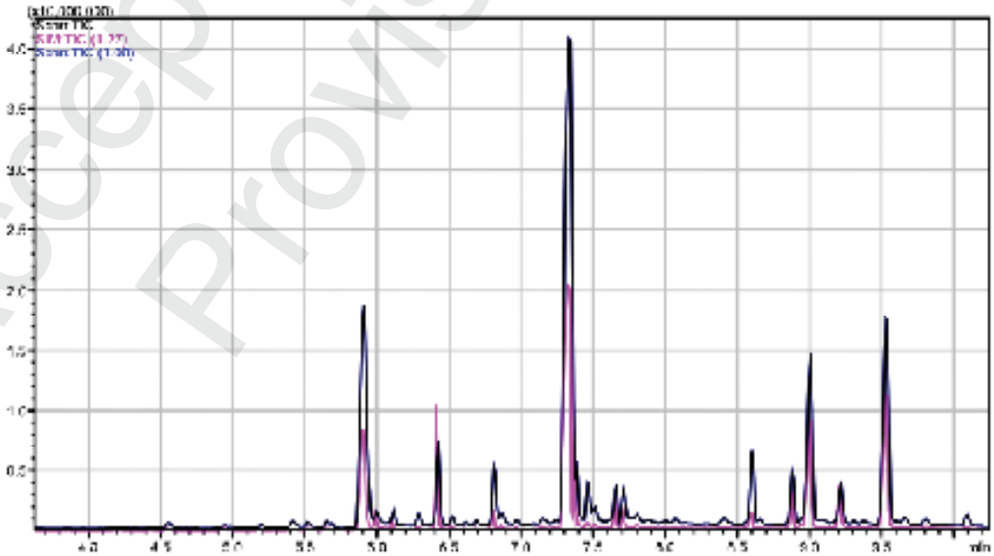

Figure 9- *Total ion chromatogram for all the drugs and internal standard.*

Table 2- *Retention times and target ions for all the drugs detected in real sample*

Drug	Retention Time (RT)	Mass to Charge ratio Quantifier Ion	Reference ion(s)/Qualifier Ions
Tapentadol	5.03	58	221, 59
Ketamine	6.08	180	182, 209
Tramadol	6.43	58	263, 46
Venlafaxine	6.99	58	134, 179
Dextromethorphan	7.33	59	271, 150
Trihexyphenidyl (ISTD)	7.75	98	99,218
Codeine	8.41	299	162, 299
Morphine	8.76	285	162, 215
6-MAM	9.11	327	268, 215
Diacetylmorphine	9.66	327	369, 268

3.1.2. Specificity

The detection of drugs of interest becomes a challenging task in the presence of other conventional drugs, impurities, and other excipient components or Active Pharmaceutical Ingredients (API) present in pharmaceutical medicines. Therefore, the detection of the prime drugs in this study was done in the presence of morphine, 6-MAM, codeine, Dextromethorphan, and Diacetylmorphine. Furthermore, the blank sample and the research samples made using the methodology were compared to determine the specificity. Since no peak was observed at the same retention time of the prime drugs of interest in this study, it is concluded that there was no indication of interference from morphine, codeine, dextromethorphan, diacetylmorphine, or any other contaminants.

3.1.3 Linearity

The GC-MS was used to plot the seven-point non-zero calibration curve. The peak areas of the drug obtained from GC-MS analysis were plotted against the concentration for all the drugs in the concentration range of 10-1500ng/ml. The linear regression analysis was performed on the calibration data and a linear regression line was obtained. The concentration ranges, correlation coefficient (r), and linear regression equation are the suggested method's statistical parameters shown in Table 3. The calibration curves for tramkadol, tapentadol, venlafaxine, and etamine are shown in Figures 10, 11, 12, and 13 respectively. The results indicate a reliable peak area response for all concentrations evaluated.

3.1.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The method's sensitivity was evaluated by determining each analyte's LOD and LOQ. The specific values of LOD and LOQ for the selected drugs are specified in Table 3.

