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Assessing Alternative Specimens for Toxicological Analysis: A Review

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ABSTRACT

The use of alternative matrices in toxicological analyses has increased in forensic toxicological analysis. Alternative samples to blood, visceral organs, and urine are useful for reliability and comprehensiveness regarding generating toxicological profiles. The article critically reviews recent literature on alternative matrices like oral fluid, hair, skeletal tissue, sweat, cerebrospinal fluid, meconium, breast milk, and vitreous humor and their applications in forensic toxicology. It analyzes the characteristics, advantages, limitations, and applications of alternative matrices in drug and poison analysis. This review article further discusses the characteristics of biological matrices used in forensic analyses as substitutes for whole blood, urine, and viscera samples. Each matrix type has advantages regarding sampling, preservation, extraction methods, detection time frame, drug levels, and other factors. However, these matrices also have limitations, such as limited drug incorporation, correlation with concentrations for effects, low xenobiotic levels, and the need for proper sample preparation and analysis. Although there is data on detecting traditional drugs of abuse in alternative matrices, information on the detection of emerging illicit drugs in these matrices is scarcer. Alternative biological specimens play a crucial role in forensic toxicology, but they come with distinct limitations and characteristics that must be considered. The results from analyzing these samples may differ based on the type of specimen. Alternative matrices will remain a focus in forensic toxicology due to their benefits and potential use when blood, urine, or visceral organs are inaccessible or impractical, particularly in cases like drug-facilitated sexual assault, burnt bodies, or decomposed remains.

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Introduction

The domain of forensic toxicology aims to apply methods of analytical chemistry, pharmacological science, and toxicology to investigate forensic casework[1]. The main step of forensic toxicological analysis is to test for the presence of any drugs or any other xenobiotics in cases like death due to poison, driving under the influence of any drug of abuse like alcohol, drug-facilitated sexual assault, death due to drug abuse, and many more from the traditional biological samples that are received in the forensic science laboratory, like whole blood, visceral organs, and urine samples. Alternative specimens are being explored mainly for their advantages, such as larger detection windows, easy collection, and the availability of conventional biological specimens[2]. However, the drug concentration may be low compared to urine or blood due to postmortem redistribution factors or toxicokinetics due to absorption, distribution, or elimination. Modern instruments have advanced in being highly sensitive, detecting even deficient levels of drugs, enabling xenobiotic detection in the alternative samples[3]. Some samples, like oral fluid and hair, have already been established and used in drug testing by forensic laboratories. Recent studies have applied these specimens to the analytical detection of novel abuse substances.

This article discusses less-reported biological samples like oral fluid, hair, skeletal tissue, sweat, cerebrospinal fluid, meconium, breast milk, and vitreous humor. They are considered for their advantages and limitations in forensic toxicological analysis.

Review of Alternative Specimens for Toxicological Analysis

Oral Fluid

Oral fluid combines saliva, gingival crevicular fluid, buccal and mucosal transudates, cellular debris, bacteria, and residues of ingested products[4]. The high perfusion of salivary glands allows for the rapid transfer of drugs from the blood to the salivary glands. Oral fluid collection is simpler, easier, and safer for patients and collection staff as it is painless because of the non-invasive nature of collection and avoids privacy issues[5]. Moreover, it reduces the opportunity for adulteration and substitution compared to urine testing. These factors contribute to the success of oral fluid testing. The transfer of drugs and metabolites from blood to saliva occurs primarily by passive diffusion and depends on various factors such as the chemical properties of the drug, salivary pH, concentration of un-ionized drug (ionized drug does not passively diffuse across cellular membranes), drug-protein binding (only the free fraction can diffuse), and membrane characteristics[6]. Improvements in sample preparation methods, such as microextraction techniques, and advances in analysis procedures, including gas chromatography coupled to mass spectrometry (GC-MS) or liquid chromatography coupled to mass spectrometry (LC-MS), are efficient even when the analytes are present in low concentrations[7]. The schematic workflow of oral fluid analysis is shown in Figure 1.

