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www.commprac.com
ISSN 1462 2815

EFFECTS OF TEMPERATURES AND TIME ON THE STABILITY OF KETAMINE IN URINE SAMPLES

Mohammed Ali Ahmed Alwaeel [1], Noora Mohammed Juma [2], Naser Ansari [3] and Nrashant Singh [4]

[1,2,3] Toxicology Department, General Department of Forensic Science and Criminology, Dubai Police, Dubai, UAE.
[1,4] Department of Forensic Science, Amity University Dubai, Dubai, UAE.
Email: [1]alwaeeliphone7@gmail.com

DOI: 10.5281/zenodo.13842845

Abstract

During the pre-analytical stage, sample handling is a crucial factor to take into account. Significant delays are frequent in a forensic setting, in contrast to a clinical one where the interval between sample collection and testing is frequently relatively brief. The time between the victim's death and / or discovery, the autopsy and collection of specimens, sample storage, transport to the lab, and further storage before analytical testing may all fall within the pre-analytical phase, which can be rather lengthy. Specimens must be kept at the proper temperature; the majority of samples should be stored either frozen (-20 ° C or below) during short-term storage (less than 2 weeks) or at a refrigerated temperature (4 ° C). Hair, nails, or dried blood swatches on filter paper are exceptions to this rule and can be kept at room temperature.

Keywords: Drug of abuse, Screening Analysis, Urine sample, Drug test, GC-MS, Ketamine, Stability, Temperatures.

INTRODUCTION

Hospitals and clinics require a urine sample in order to conduct medical evaluations. When drug abuse is suspected, a urine sample is also obtained in order to conduct drug tests. 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 ask for a re-examination if the sample is retained for analysis at a later date or if the samples are retained for a specific amount of time in accordance with the Law on Combating Narcotic Drugs and Psychotropic Substances. or more analysis of poisonous and narcotic materials. (Wu et al., 2015)

The urine sample should be kept in a closed plastic container and refrigerated at about 4°C if you are unable to analyze it within an hour. It should not be kept for more than a day. If the urine sample is not refrigerated, bacteria in it may grow. The test results may be impacted if this takes place.

Therefore, in order to stop bacterial development and preserve drug concentrations in the urine sample, preservatives need to be added and employed when samples are being stored for a specific amount of time. (Ercan et al., 2015) Analysis of urine samples provides useful information regarding drug toxicity screening. When compared with other biological samples, urine samples provided a smaller window for drug detection but are easy to collect, available in more quantity, have a higher concentration of drug than any other sample from the same specimen and has an easy detection of adulteration. (Ercan et al., 2015)

Three types of samples were prepared, with **preservatives (SM)**, **without preservatives**, and with **formaldehyde**. The samples were kept at different temperatures **(RT, 40° C ,4° C, -20° C)** for different periods of time **(1, 3, 6 Months),** and extraction was performed in three stages to determine changes in drug concentrations in urine samples.

The street names of ketamine include; Cat valium, Super acid, Lady K, Special K, K, Vitamin K, Purple, Green, Ketaset, Jet, Cat tranquilizers, Kit Kat, K2, Super C, Special LA coke, Super K, Mauve and Ketaset.

Its brand name is Ketalar. (B. Levine, 2003) Since the 1970s, ketamine has been prescribed in the US for both veterinary and human use as a dissociative anesthetic. Phencyclidine (PCP) and dextromethorphan (DXM) are two examples of dissociative anesthetics that inhibit sensory experience and can distort sounds, sight, colors, and surroundings. It is available as a clear liquid or as a white powder for intravenous injection. (Broussard, 2005)

Ketamine is used as a pain reliever and for temporary memory loss, such as forgetfulness of medical procedures. In addition to providing general anesthesia, it serves as an induction and maintenance sedative during surgery. It is also used in burn therapy to manage discomfort from war injuries. Ketamine is also administered to children who cannot take anesthetics because to allergies or negative effects. Due to its decreased likelihood of causing respiratory depression, it is favored over opioids. (Mycek et al., 2000)

Because of its hallucinatory qualities, ketamine has been abused as a recreational drug in addition to its legitimate uses. Hazardous designer medicines that are analogues of ketamine have been created. Ketamine is obtained illegally by distorting prescription drugs for use in recreational activities.

It is typically snorted in social settings. In addition, it can be consumed orally as a liquid, smoked with marijuana, or injected intravenously or subcutaneously. In the United States, ketamine is categorized as Schedule 111 under the DEA Controlled Substances Act. However, it is not considered a narcotic. (B. Levine, 2003)

Potent visual hallucinations that are increased by environmental stimuli are associated with the abuse of large doses of ketamine. In severe cases, deep unconsciousness may occur. When ketamine is abused in high doses or during an emergency, it causes near-death hallucinogenic experiences, which are often said to be terrifying. (Broussard, 2005) (Mycek et al., 2000)

A urine preservative system:

In forensic toxicology, appropriate biological evidence selection, sampling, and storage are crucial but sometimes disregarded processes. These elements, along with drug stability, can have a significant impact on how results are interpreted and how forensic cases turn out. (Goodman et al., 1985)

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 in order to perform a quantitative analysis for microorganisms. (Goodman et al., 1985)

Role of urine preservatives: (Goodman et al., 1985)

1. These preservatives are added to the following:
    1) Reduce bacterial growth.
    2) Decrease the decomposition of the chemicals.
    3) Keep the substance in solute form.
    4) Decreases the atmospheric oxidation of unstable compounds.
    5) Refrigeration is the most useful for the collection and its utility increases with the preservatives.

The aim of this study is to assess the stability of Ketamine in urine samples stored at different temperature and time period by adding the preservatives and without the preservatives.

Aims:

1) Determine the stability of Ketamine in urine samples stored at different temperature.
2) Determine the stability of Ketamine adding the preservatives and without the preservatives.
3) Calculate the quantity of Ketamine in urine samples.