3.1.5. Recovery and Accuracy

The method's accuracy was tested for every analyte using three replicate evaluations at three concentrations (low, medium, and high). The percentage recoveries along with %RSD were calculated and found to be within the range of 75.18-

Table 3- *Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)*

Analyte	Linearity range (ng/ml)	Regression equation	Correlation Coefficient (R^2)	LOD (ng/ml)	LOQ (ng/ml)
Tapentadol	10-1500	Y = (0.43274)X + (-0.03435)	0.998	22.42	67.94
Ketamine	10-1500	Y = (0.16173)X + (-0.01011)	0.999	35.54	107.72
Tramadol	10-1500	Y = (0.48936)X + (-0.03674)	0.998	35.44	107.39
Venlafaxine	10-1500	Y = (0.48936)X + (-0.03674)	0.999	23.98	72.69

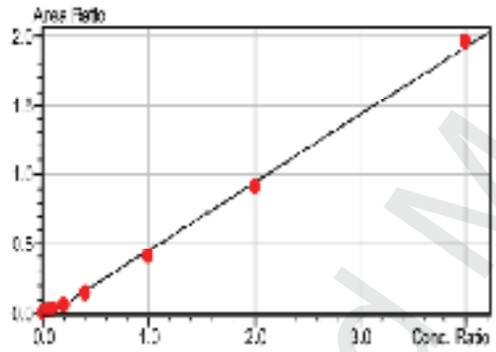

Figure 10- *Calibration curve for Tramadol.*

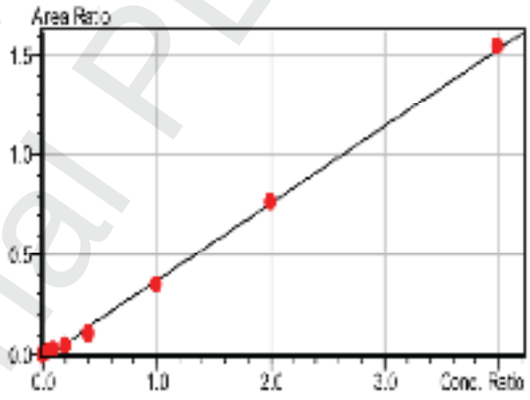

Figure 12- *Calibration curve for Venlafaxine.*

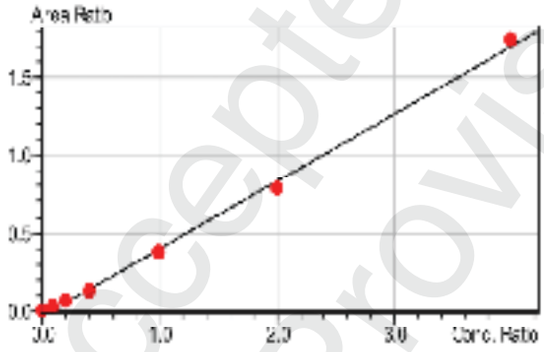

Figure 11- Calibration curve for Tapentadol.

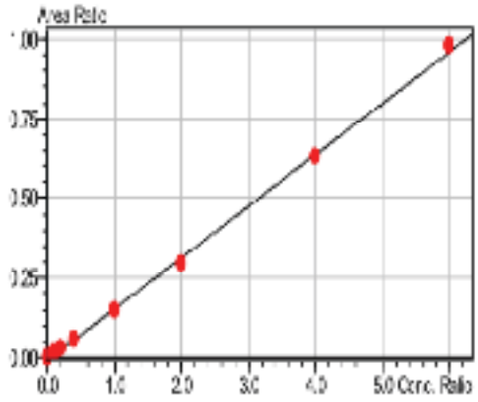

Figure 13- *Calibration curve for Ketamine.*

89.30% with RSD below 15%. The results are given in Tables 4 and 5.

3.1.6. Precision

Precision was ascertained by running three replicate samples of the sample prepared by methodology under the proposed chromatographic parameters and calculated by %RSD. The intraday and inter-day precision studies were conducted for all the drugs. The inter and intra-day RSD%

Table 4- *Recovery percentage for all analyte in proposed method*

Analyte	Low concentration 80 ng/ml	Medium concentration 600 ng/ml	High concentration 1200ng/ml	Mean Recovery%	% RSD
Tapentadol	87.79	96.05	100.55	94.79	5.97
Tramadol	84.50	95.05	100.81	93.45	7.85
Ketamine	84.29	94.31	101.57	93.39	8.19
Venlafaxine	85.55	95.60	98.99	93.05	6.53