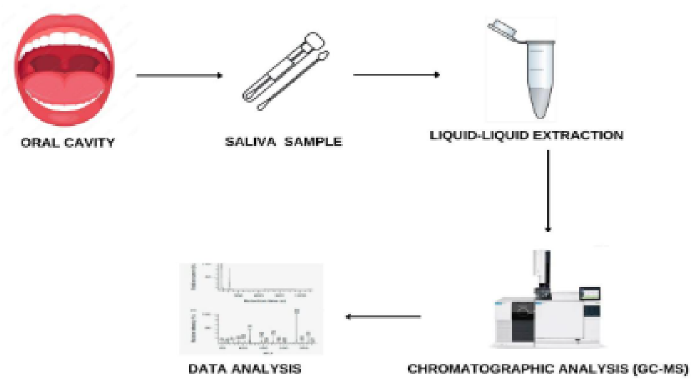

Fig. 1: Schematic workflow of oral fluid sample analysis

Da Cunha et al. demonstrated another use of oral fluid by screening 104 drugs of abuse, such as synthetic cannabinoids, synthetic cathinones, fentanyl analogs, phenethylamines, and other abused psychoactive compounds using liquid chromatography-tandem mass spectrometry (LC-MS-MS) for the screening[8]. In another application, oral fluid can be used for on-site or real-time monitoring for doping purposes. Bessonneau et al. collected oral fluid to monitor 49 prohibited substances in sports in vivo, including psychoactive drugs such as cannabidiol (CBD) and cannabinol (CBN), as well as methadone, heroin, strychnine, and fentanyl.[9]

Hair

Hair includes a shaft and a root. [10] The cross-section of a hair strand shows the cuticle, cortex, and medulla. The cortex contains keratin and melanin, making up 80–90% of the hair's weight. [11] Melanin has a hydrophobic and acidic nature, which makes this pigment responsible for the attraction of hair to basic drugs such as cocaine, codeine, and ketamine[12]. The exact processes involved in how drugs are incorporated into hair are not fully understood. The most preferred mechanism is that the drugs and their byproducts or metabolites enter the hair through passive diffusion via blood capillaries into the cells of the growing matrix at the base of the hair follicle[14]. These cells become keratinized as they grow, allowing drugs and toxins to deposit in the hair. The rate of hair growth varies according to age, sex, race, and health conditions, with growth rates of about 10 mm and 6 mm per month for scalp and pubic hair, respectively[15].

The advantages of hair analysis over other conventional biological samples are that it is an easy and non-invasive collection, requiring no specialized training. Therefore not infringing on an individual's privacy. The solid and durable hair structure ensures long-term stability during transportation and storage. Additionally, hair analysis poses a negligible risk of infection[16]. It allows for the assessment of retrospective and cumulative drug exposure over months to years because of its large window of detection for drugs incorporated into hair, helpful in cases like Drug facilitated sexual assault (DFSA) cases where it takes time for the victim to lodge complain out fear and trauma[17]. Furthermore, it enables the evaluation of chronic drug use through segmental analysis, where hair is cut into smaller pieces and analyzed separately. Forensic Hair Analysis has its limitations. It is unable to detect recent drug use within the past seven days[18]. Detecting very low drug concentrations requires accurate and sensitive methods. The schematic hair analysis workflow is shown in Figure 2. Additionally, the cost of analysis is higher compared to other biological samples. Hair is a significant alternative sample in forensic toxicology, allowing to examine the past drug use, depending on the length of the hair.

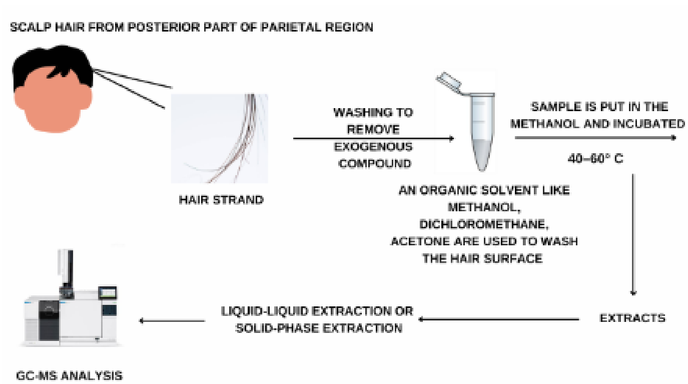

Fig. 2: Schematic workflow of hair sample analysis.

