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Research Paper

Impact of Temperature, Storage Duration, and Preservative Addition on the Stability of Tramadol in Urine Samples

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ABSTRACT

Background: Precise detection of drugs in urine samples is crucial in both clinical and forensic settings. However, the pre-analytical phase, which encompasses sample handling before testing, can significantly affect results. Additionally, Forensic investigations may involve longer storage periods than traditional clinical scenarios with immediate testing; therefore, proper sample collection and storage are essential to ensure accurate analysis. This study aims to evaluate the impact of temperature and storage duration on the stability of target compounds in urine samples.

Methods: Specifically, the research paper analyzes the impact of different storage conditions, i.e., freezing vs. refrigeration, on the detectability of Tramadol. The stability of Tramadol under the following temperatures (room temperature, 40°C, 4°C, and -20°C) with the preservatives in place and without them in place in the presence of urine samples (n= 240) was established. Findings indicate the nature of the interconnection among Tramadol stability, temperature, and storage time.

Results: We have determined that the conditions that gave the most stable state with Tramadol were those that provided a reasonable degree of stability over time after one month, mild loss of concentration after three months, and comparative degradation after six months, under storage at -20°C and with the preservative. The reason is that the sample can also be kept in Formaldehyde, as it is an antibacterial agent in addition to carbon, hydrogen, and oxygen.

Conclusion: These findings imply that one must be careful when measuring urine temperatures to maintain the integrity of the samples used for proper drug testing. The impact of such a discovery extends widely to health care professionals, toxicologists, and laboratory analysts within the urine drug testing industry. These findings hold significant implications for healthcare professionals, toxicologists, and laboratory analysts involved in urine drug testing. Ultimately, the present research seeks to minimize errors and misinterpretations caused by mishandling during the pre-analytical phase and storage, leading to more reliable drug testing outcomes.

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Introduction

Hospitals and clinics require urine samples for medical evaluations. When drug abuse is suspected, a urine sample is also obtained to conduct drug tests [1].

Waste products that the body filters out are found in urine. As a result, the urine sample is thought to be the main source of bacterial growth, which alters the sample's narcotic drug contents. The public prosecutor in court may request a re-examination if the sample is retained for analysis later, or if the samples are retained for a specific period in accordance with the Law on Combating Narcotic Drugs and Psychotropic Substances, or for further study of poisonous and narcotic materials [1].

The urine sample should be kept in a closed plastic container and refrigerated at about 4°C if it will be analyzed within an hour. It should not be kept at room temperature for more than a day. If the urine sample is not refrigerated, bacteria in it may grow. The test results may be affected if this occurs [1].

Therefore, to prevent bacterial growth and maintain drug concentrations in the urine sample, preservatives must be added and used when samples are stored for a specific period of time [2].

Analysis of urine samples provides valuable information regarding drug toxicity screening. Compared with other biological samples, urine samples provide a smaller window for drug detection but are easy to collect, available in larger quantities, have higher drug concentrations than other samples from the same specimen, and are easier to detect for adulteration [2].

Tramadol is a prescribed pain medication, and it's classified as an analgesic opioid. Mostly it's used to treat moderate to severe pain, and it's often prescribed for chronic pain conditions or after surgery. Tramadol binds to opioid receptors in the brain, which helps to alter the perception of pain. It may also help relieve pain and influence mood by affecting serotonin and norepinephrine in the body.

Although Tramadol is not as potent as other opioids such as Morphine, the drug can still be addictive, and it may trigger dependence when taken improperly. Tramadol is associated with numerous side effects, including dizziness, nausea, constipation, drowsiness, and, in a few instances, more serious side effects, including seizures or respiratory depression. Tramadol is frequently prescribed with discretion due to its ability to be abused and also addictive, particularly to individuals with a history of drug abuse [3].

The use of Tramadol is either by prescription or illegal. In the United Kingdom, it is a Class C drug under the Misuse of Drugs Act 1971, and therefore, the illegal possession, distribution, or production of Tramadol is illegal. Possession of Tramadol without a prescription is punishable by as many as 2 years of imprisonment and a lifelong fine [4]. Providing or producing Tramadol attracts greater penalties, like those relating to Class B drugs, including a maximum of 14 years in prison and an unlimited fine [4].

The effects of Tramadol are similar, although less intense than those of heroin. Users often describe the experience as calm, happy, and relaxed. Tramadol comes in different forms, affecting how long its effects last. Immediate-release tablets typically last 4–6hours after ingestion, and extended-release tablets last 12–24 hours [5].

Although the effects of Tramadol do last for up to 24 hours, traces of the drug can stay in the system for much longer than that. The type of test determines the detection window [5].

Here is a breakdown of the various tests, along with detection time: - Blood: 12-24 hours - Urine: 1-2 days - Saliva: Up to 48 hours - Fingernails: 3-6 months - Hair: Up to 12 months [6].

Detecting Tramadol in the body is primarily done through 5 types of testing:

-Blood Test (Detection: 12-24 Hours) Blood tests are efficient in identifying Tramadol if taken within the first 12 to 24 hours after drug administration. They are used in situations where an offense is suspected because they can provide an instant result. For greater accuracy in legal and medical contexts, blood tests are often used in conjunction with urine tests [6].

-Urine Test (Time it Stays in your system: 1- 4 Days) Tramadol can be detected in the urine 4 days after the drug was used through drug screening and urine tests. Depends on the dosage; the larger the dose, the larger the detection window. Urine tests are an accurate way to detect recent Tramadol use and are often mandated in court cases, routinely in conjunction with blood tests [6].