Table 5- *Accuracy (Bias %) for drug analytes*

Drug name	Low concentration 80 ng/ml (n=6)		Medium concentration 600 ng/ml (n=6)		High concentration 1200 ng/ml (n=6)	
	Calculated Mean	% Bias	Calculated Mean	% Bias	Calculated Mean	% Bias
Tapentadol	70.23	-13.91	576.32	-4.11	1206.54	0.542
Tramadol	67.60	-18.33	570.3	-5.21	1209.66	0.798
Ketamine	67.43	-18.65	565.87	-6.03	1218.93	1.553
Venlafaxine	68.44	-16.89	567.61	-5.721	1187.88	-1.020

was calculated and found to be in the admissible ranges; indicating substantial precision of the proposed method for simultaneous detection of all the selected drugs in the syringe and the metallic bottle cap.

The results indicate that the developed method was precise with an intraday variation that ranged from 0.08 to 4.36%, and an interday variation that ranged from 0.01 to 3.83%. Thus, the developed method was considered to be reproducible and accurate, and pertaining results are mentioned in Table 6.

3.1.7. Stability Studies

The stability of the stock, working solution, and the samples prepared was determined under two different conditions:

a) Benchtop at room temperature (25°C)

b) Cold storage condition at -10°C

No deterioration in the initial concentration of the drug analytes was noticed, leading to the conclusion that the stock and working solutions were stable in condition (a) for a week. In contrast, condition (b) observed the solutions stay stable for longer periods of months indicating that low temperatures promote drug analytes' stability whereas high temperatures can lead to their deterioration. Analytes from the metallic bottle cap spiked with drugs also showed a decrease in recovery when heated, compared to analytes from the syringe matrix that were not exposed to the heat effect. The sample prepared for GC-MS analysis showed stability for a week on a benchtop at room temperature and for six months at -10°C. Therefore, excellent stability for all of the compounds was observed under normal working and handling conditions.

3.1.8 Robustness

The robustness is the method's resilience towards the modifications occurring in the chromatographic parameters or other conditions like

Table 6- *Validation data of accuracy and precision using the proposed method*

Analyte	Concentration level (n=3) ng/ml	Intraday Precision (n=3)		Inter day Precision (n=3)	
		Mean ± SD	RSD %	Mean ± SD	RSD %
Tapentadol	80	68.61±2.72	3.96	71.85±2.26	3.14
	600	574.92±4.60	0.80	577.73±4.33	0.75
	1200	1210.67±15.57	1.29	1202.42±22.42	1.86
Tramadol	80	63.49±1.86	2.93	71.72±3.52	4.91
	600	562.87±6.27	1.11	577.73±4.33	0.75
	1200	1213.89±49.63	4.09	1205.43±26.79	2.22
Ketamine	80	68.41±2.29	3.34	66.45±2.86	4.31
	600	560.48±14.94	2.66	571.27±7.85	1.37
	1200	1222.97±22.89`	1.87	1214.89±25.67	2.11
Venlafaxine	80	69.04±4.25	6.15	67.85±4.26	6.29
	600	568.41±3.21	0.57	566.81±14.25	2.51
	1200	1183.04±27.49	2.32	1192.72±22.63	1.89

Table 7- *Data on ruggedness for all analytes*

Analyte	Analyst 1 (n=3)	Analyst 2 (n=3)
	Mean Recover % ± %RSD	Mean Recover % ± %RSD
Tapentadol	94.15±2.02	95.44±1.92
Ketamine	93.61±2.62	93.17±2.69
Tramadol	91.44±2.71	95.46±2.63
Venlafaxine	93.21±3.01	92.89±5.57

room temperature, humidity, etc. This was studied by measuring the influence of changes introduced in the mobile phase helium flow rate (±0.1 ml) and ramp temperature (±20C) for the GC-MS method. These deliberate variations have not impacted the resolution of the drug analytes. So, the proposed method was found to be significantly robust.

3.1.9 Ruggedness

The Ruggedness of the proposed method was evaluated by analyzing the three samples each of 80, 600, and 1200 ng/ml concentrations by two analytical experts in the same laboratory to ascertain the reproducibility of the observations. The % recovery and SD were calculated, Table7.