Skeletal tissue

The skeletal tissue is divided into two main categories: bone tissue and bone marrow, both of which can withstand putrefaction[19]. Bone marrow can be used as an alternative when blood is unavailable[17]. In a study, skeletal tissue was shown to be a viable alternative matrix. It was found that bone marrow showed significant potential, demonstrating an 80.5% concordance with blood for evaluating xenobiotic compounds, and for the study, blood, bone tissue, and bone marrow from various forensic cases were analyzed for 415 compounds of forensic interest. Sample preparation included solid-phase extraction (SPE) for whole blood and bone marrow, methanol extraction for bone sections, and protein precipitation for whole blood, and samples were analyzed using liquid chromatography coupled to a triple quadrupole mass spectrometer[20]. The bone marrow offers a viable alternative when sampling body fluids is not possible, or even in cases of mutilated and dismembered bodies.

Cerebrospinal fluid

Cerebrospinal fluid (CSF) is a colorless and clear body fluid found within the tissues that enclose the spinal cord and the brain[21]. There is about 125 mL of CSF at one time, and approximately 500 mL of CSF is generated daily[22]. CSF is almost protein-free. It is derived from blood plasma and is largely similar to it, except that it has some distinctive levels of electrolytes; CSF has a higher sodium level and a lower chloride level[23]. Wachholz et al. in their study on autopsy cases demonstrated that ethyl alcohol was detected in CSF using gas chromatography with a flame ionization detector (HS-GC-FID), and a high positive correlation was also found between blood ethyl alcohol concentration and cerebrospinal fluid collected from the spinal canal ethyl alcohol concentration[24]. It may be collected during autopsies, especially when other bodily fluids are unavailable. Collecting cerebrospinal fluid from the spinal canal is preferred because it is less likely to undergo postmortem changes until it becomes contaminated with blood due to the breakdown of the membrane surrounding the spine. Most significant xenobiotics like ethanol, benzodiazepines, opioids, cocaine, and other plant alkaloids have lipophilic properties, and they easily pass through the blood-brain barrier, resulting in the presence of these xenobiotics in the CSF[25].

Breast milk

Breast milk is the major source of nutrition for newborn babies. Human breast milk contains 0.8–0.9% protein, 4.5% fat, 7.1% carbohydrates, and 0.2% ash (minerals)[26]. Human breast milk provides a short detection window, allowing for the investigation of recent drug use by a mother within hours[27]. It also helps assess infant exposure to substances that could harm their cognitive and motor development. It has been reported that the use of LC-MS/MS for detecting human breast milk and the most common procedures for sample preparation techniques are SPE, protein precipitation, liquid-liquid extraction (LLE), and some miniaturized techniques[28].

The cannabinoids CBD and Delta-9-tetrahydrocannabinol (THC) were found using LC-MS/MS, accumulate in breast milk after maternal cannabis use for medicinal purposes, whether by inhalation or edible ingestion, and appear rapidly after exposure and may remain present for at least one to two days[29]. Recently, it was reported that microextraction techniques such as liquid-phase microextraction and electro-membrane extraction could be utilized for the determination of amphetamines and synthetic cathinones in human breast milk, providing good recovery (81–91% and 40–89% for electro-membrane extraction and liquid-phase microextraction, respectively)[30]. An illustration of human breast milk analysis is shown in Figure 3.

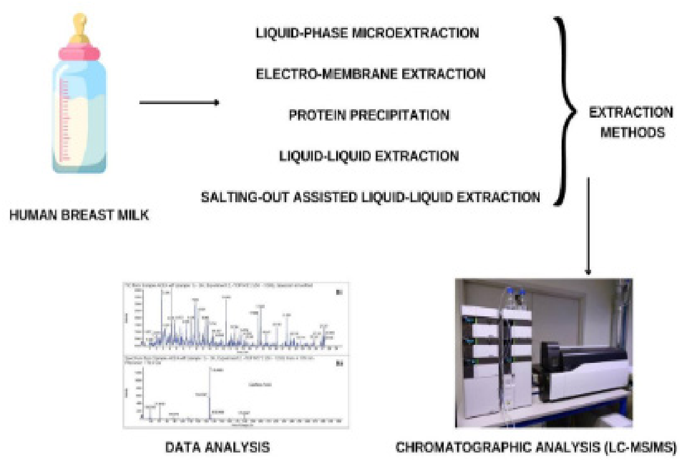

Fig. 3: Schematic workflow of human breast milk analysis.