METHOD

A total of 960 urine samples collected from adult humans with age range between 15 – 50 years and tests will be performed as follows:

Two types of urine samples were prepared with and without preservatives. Figure 1 Figure 2 Stored at different temperature and for different time period. Screening by using Atellica (Siemens) Randox Evidence & Evidence MultiSTAT and One-Step test (Cup Test). Confirm results of screening techniques by using GC-MMS After a different time from collection/preparation to look for eventual analyte degradation.

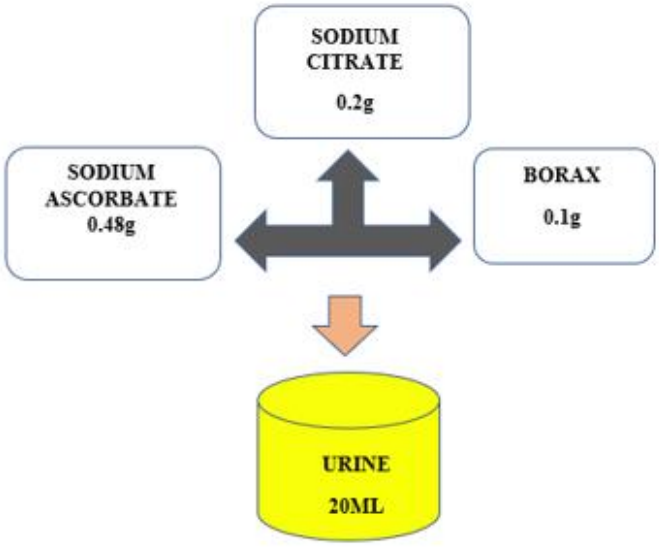


Figure 1: Sample preparation using salt mixture

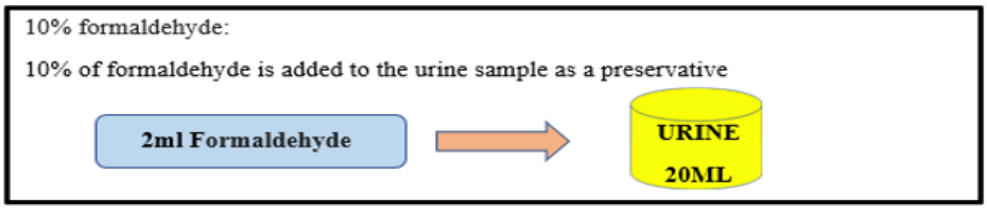


Figure 2: Sample preparation using Formaldehyde

Storage and Analyses of Samples (Table 1):

Storage of Samples					
Ketamine					
240 Positive Samples					
80 samples Without preservatives		**80 samples with preservatives (Salt Mixture)**		**80 samples with preservatives (formaldehyde)**	
20	R. T	20	R. T	20	R. T
20	4 °C	20	4 °C	20	4 °C
20	-20 °C	20	-20 °C	20	-20 °C
20	40°C	20	40°C	20	40°C
Samples Analyses					
First Daye	240 Samples	Screening by Using Immunoassay	Extraction	Confirm results of screening techniques by using GC-MMS	
One Month	240 Samples				
Three Months	240 Samples				
Sex Months	240 Samples				
Total samples analyzed	960 Samples				

Toxi-Tubes (A) General Procedure: Add up to 5 mL of sample to the desired Toxi-tube, mix it for 2 minutes, add 50 µl appropriate internal standard, centrifuge for 2 minutes, and remove organic extract for analysis. Figure 3

Direct Extraction Method:

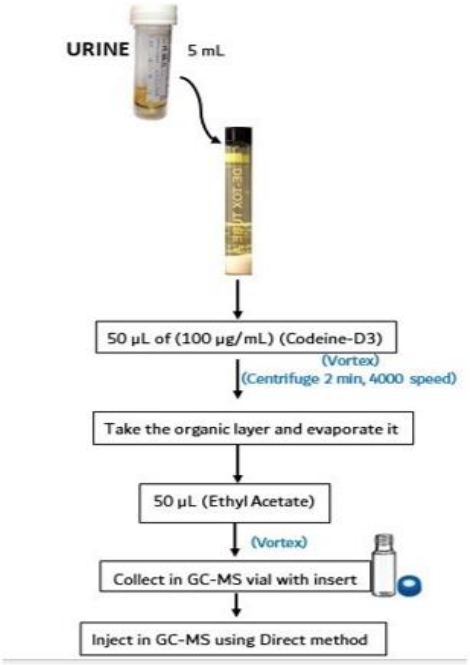


Figure 3: Toxi-Tubes (A) Direct Extraction Method

RESULTS AND DISCUSSION

In this study, Ketamine was examined in 960 urine samples using four different testing methods. Tests were performed on four separate instruments that are known to be widely applied in toxicology and forensic laboratories around the world. These kits are known to offer screening using Enzyme Multiplex Immunoassay (EMIT) and Biochip Array – Linked Immunosorbent Assay technology. Using preliminary screening techniques, a positive result for the drug Ketamine was found

Urine samples that were tested positive by any method were confirmed and quantitated by gas chromatography/mass spectrometry (GC/MS). The concentrations were calculated after a different time and preservation at a different temperature to determine the percentage change in the concentrations of narcotic substances in the urine samples. according to the following tables.

As we can see from the tables above we tested 20 samples that contain Ketamine in different conditions then we measured the concentrations for each sample so in the beginning you can see the concentration in the start day then the concentration with preservative , with formaldehyde and without preservative and all of these samples were tested in a different temperatures such as (Room temperature, 40° C , 4 ° C, -20 ° C) and in different period of time starting from one month up to six month.

