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Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

REVIEW ARTICLE

IMPACT OF SAMPLE STORAGE CONDITIONS ON FORENSIC TOXICOLOGY ANALYSIS – A REVIEW

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Article Info:

Article History:
Received: 3 December 2022
Reviewed: 8 January 2023
Accepted: 21 February 2023
Published: 15 March 2023

Cite this article
ADOUANI B, RAHMOUNE I, FILALI H. Impact of sample storage conditions on forensic toxicology analysis – A review. Universal Journal of Pharmaceutical Research 2023; 8(1):69-73.
https://doi.org/10.22270/ujpr.v8i1.901

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Abstract

In forensic toxicology, the preservation of matrices is a crucial stage in the analysis that affects the accuracy and reliability of the findings. To reduce interference and improve the findings' interpretation, specimens should be maintained in the right environment. For analytical objectives in forensic toxicology, we aim to characterize the modalities of preservation of the distinct matrices for each incriminated toxin. A comprehensive literature search in both English and French languages was conducted using the keywords "forensic samples preservation" to find pertinent papers in the PubMed and Science Direct databases. The results were then carefully examined. The preservation method based on the biological matrix and the required harmful substances was the major focus of the investigation. This bibliographic research's objective is to understand the sample preservation methods used in diverse studies as well as each method's limitations in light of the biological matrix and xenobiotics being investigated. The mode and duration of conservation depend closely on the matrix and the toxins to be sought. Certain hazardous compounds' chemical stability in biological matrices is influenced by storage conditions compliance. Therefore, it is apparent that forensic scientists would benefit from knowledge of the sampling and storage procedures for substances suspected of posing a threat.

Keywords: Forensic, preservation, sample.

INTRODUCTION

Even though forensic toxicology is based on significant improvements in identification and assay methods, it should not be forgotten that the quality of these analyses and the interpretations that follow will depend a lot on the quality of the sample. This makes it very important whether the sample is taken after the person has died or while they are still alive[1] . The conservation of matrices is a crucial step in forensic toxicology that impacts the quality and accuracy of analytical findings. Specimens should be kept in the right environment to reduce interference and make it easier to understand the data[2]. The process of choosing and gathering samples for toxicological examination both ante-mortem and post-mortem presents a number of unique difficulties. Peripheral and cardiac blood, urine, stomach contents, vitreous humour, bile, viscera (and more specifically the lung, liver, kidney, heart, and brain), and lastly hair are samples that must be routinely obtained at each autopsy when they are available. Additionally, there are optional or substitute samples, such as nasopharyngeal samples, putrefactive liquids, and sometimes even larvae or insects discovered on putrefied corpses, as well as bones when just the skeleton is still there. Therefore, it is crucial that the toxicologist stores these samples properly[3-5]. For forensic toxicology analysis, our goal is to describe how the different matrices for each incriminated toxicant stay stable over time.

METHODS

Using the keywords "forensic sample preservation," a thorough literature search in English and French was conducted to find relevant papers on the PubMed and Science Direct databases. The findings were then thoroughly analysed. The study's main emphasis was on the preservation strategy based on the biological matrix and the desired hazardous chemicals. The goal of this bibliographic research is to understand the methods used to keep samples safe in different studies, as well as the limits of each method based on the biological matrix and xenobiotics being studied.

RESULTS AND DISCUSSION

The most investigated biological matrices in papers describing the manner of preservation of samples for toxicological investigation were blood, vitreous humour, urine, and bile. The other matrices showed all of the information that had been gathered, such as the contents of the digestive system, hair, insects, viscera, putrid liquids, bone, and muscle.

The mechanism of conservation and its duration are both determined by the toxicants that may be present in the matrix. In an effort to increase the chemical stability of the toxins, all other samples must be stored at temperatures as low as 4 degrees Celsius (short term), as low as -20 degrees Celsius (medium term), or as low as -80 degrees Celsius (long term), with the exception of hair, which must be stored at room temperature and kept dry atmosphere. Because they remain unchanged even when exposed to ambient temperature, hair and nails are an exception to this rule. For blood, bile, and vitreous fluid, sodium or potassium fluoride, EDTA for blood, potassium oxalate for vitreous humour, and 80% ethanol for larvae and insects were the most often used preservatives.

Several factors impact the change of samples in toxicology, which may significantly affect the quantities measured for various analytes. Other changes brought on by inadequacies *in vitro* conservation and preservation are less tolerable, despite the fact that these effects are almost unavoidable.

As a consequence of this, the preservation of samples and the physical circumstances (such as temperature) that exist during storage should not be ignored, given that fluctuations in analyte concentrations might occur even *in vitro*[6]. General techniques for sample preservation and storage are highlighted in the following section.