Meconium

Meconium, the newborn's first stool, contains a variety of substances, including lipids, plasma proteins, tissue debris, enzymes, ions, hemoglobin metabolites (such as bilirubin and porphyrins), steroids, bile acids, sterols, and mucus glycoproteins[31]. These substances come from secretions of the fetal digestive system, including bile, pancreatic, and intestinal secretions, swallowed amniotic fluid, and shedding of cells from the mouth, digestive system, skin, lanugo hair, and vernix. It is thick, sticky, greenish-black in color, and lacks odor, accumulating in a baby's large intestine before birth and being excreted within 1–3 days after birth. (30) Drugs administered to pregnant women can be detected in meconium, providing valuable information about fetal exposure[31]. Meconium is easy to collect from diapers, and typical analysis involves LLE or SPE.[32] Drugs, whether illicit or licit, used by the mother during pregnancy can pass to the baby through the placenta mainly by passive diffusion[33]. These drugs can accumulate in meconium through bile deposition and swallowing of amniotic fluid[34]. Meconium has been used as an alternative way to test for prenatal exposure to drugs. However, it does not provide information about fetal drug exposure in the first trimester of pregnancy. Meconium can be contaminated by urine or traditional feces (milk stool). Meconium may be lost if it is excreted in utero. The author concluded that an analytical method for the determination of 137 new psychoactive substances (NPS) and other drugs of abuse in meconium by UHPLC-QTOF was developed and validated for semi-quantitative purposes. The method was applied to 30 meconium specimens from cases in which fentanyl had been administered as anesthesia during delivery or cases in which the maternal hair was positive for other drugs of abuse. Among them, four meconium samples tested positive for fentanyl, and two samples tested positive for acetyl fentanyl[35].

Vitreous humor

Vitreous humor (VH) is the gel-like, clear, colorless substance found between the crystalline lens and the retina, covering the center of the eyeball, providing shape, and maintaining eye pressure[36]. It comprises a transparent liquid containing between 98% and 99.7% water. The remaining part comprises sugars, salts, phagocytes, proteins, and a collagen network. Examining the eye's VH can provide information on exposure to drugs and chemicals orally, inhaled, parenterally, or through the skin. The anatomical location of the fluid in the eyeball protects it against the effects of changes occurring in the body after death. Based on experiments with many bacterial strains requiring different culture conditions, including Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Staphylococcus epidermidis, the sterility of cultures with vitreous fluid was evaluated[37]. This experiment investigated the properties of human vitreous as a substrate for supporting bacterial growth. The differences in the growth kinetics of Gram-positive and Gram-negative bacteria were checked. Based on this experiment, it has been proven that vitreous humor in vitro has special, natural antibacterial properties. The eye's VH is not exposed to the influence of other biological fluids; hence, it is useful for toxicological studies, as it can provide information on the concentration of some biologically active substances present in the blood 1–2 hours before death. An illustration of vitreous humor examination is shown in Figure 4. Cases involving highly decomposed or burned bodies are such examples where VH provides an alternate sample for blood or urine[13].

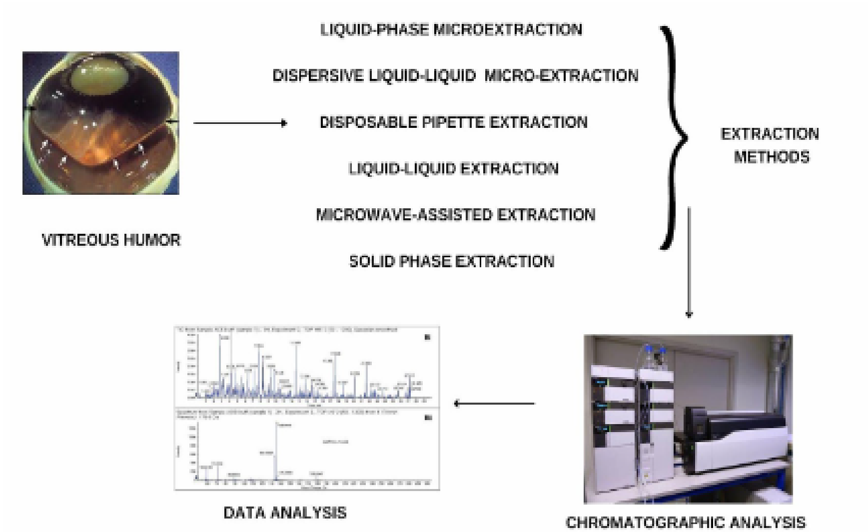

Fig. 4: Illustration showing analysis of vitreous humor.