The chart provides comparison underscores the integral role of internal standards like Cod-D3 in maintaining the reliability of analytical procedures involving methadone, ensuring precise measurement and analysis in clinical and research settings. It also includes graphical representations showing the correlation between Ketamine concentrations and the internal standard Cod-D3, calibration curves, and response ratios. Figure 4

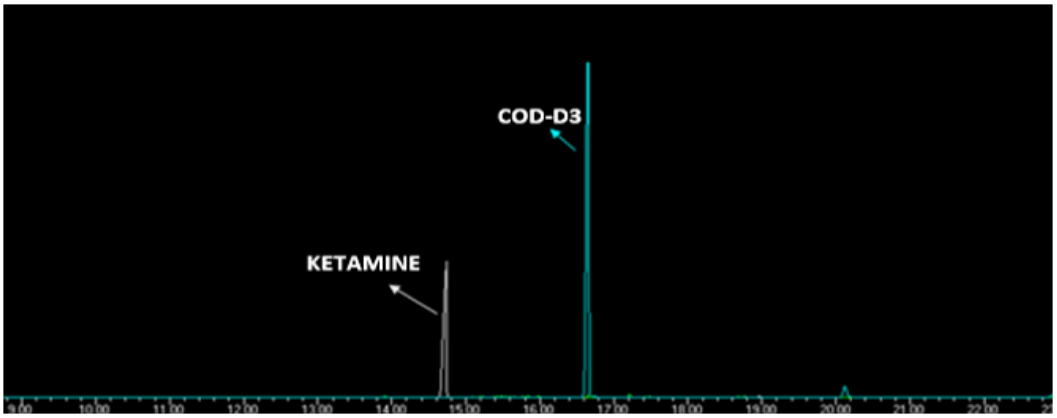


Figure 4: Complete Chart of Ketamine and Internal Standard (Cod-D3)

The chart presents the results from analyzing Ketamine standard samples using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS technique is employed to identify and quantify Ketamine and ensure the accuracy of its concentration in Standard Samples. Figure 5

The chart displays a prominent peak at the retention time specific to Ketamine, which is used for identification and quantification. The height and area of this peak correlate with the concentration of Ketamine in the Standard Samples. Figure 5

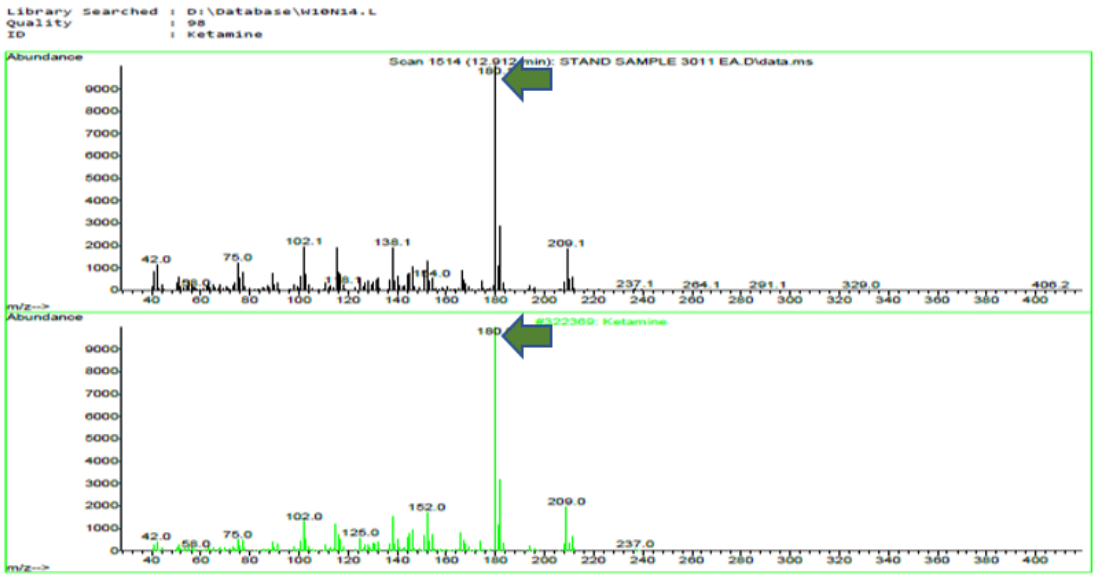


Figure 5: GC-MS Result of Ketamine Standard Samples

The Chromatogram presents the results of analyzing Ketamine in urine samples using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis aims to detect and quantify Ketamine to monitor its presence and concentration in urine samples. Figure 6

The primary peak in the chromatogram at the retention time corresponding to Ketamine. This peak signifies the presence of Ketamine in the urine sample. The GC-MS results chart for Ketamine in urine samples provides a detailed analysis of Ketamine concentration, allowing for accurate detection and quantification. By interpreting the chromatogram peaks, mass spectra, and calibration data, analysts can effectively monitor Ketamine levels and ensure the reliability of the analytical process. Figure 6