-Saliva Test (Detection: Up to 48 Hours) A rapid saliva test provides the fastest results, identifying tramadol consumption within 48 hours. You may see positive results as soon as 30 minutes after consumption.

However, these tests are not as commonly used for detecting Tramadol, as not all saliva test kits are designed to identify the drug [6].

- Hair Test (Detection: Up to 12 Months) A hair drug test provides the longest detection window, with traces of Tramadol remaining in hair for up to a year. This test analyzes hair keratin fibers and segments the sample to identify when Tramadol was consumed. The accuracy of this test depends on the length of the hair, as hair grows approximately 1 cm per month. There is, however, a 3-week delay before tramadol use will show in hair samples [6].

- Fingernail Test (Detection: 3-6 Months) Similar to hair testing, a fingernail test can detect Tramadol for up to six months. Once Tramadol enters the bloodstream, it travels to the keratin in the fingernails, where it remains trapped until the nail grows out. This test is often used in court-ordered cases alongside hair testing [7].

The time period of the Tramadol in the body depends on many factors, including: -Tramadol regularity- The interval since the last dose -The metabolism rate -The overall health condition -The degree of hydration -Age- The dosage, and the intensity of the Tramadol. All this may compromise the detection windows and accuracy of different tests.

Urine metabolite variables are also sensitive to many factors, including temperature, storage time, and bacteria, which can influence study results. The substance of the matter is that good conditions of the urine collection and storage must be used in such a way that the integrity of the metabolites and the fidelity of the results can be ensured at the time. The urine either will dry up as it evaporates or will be infected by bacteria and/or contaminated when stored under inappropriate conditions, such as very high temperatures or prolonged exposure during transportation [9].

This gap is addressed by the proposed study, which systematically examined the stability and detectability of Tramadol across different temperatures and environmental conditions. The issue of analyte stability in forensic samples is recognized as a precondition in forensic toxicology. It is often due to other factors, such as enzymatic degradation, chemical reactivity, autoxidation, and the breakdown of N-oxides, that result in instability or artifactual formation. Usually, urine contains no proteins, lipids, or enzymes, yet it exhibits a wide pH range and considerable compositional diversity. Forensic toxicologists need to take into account that instability and the formation of novel compounds depend on the sample, matrix, and the particular analyte under consideration [10]. Thorough knowledge of analyte stability in samples is of utmost importance when conducting quantitative analysis in forensic toxicology. The loss of analytes can lead to inaccurate interpretation of results, potentially resulting in underestimation or overestimation of concentrations. Various studies have shown that analyte stability increases with decreasing storage temperature. Researchers have posited that analyte stability should be considered before interpreting concentration levels in forensic toxicological cases [11].

Dahlin and Petrides reported that certain benzodiazepines exhibited a range of degradation patterns over the course of their storage [12]. Nickley and team investigated the bioanalytical and methodological challenges associated with detecting cocaine exposure, highlighting the intricacies of accurate quantification in forensic contexts [13]. Aldubayyan et al. performed a comprehensive study on the stability of selected synthetic cathinones (SCts) and their metabolites in human urine under various storage conditions. Their results showed that high temperatures increased the rate of analyte degradation, while freezing greatly increased stability. It is important to note that dihydro-metabolites showed increased resilience across all conditions and may serve as reliable biomarkers for detecting SCt. Based on these findings, it was advised that urine samples found to be SCt-positive should be frozen and analyzed as soon as possible to reduce loss of their analytes [14].

Álvaro-Alonso and colleagues evaluated the physical and microbiological stability of methadone hydrochloride oral solutions—both with and without parabens—over 91 days under different temperature conditions. Their study supported the possibility of extending the beyond-use date (BUD), thus facilitating methadone maintenance therapy in clinical settings [15]. Similarly, Riggio et al. reported considerable degradation of amphetamines under various storage conditions, reinforcing the importance of controlled storage environments to preserve analyte integrity [16]. In a related study, Aldubayyan's team also examined the long-term stability of compounds in whole blood. Their results indicated that temperature had a more substantial impact on analyte degradation than the use of preservatives. While sodium fluoride (2% w/v) was effective in reducing degradation at room temperature, compounds containing aromatic halogens were found to be less stable compared to those with N-pyrrolidine structures. For accurate toxicological analysis, prompt testing is advised, and for long-term preservation, storage at or below –40°C is recommended [17].

A urine preservative system

In forensic toxicology, appropriate selection, sampling, and storage of biological evidence are crucial but are sometimes disregarded. These elements, along with drug stability, can significantly impact how results are interpreted and how forensic cases turn out [18].

Whether a patient is mobile, hospitalized, or institutionalized, the urinary system is frequently the location of infection. Urine should ideally be chilled for up to 24 hours or cultured right away to perform a quantitative analysis of microorganisms [18].

Role of urine preservatives:

The use of chemical preservatives in urine samples plays a crucial role in inhibiting bacterial proliferation, reducing analyte degradation, and preserving compound solubility. Moreover, preservatives can help prevent the oxidation of unstable analytes, thereby enhancing the overall integrity of the sample [19]. Although refrigeration is widely regarded as the gold

Table 1. Storage and Analysis of Samples.