3.2 Analysis of Spike simulated samples under different Environmental conditions:

After development and validation, the GC-MS method was applied for the quantification of Tapentadol, Tramadol, Ketamine, and Venlafaxine to evaluate the spike recovery from the simulated paraphernalia. 500ng/ml of each drug was added to simulated paraphernalia.

Table 8- *Recovery percentage of analytes in simulated spiked paraphernalia analysed instantly*

Analytes	Metallic Cap surface		Aluminum foil surface		Syringe	
	Mean Recovery %	RSD %	Mean Recovery %	RSD %	Mean Recovery %	RSD %
Tapentadol	99.20	1.792	96.6	1.035	99.67	1.175
Tramadol	99.53	1.637	96.87	0.631	96.33	3.676
Ketamine	101.13	1.208	97.2	1.63318	95.67	1.483
Venlafaxine	99.2	1.792	95.87	1.357	95.33	1.58

Table 9- *Recovery percentage of analytes in simulated spiked paraphernalia left for 24 hours at 25°C with 35-40% humidity*

Analytes	Metallic Cap surface		Aluminum foil surface		Syringe	
	Mean Recovery %	RSD %	Mean Recovery %	RSD %	Mean Recovery %	RSD %
Tapentadol	93.33	1.459	94.4	2.201	99.73	2.125
Tramadol	91.46	1.243	93.73	2.607	97.2	1.285
Ketamine	95.2	2.272	91.73	1.879	97.6	3.999
Venlafaxine	92.73	2.385	92.93	3.076	91.87	1.18

Table 10- *Recovery percentage of analytes in simulated spiked paraphernalia placed in an incubator at 50°C for 30 minutes*

Analytes	Cap surface		Aluminum foil surface	
	Mean Recovery %	RSD %	Mean Recovery %	RSD %
Tapentadol	65.67	4.952	65.07	5.091
Tramadol	67.53	3.998	64.33	3.265
Ketamine	63.67	2.708	63.33	4.109
Venlafaxine	65.20	3.681	64.13	4.302

Table 11- *Recovery percentages of analytes in simulated spiked paraphernalia placed in an incubator at 70°C for 30 minutes*

Analytes	Cap surface		Aluminum foil surface	
	Mean Recovery %	RSD %	Mean Recovery %	RSD %
Tapentadol	44.8	5.263	43.8	6.277
Tramadol	46.53	5.961	44.13	3.017
Ketamine	43.6	5.290	41.47	2.742
Venlafaxine	42.33	4.755	42.53	4.939

Table 12- *Data of analysis of samples of suspected abuse of diacetylmorphine, collected from crime scene*

Analytes	Used Cap surface (n=02)		Used Aluminum foil surface (n=15)		Used Syringes (n=28)	
	Positive	Concentration Range (ng/ml)	Positive	Concentration Range	Positive	Concentration Range (ng/ml)
Tapentadol	02	281-567	15	311-979	23	152-691
Tramadol	02	196 -732	14	473-814	27	219-1086
Ketamine	02	237-648	11	291-731	18	186-592
Venlafaxine	00	00	03	108-157	00	00

The accuracy of the simulated paraphernalia analyzed by GC-MS, as reflected in the recovery percentages of the analytes, showed a clear pattern of how environmental conditions impact the stability and detectability of the substances. The results are provided in Tables 8, 9, 10, and 11.

The accuracy of simulated paraphernalia analyzed by GC-MS reveals critical insights into the stability and detectability of various drugs under different environmental conditions. The immediate analysis of spiked paraphernalia samples yielded exceptionally high recovery percentages for all analytes, ranging from 95.33% to 101.13%, with relative standard deviations (RSD) below 5%. This indicates minimal degradation or loss, demonstrating the high precision and reliability of the method. Drugs such as Tapentadol, Tramadol, Ketamine, and Venlafaxine were effectively recovered across different surfaces, affirming the robustness of the analytical procedure.

However, when the analysis was delayed by 24 hours under ambient conditions (25°C with 35-40% humidity), a slight decline in recovery percentages was observed, particularly on the metallic cap and aluminium foil surfaces. This suggests that even mild environmental exposure can initiate degradation or loss of analytes over time. Notably, the syringe surface maintained a high recovery for Tapentadol (99.73%), indicating that the drug spiked into the syringe barrel and remained unexposed to environmental factors. Therefore, the recovery of the drug is higher in the syringe relative to other selected paraphernalia. This suggests that the material of the paraphernalia plays a significant role in the stability of the drugs.