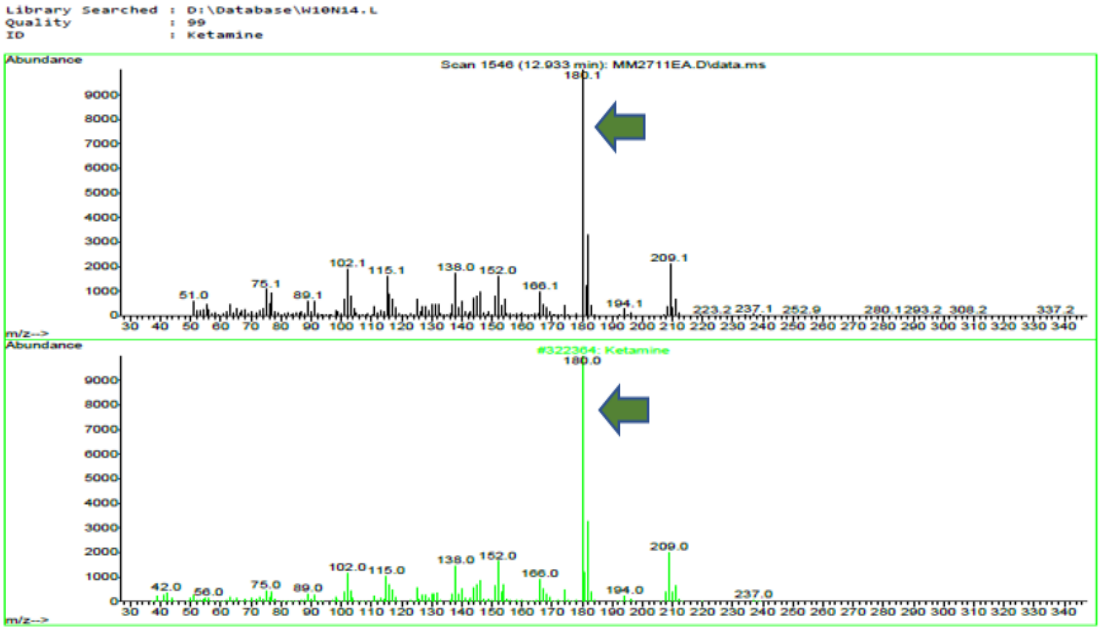


Figure 6: GC-MS Result of Ketamine Urine Samples

The chart shows the analysis of Cod-D3, an internal standard used in GC-MS for calibrating and validating the measurement of target analytes, such as Ketamine. The internal standard helps correct for variations in the analytical process. Figure 7 The GC-MS results for the internal standard Cod-D3 provide crucial data for calibrating and validating the analytical process. By examining the chromatogram and mass spectra of Cod-D3, analysts can ensure accurate quantification of target analytes, such as Ketamine, and maintain high-quality standards in analytical testing. Figure 7

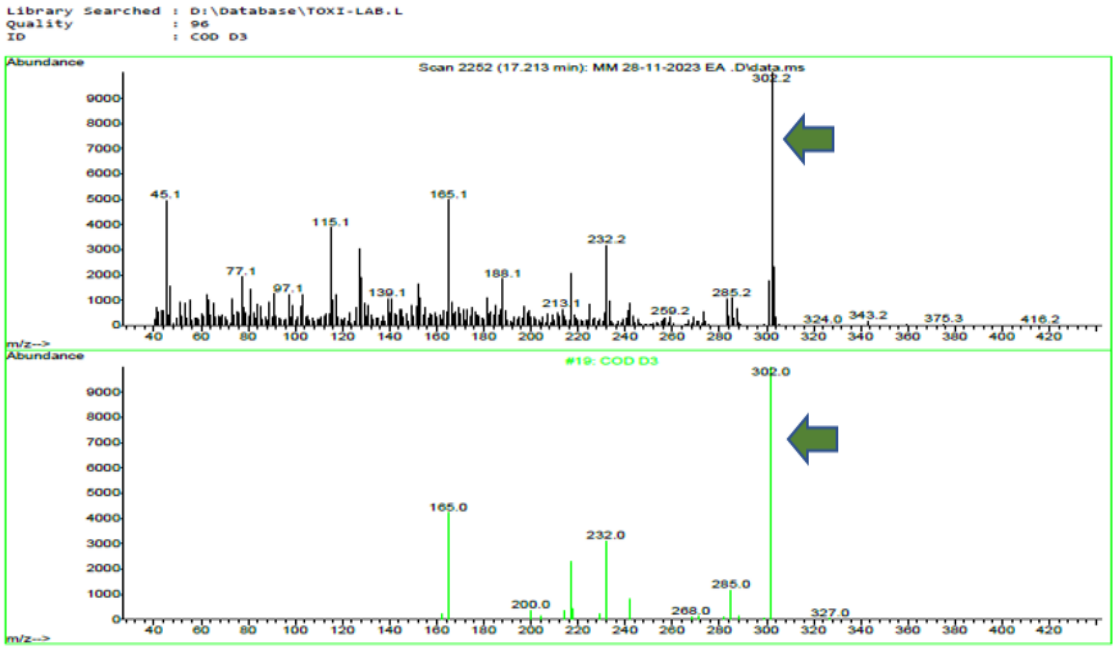
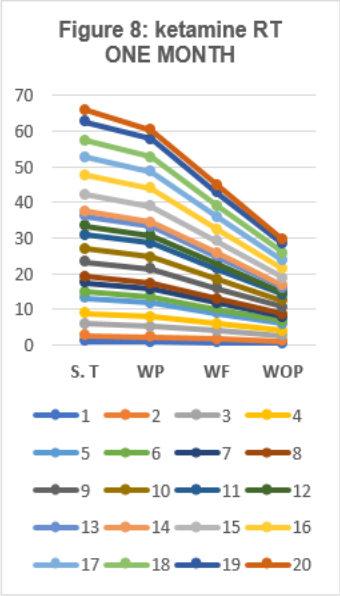


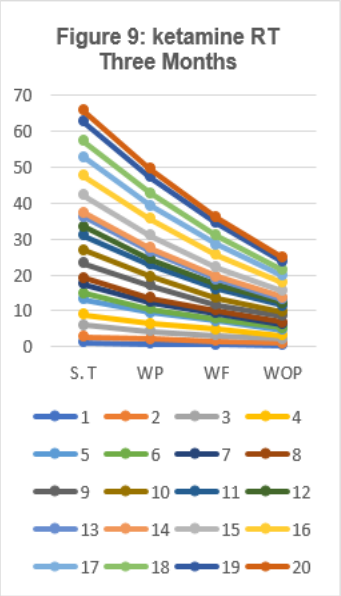
Figure 7: GC-MS Result Internal Standard (Cod-D3)

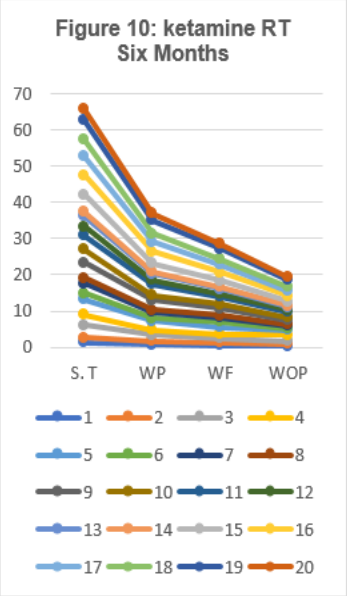
Table 2: ketamine one Months				
RT				
S N	S. T	WP	WF	WOP
1	1.2	1.0	0.75	0.5
2	1.5	1.3	0.97	0.65
3	3.3	3.0	2.25	1.5
4	3.0	2.6	1.95	1.3
5	4.1	3.9	2.92	1.95
6	1.8	1.7	1.27	0.85
7	2.6	2.3	1.72	1.15
8	1.7	1.5	1.12	0.75
9	4.1	4.0	3.0	2.0
10	3.6	3.4	2.55	1.7
11	4.0	3.8	2.85	1.9
12	2.5	2.2	1.65	0.55
13	2.8	2.6	1.95	1.3
14	1.2	1.1	0.82	0.55
15	4.8	4.5	3.37	2.25
16	5.4	5.0	3.37	2.5
17	5.1	4.7	3.52	2.35
18	4.6	4.1	3.07	2.05
19	5.3	5.0	3.75	2.5
20	3.2	2.6	1.95	1.3