Tramadol Samples	Tramadol Samples	Tramadol Samples	Tramadol Samples	Tramadol Samples	Tramadol Samples
240 Positive Samples	240 Positive Samples	240 Positive Samples	240 Positive Samples	240 Positive Samples	240 Positive Samples
80 samples without preservatives	80 samples without preservatives	80 samples with preservatives (Salt Mixture)	80 samples with preservatives (Salt Mixture)	80 samples with preservatives (Formaldehyde)	80 samples with preservatives (Formaldehyde)
20 No.	R. T	20 No.	R. T	20 No.	R. T
20 No.	4°C	20 No.	4°C	20 No.	4°C
20 No.	-20°C	20 No.	-20°C	20 No.	-20°C
20 No.	40°C	20 No.	40°C	20 No.	40°C
Samples Analyses	Samples Analyses	Samples Analyses	Samples Analyses	Samples Analyses	Samples Analyses
First Daye	240 Samples	Screening by Using Immunoassay	Extraction	Confirm the results of screening techniques by using GC-MS.	Confirm the results of screening techniques by using GC-MS.
One Month	240 Samples	Screening by Using Immunoassay	Extraction	Confirm the results of screening techniques by using GC-MS.	Confirm the results of screening techniques by using GC-MS.
Three Months	240 Samples	Screening by Using Immunoassay	Extraction	Confirm the results of screening techniques by using GC-MS.	Confirm the results of screening techniques by using GC-MS.
Sex Months	240 Samples	Screening by Using Immunoassay	Extraction	Confirm the results of screening techniques by using GC-MS.	Confirm the results of screening techniques by using GC-MS.
Total samples analyzed	960 Samples	960 Samples	960 Samples	960 Samples	960 Samples

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standard for urine sample preservation, its effectiveness can be significantly enhanced by concurrent use of appropriate chemical preservatives. Kolb and colleagues emphasized the critical role of chemical preservation in maintaining urine sample stability and preventing microbial contamination. Commonly employed preservatives include boric acid, hydrochloric acid, acetic acid, and oxalic acid. In addition, buffers and bicarbonate salts may be utilized, depending on the specific analytical requirements. It is imperative that laboratories thoroughly assess the efficacy of these preservatives and evaluate their potential to interfere with analytical outcomes [20].

The present study aims to assess the stability of Tramadol in urine samples stored under varying temperature conditions and time intervals, both with and without chemical preservatives.

Materials and Methods

Sample preparation

A total of 240 urine samples were collected from randomly selected individuals aged 15-50 years as part of routine drug abuse screening conducted at various times and locations under the supervision of the Dubai Police. The sample collection procedures and study methodology received approval from the institutional ethics committee. All data was anonymized and utilized solely for research purposes. Three types of urine samples were prepared for analysis: (i) samples treated with a salt-based preservative mixture, (ii) samples preserved with Formaldehyde, and (iii) samples without any preservatives. The preparation process for each type is illustrated in Figures 1 and 2.

- The groups were subsequently stored under different temperature conditions and for varying durations, as detailed in Table 1.

- An initial screening employed Atellica (Siemens) Randox Evidence & Evidence MultiSTAT alongside One-Step cup tests.

After designated storage periods, samples with positive initial screens underwent confirmatory GC-MS analysis to assess potential analyte degradation over time.

GC-MS Instrument Conditions

Specific safety protocols were followed for each instrument in accordance with the manufacturer's safety guidelines. Additionally, periodic maintenance was conducted to ensure the safety and proper functioning of each instrument. An Agilent GC-MS system equipped with a DB-5MS Ultra Inert column (30 m × 0.25 mm × 0.25 µm) was utilized in splitless mode. The initial oven temperature was set to 70°C, then increased to 280°C at 12°C/min, where it was held constant for 11 minutes. The total flow rate, purge flow rate, and pressure were set at 24 mL/min, 3 mL/min, and 8.8085 psi, respectively. For detection, the mass spectrometer was set to a scan range of 50–550 m/z, with the ion source temperature maintained at 230°C and the quadrupole temperature set at 150°C.

Toxi-Tubes (A) General Procedure: Add up to 5 mL of sample to the desired Toxi-tube, mix it for 2

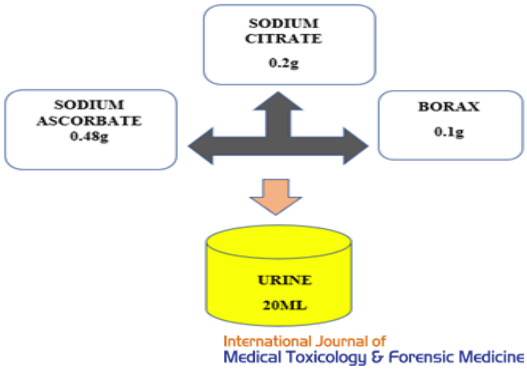

Figure 1. Sample preparation using a Salt mixture as a preservative.

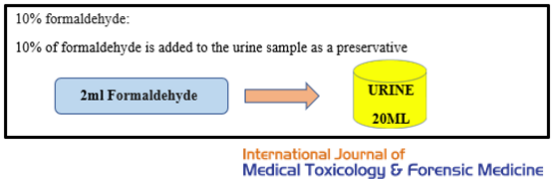

Figure 2. Sample preparation using Formaldehyde as a preservative.

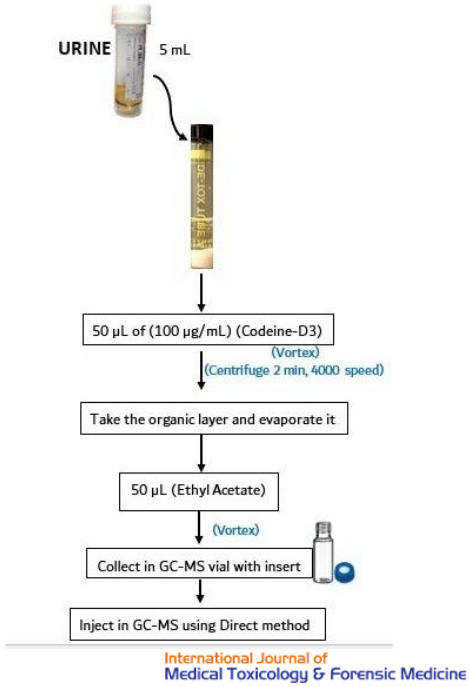

Figure 3. Toxi-Tubes (A) Direct Extraction Method.

minutes, add 50 µl of the appropriate internal standard, centrifuge for 2 minutes, and remove the organic extract for analysis (Figure 3).