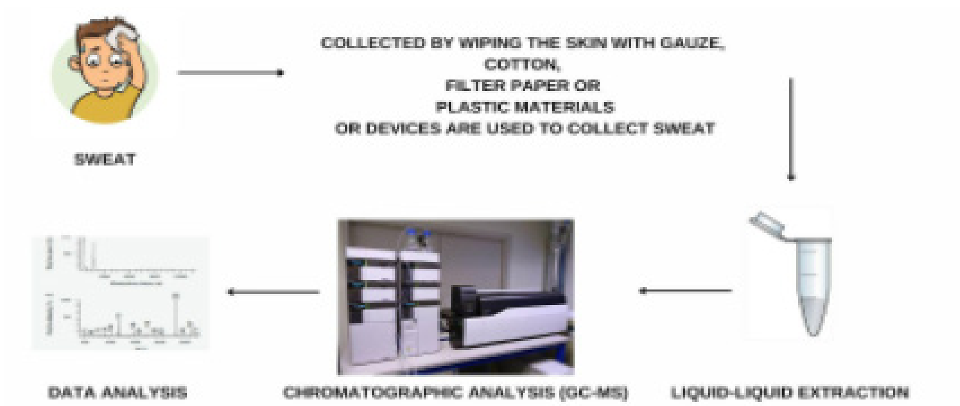

Fig. 5: Illustration showing analysis of sweat sample.

Sweat

The eccrine and apocrine sweat glands secrete sweat. It comprises electrolytes, water (99%), amino acids, lactate, carbohydrates, urea, and other organic compounds. The transfer of substances from the bloodstream to the sweat occurs via passive diffusion. However, substances can also migrate to the skin surface through skin layer transfer, known as transdermal migration. Sweat collection is simple and non-invasive. Sweat collection mainly involves swabbing the skin with sterilized cotton, gauze cloth, and filter paper[38]. The volume of samples is regulated by external factors like room temperature and physical exercise, and it is also regulated by internal factors like the variability of sweat production due to age, gender, or any underlying medical condition. To obtain the standard volume of sweat sample, specialized devices are also available to collect the sample. Sample preparation mainly involves liquid-liquid extraction with organic solvents. However, immunoassays can be performed on the sample subjected to confirmation by conventional chromatographic techniques[39]. The schematic workflow of the analysis of the sweat sample is shown in Figure 5. After oral administration, codeine and cocaine were detected after 4.5h-24h of drug intake, and the method developed presented limits of quantitation ranging from 1.25 to 2.5 ng/patch for all analytes[38].

Conclusions

The process of selecting specimens for toxicological analyses is critical. Understanding the target analyte and the matrix's properties is important for choosing the most suitable biological fluid or tissue for the forensic toxicological investigation. Alternative biological matrices are fluids or tissues that can offer additional information and advantages compared to blood, viscera, and urine examination, particularly in terms of simple and non-invasive sample collection, longer detection windows, and sample preparation and analysis complexity. Moreover, these samples can be collected and examined when traditional matrices are unavailable. However, each of these alternative matrices has unique characteristics, advantages, and limitations that must be considered. Forensic toxicology is expected to explore the use of alternative matrices increasingly in the future. Additional research is still needed to specify analytical procedures and to achieve a better understanding of the behavior of xenobiotics in these alternative specimens.

List of Abbreviations

DFSA: Drug facilitated Sexual Assault
GC-MS: Gas chromatography - Mass spectrometry
LC-MS: Liquid chromatography - Mass spectrometry
LC-TMS: Liquid chromatography–tandem mass spectrometry
CBD: Cannabidiol
CBN: Cannabinol
THC: Delta-9-tetrahydrocannabinol
SPE: Solid- phase extraction
CSF: Cerebrospinal fluid
HS-GC-FID: Head space gas chromatography with flame ionization detector
LLE: Liquid-liquid Extraction
UHPLC-QTOF: Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry
NPS: New psychoactive substances
VH: Vitreous humor

Author contributions

All authors contributed to the study design and conception, manuscript drafting, and approved the final draft of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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None

Ethical Clearance

Not applicable

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