Table 3: ketamine Three Months				
RT				
S N	S. T	WP	WF	WOP
1	1.2	0.9	0.67	0.45
2	1.5	1.3	0.97	0.65
3	3.3	2	1.5	1.0
4	3.0	2.2	1.65	1.1
5	4.1	3.1	2.32	1.55
6	1.8	0.9	0.67	0.45
7	2.6	2.0	1.5	1.0
8	1.7	1.1	0.82	0.55
9	4.1	3.5	1.62	1.75
10	3.6	2.5	1.87	1.25
11	4.0	3.3	2.47	1.65
12	2.5	1.5	1.12	0.75
13	2.8	2.2	1.65	1.1
14	1.2	0.9	0.67	0.45
15	4.8	3.5	2.62	1.75
16	5.4	4.6	3.45	2.3
17	5.1	3.8	2.85	1.9
18	4.6	3.4	2.55	1.7
19	5.3	4.5	3.37	2.25
20	3.2	2.2	1.65	1.1

Table 4: Ketamine Six Months				
RT				
SN	S. T	WP	WF	WOP
1	1.2	0.71	0.53	0.35
2	1.5	1.01	0.75	0.50
3	3.3	1.60	1.20	0.8
4	3.0	1.5	1.12	1.75
5	4.1	2.46	1.8	1.23
6	1.8	0.76	1.57	0.38
7	2.6	1.61	1.20	0.80
8	1.7	0.72	0.54	0.36
9	4.1	2.53	1.89	1.26
10	3.6	1.52	1.14	0.76
11	4.0	2.88	2.16	1.44
12	2.5	1.28	0.9	0.64
13	2.8	1.61	1.20	0.80
14	1.2	0.72	0.54	0.36
15	4.8	2.31	1.73	1.15
16	5.4	3.21	2.40	1.60
17	5.1	2.73	2.04	1.36
18	4.6	2.21	1.65	1.10
19	5.3	3.71	2.78	1.85
20	3.2	1.82	1.36	0.91







After a one-month period of sample incubation, the results reveal a consistent trend in the concentrations observed. Notably, the inclusion of a preservative agent maintained the initial concentration levels, reflecting its intended function of stabilizing sample chemistry and mitigating degradation or contamination risks. This is evident in Table 2 and Figure 8, where sample number 9, initially at a concentration of 4.1, remained unchanged after exposure to room temperature for one month with the addition of the preservative. Conversely, the introduction of formaldehyde resulted in a reduction of concentration to 3.0, underscoring its role in preventing microbial-induced deterioration. Interestingly, despite the efficacy of formaldehyde in this regard, the preservative effectively counteracted any decline in concentration, preserving the sample integrity. Moreover, samples lacking any preservative exhibited a marked decrease in concentration, dropping from 4.1 to 2.0 over the same one-month period at room temperature. This stark contrast emphasizes the crucial role of preservatives in maintaining sample stability and ensuring reliable analytical outcomes.

Table 5: ketamine One Month

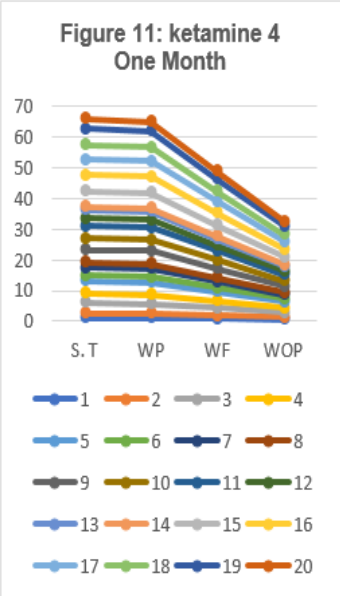
4				
S N	S. T	WP	WF	WOP
1	1.2	1.1	0.82	0.55
2	1.5	1.4	1.05	0.7
3	3.3	3.2	2.4	1.6
4	3.0	3.0	2.25	1.5
5	4.1	4.0	3.0	2.0
6	1.8	1.8	1.35	0.9
7	2.6	2.6	1.95	1.3
8	1.7	1.7	1.27	0.85
9	4.1	4.1	3.07	2.05
10	3.6	3.6	2.7	1.8
11	4.0	4.0	3.0	2.0
12	2.5	2.5	1.87	1.25
13	2.8	2.7	2.02	1.35
14	1.2	1.2	0.9	0.6
15	4.8	4.8	3.6	2.4
16	5.4	5.4	4.05	2.7
17	5.1	5.0	3.75	2.5
18	4.6	4.5	3.37	2.25
19	5.3	5.3	3.97	2.65
20	3.2	3.1	2.32	1.55