Statistical Analysis

The statistical analysis aims to evaluate differences in the mean values of physical parameters across the study groups. Comparisons between groups were performed using One-Way ANOVA, with a 95% confidence interval (CI). The analysis was based on the formulation of two mutually exclusive hypotheses: the null and the alternative. Equal variances were assumed for the study.

Null hypothesis: All means are equal.

Alternative hypothesis: Not all means are equal.

Significance level: $\alpha = 0.05$.

Results

In the present study, Tramadol was examined in 960 urine samples using four different testing conditions (temperatures: room temperature, 40°C, 4°C, and -20°C). The results of the present study were summarized as follows:

One Month Analysis

- Room temperature (RT): The stability of Tramadol decreases slightly by 10-20% over time, with minor variations observed across the different categories: initial concentration (IC), with preservatives (WP), with formaldehyde preservatives (WF), and without preservatives (WOP).
- Storage at 4°C: Similar trends were observed, with stability showing a marginal decrease over one month.
- Storage at 40°C: Significant degradation occurred, with more than 50% loss in stability across all categories.
- Storage at -20°C: The most stable condition was observed at -20°C, where Tramadol retained relatively high stability even after 1 month (Tables 2a & 2 b).
- Figures 5 & 8: The data indicate that the concentration of Tramadol at the start and after one month without preservatives showed negligible change. In contrast, the concentrations remained nearly identical in the samples with preservatives and those with Formaldehyde.

Three Months Analysis

- Room temperature and 4°C: Similar trends were observed at both temperatures, with Tramadol stability decreasing gradually by 20-30% over 3 months.
- Storage at -20°C: This condition proved to be the most stable, although slight degradation was observed compared with the one-month results.
- Storage at 40°C: Tramadol stability declined significantly, with a decrease of more than 50%, indicating substantial degradation after three months (Table 3a & b).
- With preservatives: After 3 months, Tramadol concentration declined slightly by 10-20%. Samples with formaldehyde preservatives showed greater degradation than those with other preservatives. Notably, degradation in samples without preservatives was more pronounced, with a loss exceeding 50% (Figures 5 & 8).

Six Months Analysis

- At room temperature and 4°C, Tramadol stability continued to decline by 50-60%, albeit at a slower rate than observed at earlier time points.
- Storage at -20°C: Tramadol retained the highest stability under these conditions, though some degradation was noted compared to the three-month results.
- Storage at 40°C: Significant degradation occurred, with stability declining by approximately 70%, indicating increased instability over six months.
- Temperature effects: Lower temperatures, particularly -20°C, preserved Tramadol stability over time, while higher temperatures accelerated degradation. These findings underscore the critical importance of temperature control in maintaining the integrity of urine samples for accurate Tramadol drug testing (Table 4a & b).

Figures 7 & 10: After six months, degradation of Tramadol concentration became more noticeable compared to the concentration observed after one month with preservatives. Notably, samples without preservatives showed a decline of more than 50%.

Discussion

The experiment at hand tested two variables (temperature and storage time) related to Tramadol stability in urine. Our findings clarify the critical matters that determine the accuracy of drug testing in the forensic and clinical setting, particularly in the context of Tramadol, one of the synthetic opioids that is used in the treatment of addictions and in managing pain. The graph provides a comparison that emphasizes the critical role of internal standards, such as Cod-D3, in ensuring the reliability of analytics processes for Tramadol and in measuring and analyzing data properly in clinics and research. It is also supported by

Table 2. (a) Observation of Tramadol stability over 1 month. (b) Analysis of Variance of Tramadol stability over 1 month.

(a)

Samples	N	Mean	StDev	95% CI	95% CI
IC	20	0.925	0.57	(0.735,	1.115)
WP_RT	20	0.815	0.516	(0.625,	1.005)
WF_RT	20	0.61	0.3873	(0.4197,	0.8003)
WOP_RT	20	0.4075	0.2582	(0.2172,	0.5978)
WP_4C	20	0.865	0.558	(0.675,	1.055)
WF_4C	20	0.697	0.502	(0.506,	0.887)
WOP_4C	20	0.4275	0.2835	(0.2372,	0.6178)
WP_(-)20C	20	0.902	0.559	(0.712,	1.092)
WF_(-)20C	20	0.674	0.4192	(0.4837,	0.8643)
WOP_(-)20C	20	0.451	0.2794	(0.2607,	0.6413)
WP_40C	20	0.702	0.474	(0.511,	0.892)
WF_40C	20	0.522	0.3549	(0.3317,	0.7123)
WOP_40C	20	0.3385	0.2425	(0.1482,	0.5288)

Abbreviations: IC – Initial Concentration; WP_RT – With Preservative at Room Temperature; WF_RT – With Formaldehyde Preservative at Room Temperature; WOF_RT – Without Preservative at Room Temperature; WP_4C – With Preservative at 4oC; WF_4C – With Formaldehyde Preservative at 4°C; WOF_4C – Without Preservative at 4°C; WP_(-)20C – With Preservative at -20°C; WF_(-)20C – With Formaldehyde Preservative at -20°C; WOF_(-)20C – Without Preservative at -20°C; WP_40C – With Preservative at 40°C; WF_40C – With Formaldehyde Preservative at 40°C; WOF_40C – Without Preservative at 40°C.