1. Blood: The most essential medium of all the samples obtained during an autopsy is blood. It is the only setting for which data from the literature allow for the determination of a person's amount of impregnation with a material at the time of death[3]. There are numbers about the concentration ratios of whole blood to plasma or serum[7], but all of these numbers come from research done on living people. Whole blood from a dead person is very different from whole blood from a living person.

Typically, peripheral blood is found in lesser amounts than cardiac blood. Once collected, peripheral blood is separated into two kinds of containers: half is placed in a glass or plastic jar without anticoagulant and preservative (or a dry tube without agar), and the other half is placed in a tube without agar containing 0.1% sodium fluoride (hospital grey cap tube)[3].

Ricardo Jorge Dinis-Oliveira *et al.*,[8] say that blood from the heart, thorax, or abdomen can be collected in a plastic container with a screw cap and a 30 ml capacity. No preservative is needed.

Ethanol: Temperature and storage duration are the two most crucial variables impacting blood ethanol stability, according to Fatma Emel Kocak *et al.*,[9]. This research looked at how well ethanol held up throughout different periods of storage at -20°C in plasma samples supplemented with sodium fluoride and K3EDTA (after 2, 3, 4, or 5 months). Analytically significant decreases in ethanol were only seen in samples that had been stored for 5 months. Decreases in the other groups did not reach this level.

Benzodiazepines: In the context of chemical submission, samples are sent to the laboratory for quick freezing, since a number of molecules degrade even at 4°C[10]. In research looking at diazepam's stability characteristics in sample matrices, the researchers examined the amount of diazepam that remained in blood and plasma samples that had been treated, at different temperatures and whether fluoride was present or not. Evaluations were made of sodium's effectiveness as a stabiliser and the impact that ethanol has on diazepam's stability[11]. Diazepam's stability at -20°C was shown to be affected by the presence of ethanol at either a low (0.5 g/L) or high (3 g/L) level of concentration. For this purpose, fluoride-stabilized blood samples were used, in which diazepam levels dropped by as much as 85% after being frozen for two months (12 weeks). Adding ethanol and sodium fluoride to whole blood samples causes an additional (15–25%) drop in the analyte concentration[11].

The study by Ritva Karinen *et al.*,[12] investigated the stability of benzodiazepines over a long period of time in samples of blood taken after a person has died. The samples were stored in a solution of potassium fluoride and were analyzed again after a storage period of 16-18 years at -20°C. The average and median concentrations of diazepam, nordiazepam, and flunitrazepam in samples that had been stored for a long time didn't change much when the samples were reanalyzed, but the concentrations of clonazepam tended to go down.

Opioids: Over 16-18 years of storage at -20°C in 0.3 ml potassium fluoride solution, the average and median concentrations of amphetamine, morphine, and codeine in the samples exhibited little change[12].

Cocaine: When kept at a temperature of -20 degrees Celsius, cocaine and its metabolites benzoylecgonine, benzoylecgonine ethyl ester, and ecgonine methyl ester were all stable, and after one year of storage, more than 80% of the original substance could be recovered. When, on the other hand, the samples were stored at a temperature of 4 degrees Celsius, the concentration of the four compounds decreased. Under identical conditions, the effect of the preservative was visible, with NaF-preserved samples being more stable[13].

Tetrahydrocannabinol: Although tetrahydrocannabinol (THC) in whole blood is stable during storage at temperatures as low as -20 degrees Celsius, the stability is dependent on the container. The THC content stays constant in glass vials, whereas plastic containers lose between 60 and 100 percent of their THC during storage. Storage at temperatures as low as -20°C proved effective[14].

2. Urine: Urine is an essential sample for the toxicologist in the investigation of the causes of death. By puncturing the bladder with a syringe, urine is extracted and placed in an unpreserved glass or plastic container. Today, opiates, cocaine, amphetamines, cannabis, methadone, buprenorphine, and LSD are just some of the drugs that can be found in urine[3].

In the case of chemical submission, samples are submitted to the laboratory for quick freezing, since a number of chemicals degrade even at 4°C, notably benzodiazepines, which are the most prevalent molecules implicated in chemical submission[10].

Cathinone: Cathinone is a naturally occurring stimulant that can be found in the khat plant. The purpose of this research was to determine how stable cathinone is when it is stored in urine for a period of six months at varying pH levels, temperatures, and other circumstances. According to the findings of the study, the stability of cathinone was affected by the pH level of the urine. Specifically, the researchers discovered that cathinone was more stable in alkaline urine (pH 8) at higher temperatures (32°C) than it was in acidic urine (pH 4) at lower temperatures[15].