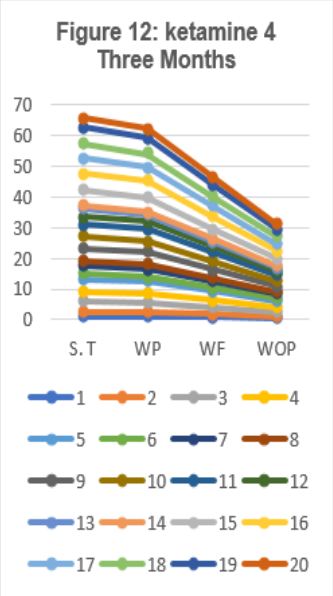
Table 6: ketamine Three Months

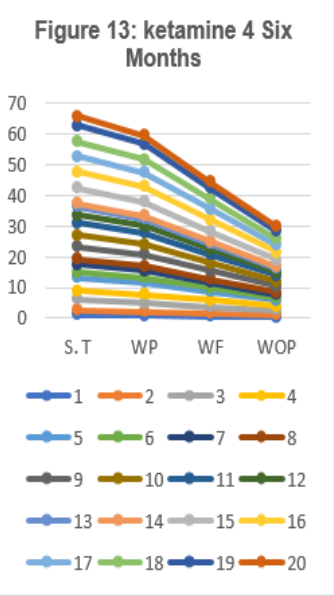
4				
S N	S. T	WP	WF	WOP
1	1.2	1.0	0.75	0.5
2	1.5	1.34	1.00	0.67
3	3.3	3.09	2.31	1.54
4	3.0	3.0	2.25	1.5
5	4.1	3.97	2.97	1.98
6	1.8	1.69	1.26	0.84
7	2.6	2.50	1.87	1.25
8	1.7	1.59	1.19	0.79
9	4.1	3.97	2.97	1.98
10	3.6	3.44	2.58	1.72
11	4.0	4.0	3.0	2.0
12	2.5	2.35	1.76	1.17
13	2.8	2.21	1.65	1.10
14	1.2	1.10	0.82	0.55
15	4.8	4.61	3.45	2.30
16	5.4	5.12	3.84	2.56
17	5.1	4.71	3.53	2.35
18	4.6	4.31	3.23	2.15
19	5.3	5.12	3.84	2.65
20	3.2	3.00	2.25	1.5

Table 7: ketamine Six Months

4				
S N	S. T	WP	WF	WOP
1	1.2	0.81	0.60	0.40
2	1.5	1.19	0.89	0.59
3	3.3	2.98	2.23	1.49
4	3.0	2.78	2.08	1.40
5	4.1	3.81	2.85	1.90
6	1.8	1.52	1.14	0.76
7	2.6	2.33	1.74	1.16
8	1.7	1.47	1.10	0.73
9	4.1	3.77	2.82	1.88
10	3.6	3.28	2.46	1.64
11	4.0	3.78	2.83	1.89
12	2.5	2.21	1.65	1.10
13	2.8	2.44	1.83	1.22
14	1.2	0.89	0.66	0.45
15	4.8	4.47	3.35	2.24
16	5.4	5.11	3.83	2.55
17	5.1	4.60	3.45	2.31
18	4.6	4.23	3.17	2.11
19	5.3	5.01	3.75	2.50
20	3.2	2.71	2.03	1.35







In contrast to the findings from the previous analysis, Table 6 and Figure 12 depicting the results of Ketamine at 4°Cover a three-month period presents a nuanced perspective. Examining sample number 16, we observe a distinctive trend in concentration variations. Initially recorded at 5.4, the introduction of preservatives resulted in a marginal decrease to 5.1 after three months of storage at 4°C. This minor decline suggests a subtle interaction between the preservative agent and the sample matrix. Conversely, the inclusion of formaldehyde led to a more pronounced reduction in concentration, dropping to 3.8 under similar storage conditions. This highlights the potent antimicrobial properties of formaldehyde, albeit at the expense of a more substantial decrease in concentration. Remarkably, samples devoid of preservatives experienced the most significant decline, with the concentration plummeting to 2.5 over the same duration at 4°C. This stark contrast underscores the indispensable role of preservatives in safeguarding sample integrity and mitigating degradation risks over prolonged storage periods.

Table 8: ketamine One Month

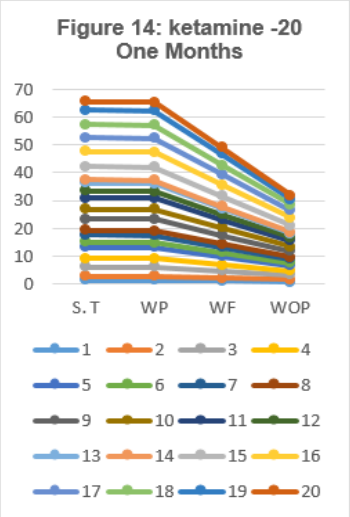
-20				
S N	S. T	WP	WF	WOP
1	1.2	1.2	0.9	0.6
2	1.5	1.5	1.12	0.75
3	3.3	3.3	2.47	1.65
4	3.0	3.0	2.25	1.5
5	4.1	4.0	3.0	2.0
6	1.8	1.8	1.35	0.9
7	2.6	2.6	1.95	1.3
8	1.7	1.47	1.27	0.85
9	4.1	4.1	3.07	2.05
10	3.6	3.6	2.7	1.8
11	4.0	4.0	3.0	2.0
12	2.5	2.5	1.87	1.25
13	2.8	2.7	2.02	1.35
14	1.2	1.2	0.9	0.6
15	4.8	4.8	3.6	2.4
16	5.4	5.4	4.05	2.7
17	5.1	5.1	3.82	2.55
18	4.6	4.6	3.45	2.3
19	5.3	5.3	3.97	1.75
20	3.2	3.1	1.55	1.55

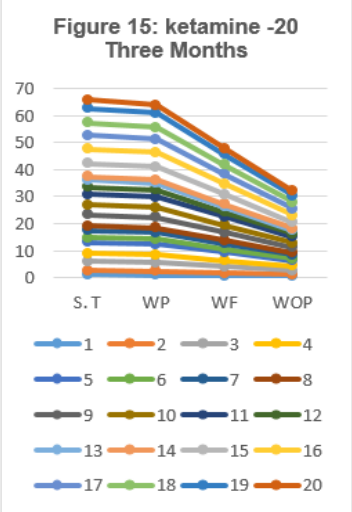
Table 9: ketamine -20 Three Months

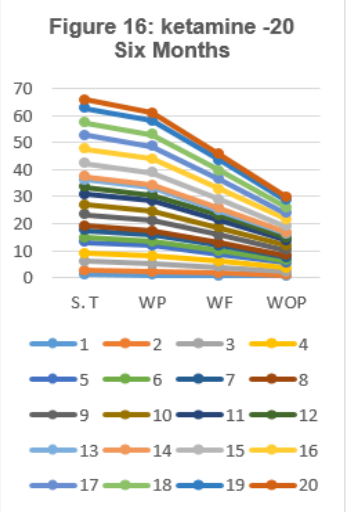
-20				
S N	S. T	WP	WF	WOP
1	1.2	1.0	0.75	0.5
2	1.5	1.34	1.00	0.67
3	3.3	3.15	2.36	1.57
4	3.0	3.0	2.25	1.5
5	4.1	4.0	3.0	2.0
6	1.8	1.75	1.31	0.87
7	2.6	2.56	1.92	1.28
8	1.7	1.65	1.23	0.82
9	4.1	4.0	3.0	2.0
10	3.6	3.54	2.65	1.77
11	4.0	4.0	3.0	2.0
12	2.5	2.44	1.83	1.22
13	2.8	2.66	1.99	1.34
14	1.2	1.18	0.88	0.59
15	4.8	4.75	3.56	2.37
16	5.4	5.33	3.99	2.66
17	5.1	4.9	3.67	2.45
18	4.6	4.47	3.35	2.23
19	5.3	5.21	3.90	2.60
20	3.2	3.05	2.28	1.52