(b)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Factor	12	9.598	17.22%	9.598	0.7998	4.28	0
Error	247	46.126	82.78%	46.126	0.1867		
Total	259	55.724	100.00%				

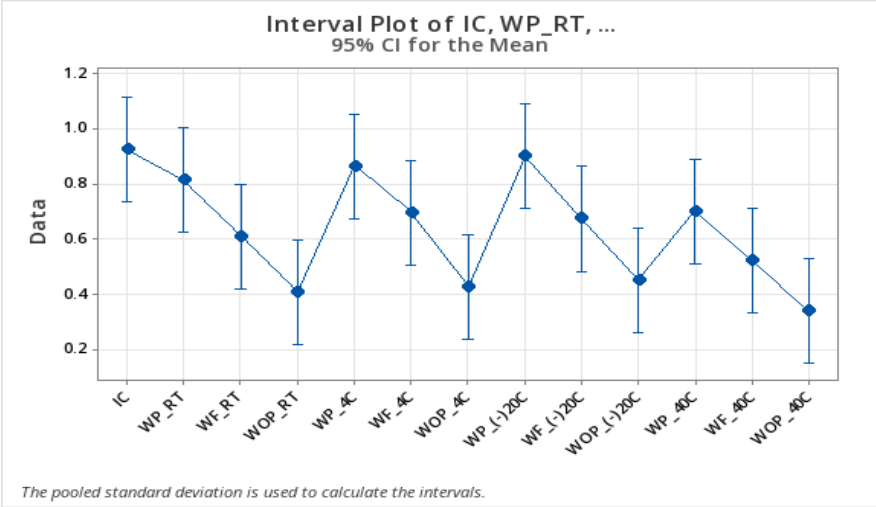

Figure 4. The 95% confidence intervals for each sample group were calculated as mean ± t* × (SD/√n) and displayed using an interval plot to compare group estimates.

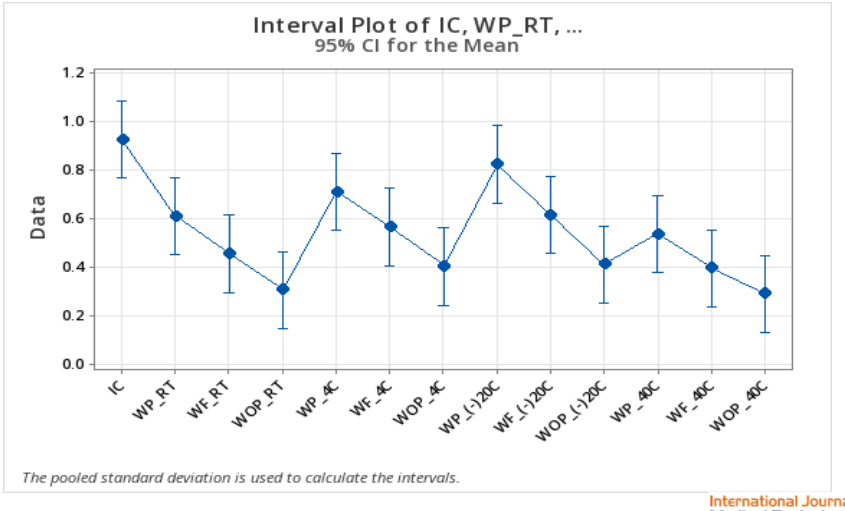

Figure 5. The 95% confidence intervals for each sample group were calculated as mean ± t* × (SD/√n) and displayed using an interval plot to compare group estimates.

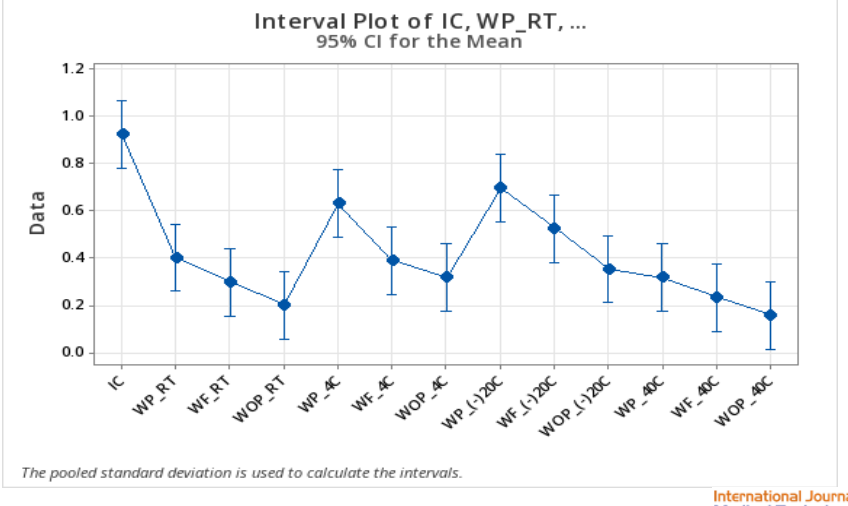

Figure 6. The 95% confidence intervals for each sample group were calculated as mean ± t* × (SD/√n) and displayed using an interval plot to compare group estimates.

graphical illustrations of the correlation between Tramadol concentrations and the internal standard Cod-

Table 3. (a) Observation of Tramadol stability over 3 months. (b) Analysis of Variance of Tramadol stability over 3 months.