Further analysis under elevated temperatures (50°C and 70°C) revealed a substantial decrease in recovery percentages, with recoveries dropping to as low as 63.33% at 50°C. The most pronounced degradation occurred at 70°C, where recovery for all analytes fell below 45%. Tapentadol, for instance, exhibited particularly low recovery on aluminum foil (43.8%), while Ketamine showed reduced recoveries of 43.6% on the metallic cap and even lower on aluminum foil (41.47%). These findings underscore the high sensitivity of these drugs to heat, which accelerates their degradation. The increased RSD values at these elevated temperatures suggest greater variability in recovery, likely due to the accelerated degradation processes. This indicates that extreme temperatures pose a significant challenge to the stability of these substances, complicating their detection and quantification in forensic investigations.

The significance of this study lies in its demonstration of how environmental factors,

particularly temperature and exposure time, can drastically affect the recovery and detection of drugs in forensic analyses. The type of surface also influenced recovery. The metallic cap surface generally yielded higher recoveries compared to aluminum foil, possibly due to differences in the surface properties affecting absorption or degradation rates. While immediate analysis provided the highest recoveries, a 24-hour delay at moderate conditions (25°C, 35-40% humidity) resulted in a slight decrease in recovery, highlighting the importance of prompt analysis in forensic investigations.

3.3. Analysis of real samples

The proposed method was applied to a real case involving evidence collected at a crime scene where the use of heroin as an incapacitating substance was suspected. The received paraphernalia from the case precisely the syringe and metallic bottle cap, were washed using 1.5 ml of methanol. The collected wash extract was filtered through a 0.45 μm nylon syringe filter. Trihexyphenidyl (25 μl of 250 ng/ml) was added as an internal standard to each extract, and the volume was adjusted to 2 ml. The suspected sample was analyzed using GC-MS. Tapentadol, Ketamine, Tramadol, and Venlafaxine were successfully detected, along with the presence of Diacetylmorphine, Morphine, 6-MAM, and Codeine in the sample suspected to be heroin. The total ion chromatogram for all the suspected drugs detected in the extract is presented above in Figure 9. Also, the RT, m/z ratio and occurrence of reference ions of the above-mentioned detected drugs are mentioned in Table 2. Table 12 represents the data of analysis of real paraphernalia samples of suspected abuse of diacetylmorphine, collected from the crime Scene.

4. Conclusion

According to ICH and UNODC guidelines, the most reliable, accurate, and robust GC-MS method was demonstrated in this study. It was validated for the simultaneous detection of Tramadol, Tapentadol, Venlafaxine, and Ketamine in the presence of other drugs, such as codeine, morphine, dextromethorphan, 6-MAM, and diacetylmorphine, in the syringe and metallic cap and aluminium foil surface. The values of the parameters validated were found to be in the admissible range. The method was also applied in the routine examination of the drugs and have shown effective results. Therefore, the proposed method can be utilized for routine examination of the drugs in similar matrices. However, further validation is required before applying the method to additional matrices..

Conflict of interest

The authors declare no conflicts of interest.

Source of funding

The authors received no financial support for the research, authorship or publication of this paper.

Acknowledgments

The author has been awarded the Senior Research Fellowship (SRF) granted by the University Grant Commission (UGC), Ministry of Education, Government of India. Also, the authors are grateful to the Regional Forensic Science Laboratory, Mandi, Himachal Pradesh for facilitating this research work.