Cocaine: Huertas T *et al.*,[13] have evaluated the stability of four different cocaine metabolites in urine samples, including benzoylecgonine, ecgonine methyl ester, and benzoylecgonine ethyl ester. The researchers found that these compounds were stable under most of the storage conditions they examined, including different storage periods (1 year) and temperatures (-20°C and 4°C). However, they found that when the samples were held at pH 8 and at 4°C, the four compounds disappeared after a certain time. Specifically, the study have found that samples held at pH 8 and 4 °C, the cocaine and benzoylecgonine disappeared after 75 days of storage and ecgonine methyl ester disappeared after 15 days. The one and only exception was benzoylecgonine, which had a recovery of 23% after being stored for a year. The stability of cocaine and its metabolites in biological samples seems to be best preserved at a temperature of -20 degrees Celsius, which was explored as a possible storage temperature. Keeping the pH of urine samples at 4 is recommended for optimal results with this.

Vitreous Humor (HV): After death, vitreous humour, a collagen-based gel, transforms into a transparent liquid. It is taken with a needle, often 2-5 ml in a plastic bottle with a screw top; there is a strong link between the amount of xenobiotics in the blood and the volume of the solution[8]. Holmgren *et al.*,[16] and Bévalot *et al.*,[17] did two experiments with forensically important compounds to show that there was a strong link between the ratio of HV/blood concentrations and the proportion of adherence to plasma proteins. Some researchers have investigated the possibility of using a preservative such as sodium fluoride (NaF) or potassium fluoride (KF) to inhibit the activity of enzymes responsible for the formation or breakdown of certain xenobiotics despite the fact that HV is typically thought to be insensitive to things that happen after death[18]. Holmgren P *et al.*,[14] investigated the potential impact of potassium fluoride on the concentrations of 46 medicines in vitreous humour after one year of storage. There were considerably lower amounts of ethanol and zopiclone in samples that lacked potassium fluoride. For 23 chemicals, correlations between vitreous humour and femoral blood concentrations were found to be significant, suggesting that vitreous humour may serve as an alternate sample when blood samples are unavailable, assuming such a connection exists for the drug in question.

Benzodiazepines: Melo *et al.*,[19] investigated the effect of temperature on the stability of benzodiazepines in HV (lorazepam, estazolam, ketazolam, chlordiazepoxide). Six months of storage at negative temperatures (-20°C, -80°C) did not result in any appreciable deterioration. At +4°C and +25°C, most benzodiazepines were stable for a few weeks, but ketazolam was completely broken down after 12 weeks.

Nordiazepam was found to be the most unstable chemical when Wójtowicz A *et al.*,[20] tested the stability of psychotropic compounds like benzodiazepines and their chosen metabolites at various thermal storage settings (-20, 4 and 20°C).

Opioids: Holmgren *et al.*,[16] investigated the impact of the addition of KF on the stability of 46-molecule blood and vitreous concentrations. The HV samples were divided in half, and only one aliquot received the preservative. All aliquots were kept at -20°C for one year. 6-MAM, an opiate metabolite, would have only been detectable in HV with preservative.

The impact of the addition of preservative (1.5% NaF) was significantly more apparent, limiting the degradation of 6-MAM at -18°C for 84 days to less than 10%, while the degradation reached 42% on day 14 and 95% on day 84 in the absence of preservative. Similarly, the degradation at +4°C with preservative was less than 10% until D35, but it was 52% beginning on D14 without preservative[18].

Cocaine: The stability of cocaine in ovine HV was investigated by Rees *et al.*,[21] over the course of 84 days at three different temperatures: room temperature, +4 degrees Celsius, and -18 degrees Celsius. The researchers compared the stability of cocaine with and without the addition of a preservative (NaF). The cocaine concentration was stable at a temperature of -18 degrees Celsius for 84 days with or without a preservative (loss of less than 15%), but unstable at a temperature of +4 degrees Celsius, with a loss of between 25% and 50% beginning on the 14th day with or without NaF.

Cocaine and its metabolites were shown to be the most unstable drugs by Wójtowicz A *et al.*,[20] who evaluated the stability of cocaine and other selected products under varying thermal storage conditions (-20, 4, and 20°C).

Cathinone derivatives: Margalho *et al.*, were interested in the stability of new psychoactive substances (n=13) including mephedrone, methcathinone, and 2C-T2, as well as ephedrine. HV samples loaded with 14 molecules at two concentration levels (5 and 500 ng/mL) were frozen at -15°C for 7 days and subjected to 3 freezing/thawing cycles. The authors found that degradation was sufficiently low to fulfil the generally recognised acceptability criteria (degradation < 20%)[22].