(a)

samples	N	Mean	StDev	95% CI	95% CI
IC	20	0.925	0.57	(0.766,	1.084)
WP_RT	20	0.6105	0.3735	(0.4519,	0.7691)
WF_RT	20	0.455	0.2799	(0.2964,	0.6136)
WOP_RT	20	0.305	0.187	(0.1464,	0.4636)
WP_4C	20	0.71	0.4328	(0.5514,	0.8686)
WF_4C	20	0.5655	0.3806	(0.4069,	0.7241)
WOP_4C	20	0.4025	0.302	(0.2439,	0.5611)
WP_(-)20C	20	0.823	0.52	(0.665,	0.982)
WF_(-)20C	20	0.6145	0.3901	(0.4559,	0.7731)
WOP_(-)20C	20	0.4115	0.2598	(0.2529,	0.5701)
WP_40C	20	0.535	0.3297	(0.3764,	0.6936)
WF_40C	20	0.395	0.2434	(0.2364,	0.5536)
WOP_40C	20	0.29	0.1643	(0.1314,	0.4486)

(b)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Factor	12	9.001	21.93%	9.001	0.7501	5.78	0
Error	247	32.034	78.07%	32.034	0.1297		
Total	259	41.035	100.00%				

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D3, calibration curves, and the response ratio (Figure 10).

The graph shows the analysis of the standard Tramadol samples using Gas Chromatography-Mass Spectrometry (GC-MS). To determine the quality of the Tramadol concentration in Standard Samples, Tramadol was identified and quantified using GC-MS (Figure 7C). The identification and quantification of Tramadol were conducted simultaneously, so it is clear that Tramadol has the highest retention time in the chart. The height and the area of this peak of Tramadol are proportional to the concentration of Tramadol in the Standard Samples (Figure 9).

The findings of the Tramadol analysis of the urine samples of the Gas Chromatography-Mass Spectrometry (GC-MS) test are shown as

Table 4. (a) Observation of Tramadol stability over the period of 6 months. (b) Analysis of Variance of Tramadol stability over 6 months.

(a)

Samples	N	Mean	StDev	95% CI	95% CI
IC	20	0.925	0.57	(0.782,	1.068)
WP_RT	20	0.4015	0.2696	(0.2587,	0.5443)
WF_RT	20	0.2975	0.202	(0.1547,	0.4403)
WOP_RT	20	0.201	0.1339	(0.0582,	0.3438)
WP_4C	20	0.63	0.481	(0.487,	0.773)
WF_4C	20	0.389	0.238	(0.2462,	0.5318)
WOP_4C	20	0.317	0.2371	(0.1742,	0.4598)
WP_(-)20C	20	0.695	0.517	(0.552,	0.838)
WF_(-)20C	20	0.524	0.3808	(0.3812,	0.6668)
WOP_(-)20C	20	0.353	0.2523	(0.2102,	0.4958)
WP_40C	20	0.316	0.2228	(0.1732,	0.4588)
WF_40C	20	0.233	0.168	(0.0902,	0.3758)
WOP_40C	20	0.1565	0.1112	(0.0137,	0.2993)

(b)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Factor	12	11.6	30.87%	11.6	0.9669	9.19	0
Error	247	25.98	69.13%	25.98	0.1052		
Total	259	37.58	100.00%				

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chromatograms. This will determine and measure Tramadol to trace its incidence and concentration in urine samples. The Tramadol chromatogram at the retention time shows a prominent peak. This peak indicates the presence of Tramadol in the urine sample. The GC-MS chromatogram of Tramadol in urine samples provides a clear breakdown of its concentration, enabling it to be traced and quantified. It will allow analysts to determine Tramadol concentrations and to ensure the analysis process is valid using chromatogram peaks, mass spectra, and calibration data (Figure 10).

This chart shows the analysis of Cod-D3, an internal standard in GC-MS, used to calibrate and validate measurements of the target analytes, including