Table 10: ketamine -20 Six Months

-20				
S N	S. T	WP	WF	WOP
1	1.2	0.89	0.66	0.45
2	1.5	0.24	0.93	0.62
3	3.3	3.00	2.25	1.05
4	3.0	2.82	2.11	1.41
5	4.1	3.87	2.90	1.93
6	1.8	1.59	1.19	0.79
7	2.6	2.41	1.80	1.21
8	1.7	1.53	1.14	0.76
9	4.1	3.86	2.89	1.93
10	3.6	3.39	2.45	1.69
11	4.0	3.83	2.87	1.91
12	2.5	2.29	1.71	1.15
13	2.8	2.51	1.77	1.25
14	1.2	1.00	0.75	0.5
15	4.8	4.53	3.39	2.25
16	5.4	5.19	3.89	2.59
17	5.1	4.72	3.54	2.36
18	4.6	4.33	3.24	2.16
19	5.3	5.09	3.81	2.54
20	3.2	2.87	2.15	1.43







In our pursuit to enhance sample preservation, we subjected the samples to a lower temperature of -20°C, effectively freezing them. The results from Table 10 and Figure 16, depicting Ketamine concentrations over a six-month period at this temperature, reveal intriguing insights. Primarily, the addition of a preservative agent proved highly effective in maintaining the stability of most samples, with concentrations remaining largely consistent over time. Notably, even after the introduction of formaldehyde, the observed decrease in concentration was minimal, indicative of the robust preservation achieved by the combination of preservatives and freezing temperatures. For instance, in sample number 3, the concentration initially measured at 3.3 experienced only a slight decrease to 3.00 with the inclusion of formaldehyde, contrasting sharply with the fluctuations observed at room temperature. Moreover, extending the storage duration to three months reaffirmed the efficacy of freezing temperatures and preservative additives. Notably, samples such as numbers 4, 12, 15, 16, and 19 exhibited virtually no change in concentration after the addition of preservatives, underscoring the reliability and longevity of sample integrity under these conditions. These findings underscore the pivotal role of both low temperatures and preservative agents in safeguarding sample stability and ensuring consistent analytical results over prolonged storage durations.

Table 11: ketamine One Month

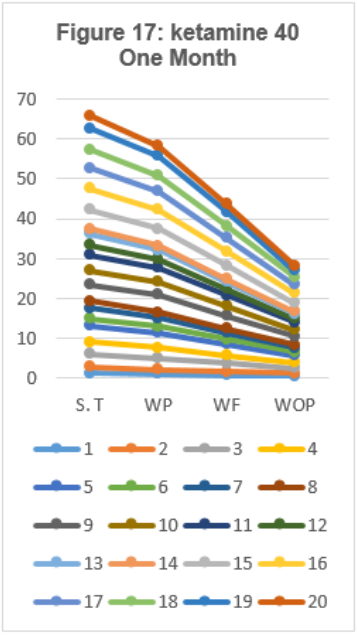
40				
S N	S. T	WP	WF	WOP
1	1.2	0.9	0.67	0.45
2	1.5	1.2	0.9	0.6
3	3.3	2.8	2.1	1.4
4	3.0	2.6	1.95	1.3
5	4.1	3.7	2.77	1.85
6	1.8	1.6	1.2	0.8
7	2.6	2.2	1.65	1.1
8	1.7	1.5	1.12	0.75
9	4.1	4.3	3.22	2.15
10	3.6	3.2	2.4	1.6
11	4.0	3.7	2.77	1.85
12	2.5	2.0	1.5	1.0
13	2.8	2.4	1.8	1.2
14	1.2	1.0	0.75	0.5
15	4.8	4.4	3.3	2.2
16	5.4	4.8	3.6	2.4
17	5.1	4.6	3.45	2.3
18	4.6	3.9	2.92	1.95
19	5.3	4.9	3.67	1.45
20	3.2	2.5	1.87	1.25