References

1. D. Basu, T. Mahintamini, A. Ghosh, R. Fazle, B.N. Subodh, S.K. Mattoo, A. Avasthi,Tapentadol, the new kid on the block in India: Is it time to worry?, Indian J. Psychiatry. 62 (6) (2020) 697-702, https://doi.org/10.4103/psychiatry.IndianJPsychiatry_332_20.
2. J. Barbosa, S. Leal, F.C. Pereira, R.J. Dinis-Oliveira, J. Faria, Tramadol and Tapentadol Induce

Conditioned Place Preference with a Differential Impact on Rewarding Memory and Incubation of Craving, Pharmaceuticals. 16 (1) (2023) 86, https://doi.org/10.3390/ph16010086

3. D. Mukherjee, L. Shukla, P. Saha, J. Mahadevan, A. Kandasamy, P. Chand, V. Benegal, P. Murthy, Tapentadol abuse and dependence in India, Asian J Psychiatr. 49 (2020) 101978, https://doi.org/10.1016/j.ajp.2020.101978
4. F. Schifano, S. Chiappini, Is There a Potential of Misuse for Venlafaxine and Bupropion?, Front Pharmacol. 21 (9) (2018) 239, doi: 10.3389/fphar.2018.00239.
5. W.J. Maloney, The Health Effects of the Abuse of Ketamine, Int J Depress Anxiety. 1 (2018) 006. doi.org/10.23937/ijda-2017/1710006
6. UNODC, Drug Market Trends: Cannabis Opioids. https://www.unodc.org/res/wdr2021/field/WDR21_Booklet_3.pdf, 2021 (accessed on 04 March 2023).
7. S. Nidhi, M. Chaudhary, Analytical Method Development and Validation of Tramadol Hydrochloride by Pharmaceutical Dosage Form by Ultraviolet Spectroscopy, Pharm Res. 5(3) (2021) 000248.DOI: 10.23880/oajpr-16000248
8. S.P. Chaudhari, K.Tawani, P.R.Mahaparale, Development and validation of UV spectrophotometric method for simultaneous estimation of Tramadol hydrochloride and Quercetin in niosomes formulation, J Scholar Research Library 7(5) (2015).
9. M. Puranik, A.Hirudkar, S.J.Wadher, P.G.Yeole, Development and Validation of spectrophotometric methods for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in tablet dosage form, Indian J of pharmaceutical sciences 68(6)(2006).
10. B.Y. Devi, K. S. Sumanth, V.B. Raju, B.M. Gandhi, K. Srinivas, A new RP-HPLC method development and validation for the simultaneous estimation of ketoroloc tromethamine and tramadol hydrochloride in pharmaceutical dosage forms, J Asian journal pharmaceutical and clinical research 10(5) (2017).
11. K. Singh, A review on Method Development and Validation of Tramadol Hydrochloride by RP HPLC Method, International journal of pharmacy and pharmaceutical research 10(3) (2017).
12. H. Tanaka, T. Naito, Y. Mino, J. Kawakami, Validated determination method of tramadol and its desmethylates in human plasma using an isocratic LC-MS/MS and its clinical application to patients with cancer pain or non cancer pain, Journal of Pharmaceutical health care and sciences 2 (2016).
13. V.S. Tambe, M. N. Deodhar, V. Prakya, LC and LC-MS study for simultaneous determination of tramadol hydrochloride and ketorolac tromethamine in bulk and formulation with their major degradation products, Bulletin of Faculty of Pharmacy, Cairo University54(1)(2016).
14. B. Singh, S. R. Nailkar, C. A. Badkhambhekar, S. Prajapati, S. Kaur, Development of qualitative GC-MS method for simultaneous identification of PM-CCM a modified illicit drugs preparation and its modern-day application in drug-facilitated crimes, J Forensic Sci Res, 7 (2023).DOI: 10.29328/journal.jfsr.1001043
15. B. Capistran, E. Sisco, Validation of a Rapid GC-MS Method for Forensic Seized Drug Screening Applications, Forensic Chemistry (2024), https://doi.org/10.1016/j.forc.2024.100609.
16. M. Bloom, E. Sisco, I. Lurie, Development & Validation of A Rapid GC-MS Method for Seized Drug Screening, Forensic Chemistry (2023) https://doi.org/10.1016/j.forc.2023.100479.https://tsapps.nist.gov/publication/get_pdf.cfm?pub_id=935899 (Accessed January 19, 2025)
17. European Medicine Agency Science Medicine Health, ICH guideline Q2(R2) on validation of analytical procedure. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r2-validation-analytical-procedures-step-2b_en.pdf, 2022 (accessed on 04 June 2024)
18. UNODC, Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens. https://www.unodc.org/unodc/en/scientists/guidance-for-the-validation-of-analytical-methodology-and-calibration-of-equipment.html, 2009 (accessed on 08 June 2024)