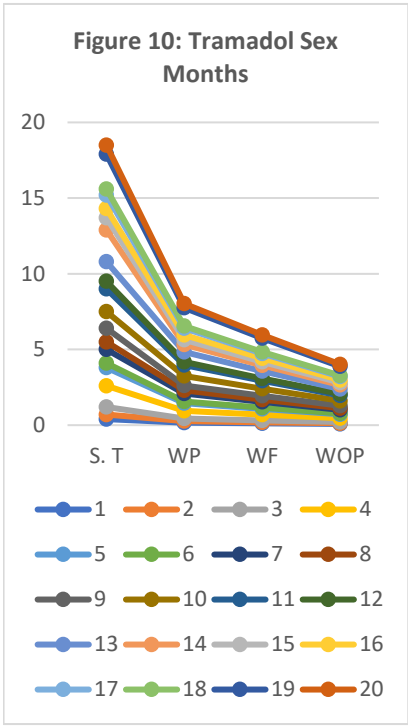

Figure 7A

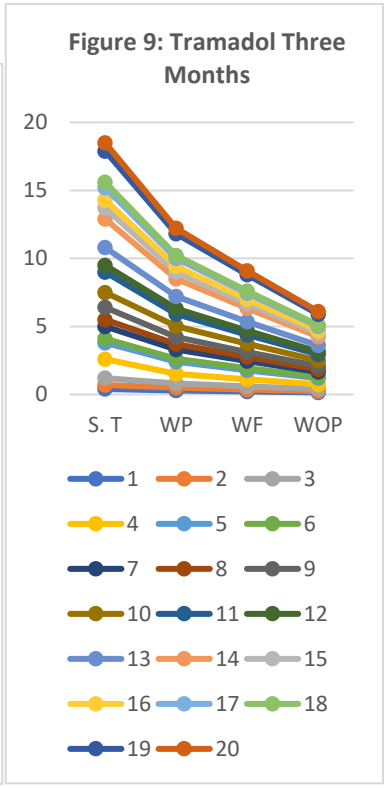

Figure 7B

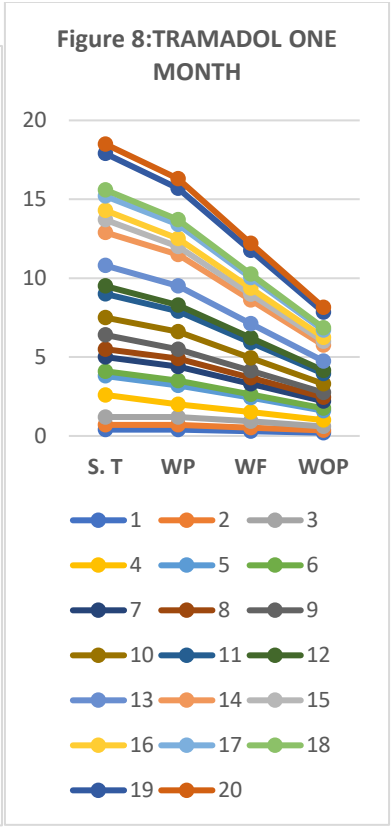

Figure 7C

Figures 7A,7B,7C. Represent the variation observed in Tramadol concentration over the period of 1, 3, and 6 months, respectively, across different categories: initial concentration (IC), with preservatives (WP), with formaldehyde preservatives (WF), and without preservatives (WOP).

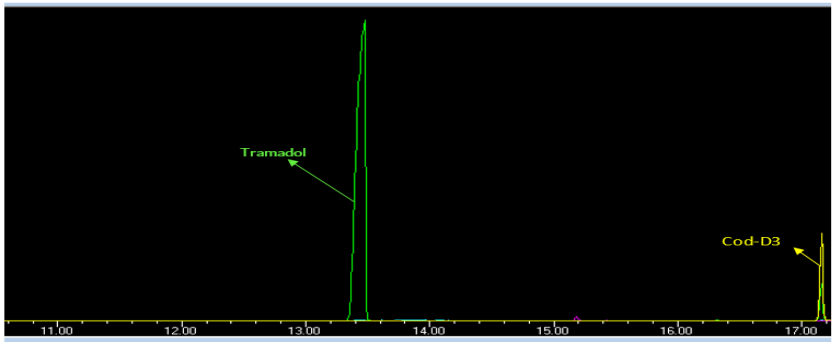

Figure 8. Complete Chart of Tramadol and Internal Standard (Cod-D3).

Tramadol. The alterations in the analysis process are corrected using an internal standard. The GC-MS

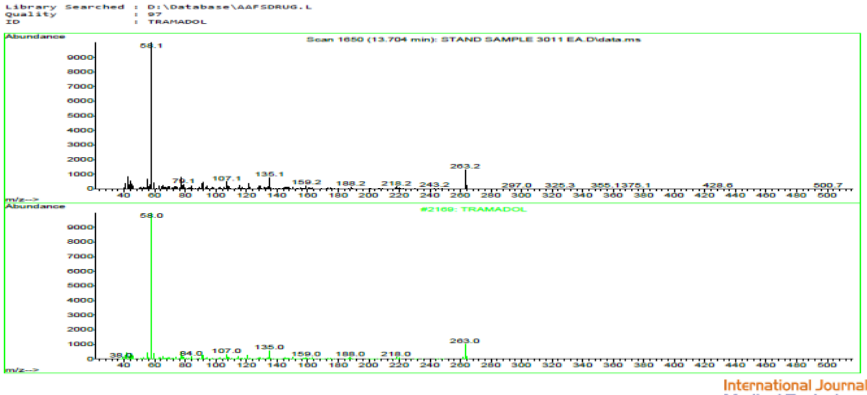

Figure 9. GC-MS Result of Tramadol Standard Samples.

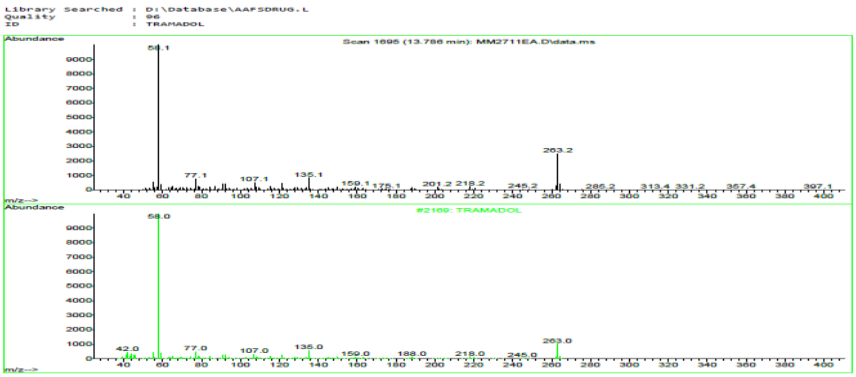

Figure 10. GC-MS Result of Tramadol Urine Samples.