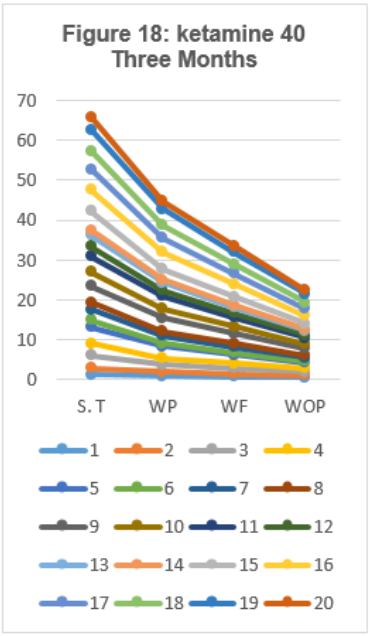
Table 12: ketamine Three Month

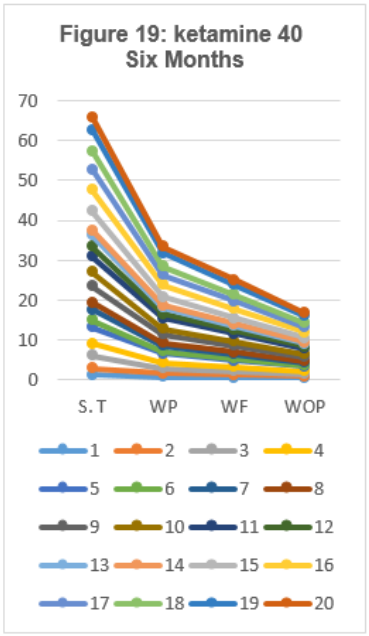
40				
S N	S. T	WP	WF	WOP
1	1.2	0.7	0.52	0.35
2	1.5	1.1	0.82	0.55
3	3.3	1.8	1.35	0.9
4	3.0	1.7	1.27	0.85
5	4.1	2.9	2.17	1.45
6	1.8	0.8	0.6	0.4
7	2.6	1.9	1.42	0.95
8	1.7	1.01	0.8	0.55
9	4.1	3.3	2.47	1.65
10	3.6	2.4	1.8	1.2
11	4.0	3.2	2.4	1.6
12	2.5	1.4	1.05	0.7
13	2.8	1.9	1.42	0.95
14	1.2	0.7	0.52	0.35
15	4.8	2.8	2.1	1.4
16	5.4	4.2	3.15	2.1
17	5.1	3.5	2.62	1.75
18	4.6	3.2	2.4	1.6
19	5.3	4.1	3.07	2.05
20	3.2	2.0	1.5	1.0

Table 13: ketamine Six Months

40				
S N	S. T	WP	WF	WOP
1	1.2	0.59	0.44	0.30
2	1.5	0.82	0.61	0.41
3	3.3	1.33	0.99	0.66
4	3.0	0.34	1.00	0.67
5	4.1	2.31	1.73	1.15
6	1.8	0.61	0.46	0.31
7	2.6	1.49	1.11	0.74
8	1.7	0.58	0.43	0.29
9	4.1	2.27	1.70	1.13
10	3.6	1.39	1.04	0.69
11	4.0	2.63	1.97	1.32
12	2.5	1.11	0.83	0.55
13	2.8	1.47	1.10	0.73
14	1.2	0.61	0.45	0.30
15	4.8	2.10	1.57	1.05
16	5.4	3.01	2.25	1.50
17	5.1	2.54	1.90	1.27
18	4.6	2.12	1.59	1.06
19	5.3	3.49	2.6	1.74
20	3.2	1.56	1.17	0.78







In our quest for comprehensive sample preservation, we explored the impact of elevated temperatures, specifically reaching 40°C, using an oven. This approach was crucial given the diverse origins of our samples, some sourced from decomposed cadavers, necessitating robust preservation techniques adaptable to varying conditions. In Table 12 and Figure 18, illustrating Ketamine concentrations over three months at 40°C, discernible differences emerged post-preservation. Notably, the concentrations exhibited a significant reduction, often surpassing half of the initial values. This underscores the profound influence of high temperatures on sample integrity and analyte stability.

Extending the duration to six months at 40°C yielded even more pronounced effects on concentration levels, exemplified by sample number 15. Initially measured at 4.8, the concentration experienced a drastic decrease to 2.10 after the addition of preservatives and six months of incubation. Subsequent introduction of formaldehyde further diminished the concentration to 1.57, underscoring the compounding impact of extended exposure to elevated temperatures. Crucially, samples lacking preservatives experienced the most substantial decline, with concentration levels plummeting to 1.05, representing more than half of the initial concentration. This stark reduction underscores the imperative of preservative use in mitigating the adverse effects of prolonged exposure to high temperatures, particularly. These findings highlight the importance of adaptable preservation strategies capable of maintaining sample integrity across diverse environmental conditions, ensuring reliable analytical outcomes despite varying temperatures and sample origins.

CONCLUSION

In conclusion, the preservative is useful to maintain the safe of the sample, so it's added to the sample to prevent spoilage caused by bacteria, molds, fungus, and yeast which can affect the reading of the results. The best concentrations that we saw was while adding the preservative most of the sample maintained the same concentrations and it didn't affect the concentrations of the samples because preservative can prevent deterioration from microorganism, enzymes, and exposure to oxygen.

On the other hand, formaldehyde was useful too so formaldehyde can be chosen as another source to preserve or save the sample because formaldehyde made up from carbon, hydrogen, and oxygen so it works as anti-bacterial, and it works by interacting with molecules on the cell membrane and in the body tissues to disrupt cellular functions.so formaldehyde can be chosen as another choice to preserve and maintain the sample. Temperatures also can impact microbial growth, so most of the microbes within certain temperature can grow optimally. So, at lower temperature microbes grow slower and at higher temperatures microbes can grow more quickly. That's why regarding the temperatures we notice the best concentrations at -20**° C followed by** 4**° C and then comes the room temperature and in the end 40°c.**

References

1) Wu, W., Yang, D., Tiselius, H., Ou, L., Mai, Z., Chen, K., Zhu, H., Xu, S., Zhao, Z., & Zeng, G. (2015). Collection and storage of urine specimens for measurement of urolithiasis risk factors. Urology, 85(2), 299–303.

2) Ercan, M., Akbulut, E. D., Abuşoğlu, S., Yılmaz, F. M., Oğuz, E. F., Topçuoğlu, C., Öztekin, V., & Boğdaycıoğlu, N. (2015). Stability of urine specimens stored with and without preservatives at room temperature and on ice prior to urinalysis. Clinical Biochemistry, 48(13–14), 919–922.

3) Levine, B. (2003). Principles of Forensic toxicology. Amer. Assoc. for Clinical Chemistry.

4) Broussard, L. (2005). Disposition of Toxic Drugs and Chemicals in Man, Seventh Edition. Randall C. Baselt. Foster City, CA: Biomedical Publications, 2004, 1250 pp., $139.50, hardcover. ISBN 09626523-6-9. Clinical Chemistry, 51(3), 680.

5) Mycek, M. J., Harvey, R. A., & Champe, P. C. (2000). Pharmacology. Lippincott Raven.

6) Goodman, L. J., Kaplan, R. L., Landau, W., Jung, E., Barrett, J. E., Levin, S., & Harris, A. A. (1985). A urine preservative system to maintain bacterial counts. Clinical Pediatrics, 24(7), 383–386.