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# ALTERNATIVE MATRICES FROM THE ORAL CAVITY AS A SOURCE FOR DRUG DETECTION

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**Abstract**

Forensic toxicology plays a crucial role in law enforcement by detecting xenobiotic substances in the human body, aiding in legal decisions. Traditional matrices like blood and urine have significant drawbacks, such as invasiveness, susceptibility to adulteration, and limited detection windows. This paper explores alternative matrices from the oral cavity, including oral fluid, teeth, dental plaque, and dental calculus, for drug detection. Oral fluid offers non-invasive collection and reliable pharmacokinetic data, while teeth provide long-term drug detection in postmortem cases. Dental plaque and dental calculus can trap substances over time, offering insights into past drug use with less invasive sampling. These matrices reflect the overall condition of the body and provide valuable forensic information. Continued research and technological advancements are necessary to optimize their application in various forensic scenarios.

***Keywords**: alternative matrices, forensic toxicology, oral cavity*

***Abstrak***

*Toksikologi forensik mempunyai peran krusial dalam penegakan hukum dengan mendeteksi zat asing di tubuh manusia, yang membantu dalam putusan hukum. Matriks yang sering digunakan seperti darah dan urin memiliki kekurangan antara lain bersifat invasif, rentan terhadap pemalsuan, dan periode deteksi yang terbatas. Jurnal ini mengulas beberapa matriks alternatif dari rongga mulut, seperti cairan rongga mulut, gigi, plak, dan kalkulus, untuk deteksi obat-obatan. Cairan rongga mulut mempunyai keunggulan, yaitu pengambilan sampel yang non-invasif dan data farmakokinetik yang baik, sementara sampel gigi menyediakan deteksi obat jangka panjang pada kasus postmortem. Plak dan kalkulus dapat menyimpan berbagai macam zat seiring berjalannya waktu, memberikan gambaran tentang penggunaan obat di masa lalu dengan pengambilan sampel yang non-invasif. Seluruh matriks tersebut dapat mencerminkan kondisi keseluruhan tubuh dan memberikan informasi forensik penting lainnya. Namun penelitian dan pengembangan teknologi lebih lanjut diperlukan untuk mengoptimalkan penerapannya dalam berbagai kasus forensik.*

***Kata Kunci**: rongga mulut, matriks alternatif, toksikologi forensik*

## 1. INTRODUCTION

Forensic toxicology plays a crucial role in law enforcement by detecting the presence of xenobiotic substances in the human body, thereby aiding in legal determinations (Dinis-Oliveira et al., 2010). Establishing the relationship between these substances and the individual is essential, whether they are prescribed or illicit, and determining the dosage, timing, and duration of exposure (Adatsi, 2005). However, forensic toxicologists face numerous challenges, such as compromised samples and the emergence of new substances (Chung & Choe, 2019).

Blood and urine are traditional matrices for drug analysis but have significant drawbacks. Blood samples require rapid analysis to prevent lysis and are invasive to collect (Jones et al., 2022). Urine samples are prone to substitution and adulteration due to the difficulty in supervising collection (Gallardo & Queiroz, 2008). Additionally, forensic toxicologists often encounter decomposed or skeletonized remains, making sample collection challenging (Dinis-Oliveira et al., 2016). Conventional matrices also have limitations in the detection window for certain substances (Greco et al., 2023).

Alternative matrices are introduced to overcome these challenges as it can provide more insight of a particular substance, stability, and the possibility of supervised sample collection (Manousi & Samanidou, 2021). With the development of technology, the process of analyzing different matrices other than conventional blood sampling is possible nowadays (Frederick, 2012). Matrices such as vitreous humor, brain, heart, bile, hair, and bones may be an alternative choice for analysis (Rodda et al., 2018). However, these matrices are not always applicable to any situation, especially sampling for the living as it requires a less invasive method while also under supervision.

The dynamic and complex relationship between individual and microbiome of the mouth may contribute to the use as a diagnostic tool for the person's overall condition (Faran Ali & Tanwir, 2012). The oral cavity has been linked as an indicator of an individual's overall health (Hasan et al., 2020). One of the most popular alternative matrices from the oral cavity is oral fluid, especially for providing easy collection and testing (Lillsunde, 2008). Normally the oral cavity will always be covered by oral fluid, thus other parts of the mouth may also contain its composition. As the beginning of the digestive system, the oral cavity plays a crucial role in the digestion of food or any other substances taken orally (Çelebi & Yörükan, 1999).

Based on these findings, the oral cavity holds a lot of information as it can reflect the overall condition of one's body and also entrapped substances passing through ingestion. Other matrices in the oral cavity may contain xenobiotics and have the potential to be used as alternative samples in forensic toxicology analysis. This paper aims to review a series of publications on the use of alternative matrices from the oral cavity for drug detection, as well as presenting some of the known advantages and drawbacks of each matrix.

## 2. METHOD

This literature review was conducted using the PubMed search engine. Keywords such as "alternative matrices," "drug detection," "oral," "forensic," and "toxicology" were used to narrow down the publications. Only English-language publications from 2013 to 2023 were included. Both authors evaluated and screened the publications for relevance, analyzing selected publications to identify similarities, gaps, advantages, and drawbacks of each method.