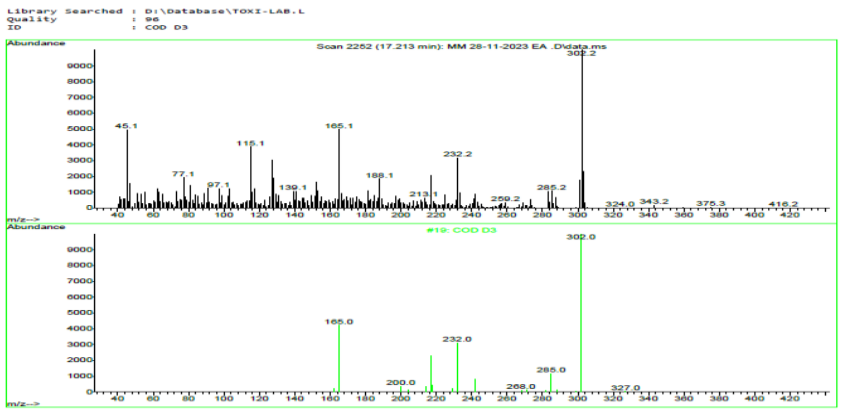

Figure 11. GC-MS Result in Internal Standard (Cod-D3).

results for the internal standard Cod-D3 are valuable data that could be used for calibration and verification of the analysis process. From the chromatogram and mass spectra of Cod-D3, an analyst can properly quantify target analytes, including Tramadol, and maintain high-quality analytical standards (Figure 11).

A multitude of studies have highlighted the significant impact of storage conditions on analyte stability in biological samples. Dahlin and Petrides demonstrated that prolonged storage (beyond 1.5 months) negatively affects analyte performance due to matrix interactions, likely caused by dynamic precipitation during freezing [12]. In another study, the stability of methamphetamine and amphetamine in highly doped urine, both with and without preservatives, was assessed. Notably, while estazolam remained stable under the same conditions, nitrazepam underwent complete degradation within 14 days at 25°C in the absence of preservatives [21]. Cocaine and 6-acetylmorphine also exhibited complete degradation after 1.5 months, even at reduced concentrations. The identified optimal storing conditions of cocaine in urine samples under controlled conditions were -16°C to -21°C, with controlled pH (approximately 5.0) in the presence of ascorbic acid, which proved to be better than hydrochloric acid to maintain sample integrity. These circumstances, however, have enhanced freezing requirements that may become an issue for space-limited labs. In addition, it was proposed that degradation during long-term storage (over 4 months) could be reduced by using dark, sanitized glass containers [13]. Riggio et al. observed that cocaine remained stable for up to 6 days at room temperature, but only 1–2 days at 37°C. Benzoylecgonine exhibited similar stability patterns, while THC remained stable for 7 days at room temperature and 2–6 days at 37°C [16]. Another study confirmed that drug concentrations in biological fluids stored at temperatures below freezing gradually declined over time, highlighting that suboptimal storage conditions can compromise analyte integrity and the overall quality of specimens [22]. The findings of the current research indicate that drug concentration degrades over time under varying temperature conditions, consistent with the results of research by Aldubayyan et al., Riggio et al., and Aldubayyan et al. [14, 16, 17].

Preservative additives have helped overcome drug concentration degradation, particularly in large-volume testing. They play a crucial role in protecting test integrity and delivering correct results by preventing bacterial growth and ensuring the test is soluble. The investigation currently underway has brought to light

the importance of the specifications of the testing protocols for conservatory training. Parameters considered as storage conditions are changes in temperature, storage period, and their influence on Tramadol stability in urine. The samples were subjected to multiple temperature conditions (-20°C, 4°C, room temperature, and 40°C) at different times (1 to 6 months) to evaluate the integrity and reliability of the drug content under real storage conditions. Our study focused on sample integrity and ensured the validity of measured results by applying industry best practices (e.g., stability of the analysed substances and pharmacokinetic considerations) and by accounting for real-life sample submission processes. The given research paper adds to the literature that deals with the specific pharmacokinetic profile of Tramadol in relation to other opioids. Tramadol has some difficulties with dosage and risk unforeseeability, though its application as an addiction treatment and as an anesthetic agent proved to be effective. The results add to the previous significance of a keen eye and careful clinical follow-up on patients under Tramadol treatment.

The stability of Tramadol under various storage conditions has also been employed to stress the importance of standard procedures for the collection, handling, and analysis of urine samples. Quality control during testing processes played a key role in ensuring genuine, valid results in Tramadol drug tests. The pharmacokinetic peculiarities of Tramadol are too complex to ignore, and an individualized treatment plan is necessary. Closely observing, as well as continuous clinical assessment, were significant for optimizing therapeutic effect and reducing the potential risks of Tramadol use. The future may offer more research opportunities to develop more specific dosing and treatment regimens, further streamlining patient management in Tramadol maintenance programmes.

Conclusion

In short, the proposed study assumes that there is a compound relationship between storage temperature and the stability and time of Tramadol in urine samples. The consequences of this finding for the work of healthcare professionals, toxicologists, and laboratory analysts specializing in urine drug testing are immense. By explaining the role of these factors in Tramadol degradation, the study will enable stakeholders to adopt optimal sample-handling protocols, thereby ensuring the quality of Tramadol metabolite screening in both clinical and forensic use.

Moreover, the study is not restricted to Tramadol, as these findings are therefore generalized to other analytes in case toxicology analysis is performed. The study suggests that evidence-based, sound recommendations can be formulated to make toxicological studies more precise and valid across settings, enhancing our understanding of how environmental variables affect drug stability. The final aim is to assist in strengthening clinical and forensic toxicology practice, improving patient outcomes, and safeguarding the sanctity of the legal process.

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We want to express our gratitude to our university for providing us with this opportunity and for the valuable resources and knowledge that support our growth.

Ethical Approval

This study was conducted in accordance with the ethical standards of the institutional research committee and the principles outlined in the Declaration of Helsinki. Urine samples were obtained from randomly selected individuals during routine drug abuse screenings conducted at various times and locations under the supervision of Dubai Police. Ethical approval for the sample collection procedures and study methodology was obtained from the relevant institutional ethics committee. All data were fully anonymized before analysis and used exclusively for research purposes.

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Conflicts of Interest

The authors report there are no competing interests to declare.

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