3. Bile: After death, bile exists as vitreous humour for a few days before drying up. Frequently available when blood and urine are no longer accessible. Comparable to the vitreous humour, it has the benefit of being in an

environment that is reasonably protected from bacterial contamination.

Few investigations on the stability of bile samples according to storage conditions have been conducted. To prevent hydrolysis of glucuronoconjugated metabolites, the sample may be mildly acidified by adding a buffer (such as 1 M ammonium acetate pH 4.0/5.5 or sodium acetate 0.1 M pH 5) or acid (acetic or hydrochloric) at the time of collection[23,24].

Melo *et al.*,[19,25] investigated the stability of four benzodiazepines (lorazepam, estazolam, ketalozam, and chlordiazepoxide) in bile for up to six months at 8ºC, 2ºC, +4ºC, and +25ºC with or without the addition of sodium fluoride (NaF) as a preservative. To keep these molecules from degrading, it is advisable to collect them on a preservative, store them at 4°C for a short time, and freeze them for an extended amount of time. It was discovered that chlordiazepoxide is more stable in bile than in blood, which the scientists attributed to the fact that bile is an adverse environment for bacterial growth. In the absence of information about how compounds behave in bile, storage rules for compounds in other matrices can be used.

4. Gastric contents: The gastric contents are also systematically used as a sample in the context of determining the reasons for death. After isolating and incising the gastric pouch, it is often administered using a spoon from a plastic container. Ten millilitres (10mL) is enough for toxicological testing[8].

For oral fluid, collect 1-2ml in a suitable plastic bottle with preservative. It should be noted that the sample may be diluted owing to the presence of buffers, preservatives, or other chemicals in the collecting devices. This sample is still relevant for most xenobiotics, especially drugs of abuse[8].

5. Hair: At each autopsy, hair must be collected methodically, although its use is optional. Their study is not required in the investigation of death causes. Sometimes, hair analysis is useful for determining the background of a dead individual. Unlike all other toxicological tests, this sample is maintained at room temperature[3].

6. Viscera: Five viscera are usually removed: the brain, heart, lungs, liver, and kidneys[3]. For laboratory analysis, specific guidelines are in place for collecting and preserving samples of these organs. 30 grams of the brain, heart, liver, and kidney are to be collected in plastic containers with screw caps and without preservatives, while 30 grams of the lung must be collected in glass containers that are sealed with Teflon or aluminum foil-lined lids, also without preservatives. The use of glass containers for lung samples is particularly useful for detecting volatile xenobiotics such as toluene and nitric oxide[8].

7. Bones: When just a skeleton is discovered, the only accessible mediums are bones and hair. Consequently, they are alternate direct debits. This bone marrow is a safe place with a lot of blood vessels that allows certain molecules in the blood to be taken in and stored[26].

Because there is a possibility of differences in the distribution of xenobiotics between the intraosseous and interosseous areas, it is essential to collect thirty grammes of tissue in a plastic container fitted with a screw lid (but no preservative)[8,27].

Save any bone marrow samples you can find in a 10-milliliter plastic vial with a screw-on lid (no preservative). Variable quantities of xenobiotics have been found in marrow extracted from the same or different bones[28].

8. Synovial Fluid: Put whatever is left over after filling each cavity of a joint that has not been damaged into a plastic container that has a screw-on top and a capacity of 5 millilitres[29]; Because it does not include alcohol dehydrogenase , and because important quantitative data for ethanol have already been collected, it can only be used for qualitative analysis[30,31].

9. Skin: Skin (from a radius of 2–4 centimetres surrounding a needle stick or chemical burn) should be collected in a screw-capped plastic container (without preservative)[8].

CONCLUSIONS

Forensic toxicologists rely heavily on knowledge of drug stability in biological specimens because estimates of the quantity of potentially hazardous substances might change over time. Consequently, the chemical stability of many harmful compounds in biological matrices is contingent on storage conditions. Forensic scientists would consequently greatly benefit from having knowledge of sampling and storage techniques for suspected dangerous chemicals.

These results show how important it is to store samples and how pre-analytical concentrations could change during transportation and normal sample handling.

ACKNOLEDGEMENTS

The authors would like to acknowledge Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, Morocco to provide necessary facilities for this work.

AUTHOR'S CONTRIBUTION

ADOUANI B: literature survey, investigation. **RAHMOUNE I:** data analysis, review. **FILALI H:** writing, review, and editing, methodology. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

None to declare.

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