## 3. RESULTS & DISCUSSION

### Oral Fluid

Oral fluid (OF) has gained popularity as an alternative matrix for forensic and clinical use due to its easy and non-invasive sample collection, which can be supervised to avoid adulteration or substitution (Langel et al., 2008). OF consists mainly of saliva mixed with gingival crevices, bacteria, food debris, blood components, and traces of drugs and/or their metabolites (Aps & Martens, 2005). OF can be collected both for antemortem and postmortem sampling. Drug detection in OF is possible due to the passive diffusion of unbound drugs from the blood to the OF (Desrosiers & Huestis, 2019). Drug concentration in oral fluid often correlates closely with blood concentrations, providing reliable data on the pharmacokinetics of various substances (Lee & Huestis, 2014). However oral or inhalation intake of substances may contaminate the oral cavity and interfere with the analysis result. Some substances may also reduce the amount of OF secretion (Bosker & Huestis, 2009).

The use of OF provides the possibility for routine prescription drug screening. Chen et al. (2019) explored the use of OF for monitoring selegiline (SG), a drug for Parkinson's disease, and its metabolites. The study found that peak concentrations of SG and its metabolite were higher in OF than blood plasma. Although SG is rapidly eliminated from OF, its metabolite can still be detected for a longer duration, and the metabolite/parent (M/P) concentration ratios can help estimate the timing of SG administration. Similarly, Soares et al. (2021) developed and validated a method for quantifying antidepressants and their metabolites in OF using dried saliva spots (DSS) method. This method simplifies sample collection and preparation, making it suitable for routine clinical and forensic toxicology applications. Understanding the pharmacokinetics of substances can benefit forensic toxicologists and drug treatment programs, as the non-invasive collection allows for easy routine drug monitoring.

OF can effectively detect recent drug use, often reflecting the presence of drugs more accurately during the immediate period following consumption compared to urine. This is particularly useful for roadside testing for driving under the influence (Martini et al., 2020). Øiestad et al. (2013) and Palmquist & Swortwood (2021) each developed a method for detecting synthetic cannabinoids and fentanyl in driving under the influence of drugs (DUID) cases. Truver et al. (2019) conducted a study comparing OF and urine samples with Drug Recognition Expert (DRE) observations in DUID cases, finding that evaluator opinions were confirmed in 90% of OF cases. These studies demonstrate the potential of OF as well as the development of useful methods for rapid toxicological analysis.

The rapid emergence of new substances presents challenges in forensic toxicology. The use of synthetic cannabinoids is growing rapidly along with the market changes and their high potency makes detection crucial. Øiestad et al. (2013) and Blandino et al. (2018) each validated a method for screening synthetic cannabinoids. Øiestad et al. (2013) was able to detect several synthetic cannabinoid parent compounds in OF using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS). Blandino et al. (2018) found positive results of AB-FUBINACA in five OF samples from HIV-positive and self-identified recent users of synthetic cannabinoids, although urine samples from the same group presented more synthetic cannabinoids. These studies show promising results, but further research is needed as detection of certain drugs are still limited and the need for optimization of methods for specific target substances.

New methods have been introduced to increase sensitivity and reliability of OF in forensic toxicology. Reinstadler et al. (2019) developed and validated a non-targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) workflow for comprehensive drug detection in OF samples. This approach allows for the identification of a wide range of compounds, including new psychoactive substances (NPS). However, difficulties in detecting very polar or apolar compounds and the reliance on reference spectra availability need improvement to provide a more accurate result. Florou et al. (2022) developed a method for quantifying brorphine, a novel synthetic opioid (NSO) in OF using fabric phase sorptive extraction (FPSE) combined with LC-MS/MS, offering a reliable approach for detecting NSOs in alternative biological matrices. These advancements aim to address current challenges and provide simple, rapid, and reliable methods for routine forensic and clinical toxicology applications.

Proper sampling, storage, and stability of substances in OF are crucial for accurate forensic toxicology analysis. Substances may undergo hydrolysis at room temperature, compromising sample quality. Miller et al. (2017) evaluates the stability of synthetic cathinones in OF under different storage conditions, while Marchei et al. (2020) investigated the stability and degradation pathways of various psychoactive drugs in OF. Their studies showed that storage in 4°C improved stability but still resulted in some quality loss, whereas storage at −20°C provided stability for all substances. The use of buffers increased stability, especially in higher temperatures, allowing OF to be stored for up to one year while maintaining reliable results.

When compared to other matrices, OF usage has mixed results. Fiorentin et al. (2018) evaluated the relationship between cocaine and its metabolites in OF, urine, and plasma collected from cocaine users, showing large variability in drug concentrations in OF.

Blandino et al. (2018) found similar results when comparing synthetic cannabinoids in OF and urine. While five OF samples tested positive for AB-FUBINACA, only one urine sample matched, and four urine samples showed multiple synthetic cannabinoids. Classic drugs were also subjected to testing and the results showed variability in OF detection results. Truver et al. (2019) compares OF and urine samples with DRE observations in DUID cases, confirming evaluator opinions with 90% occurrences of OF cases and 85% of urine cases. The differences in results could be due to the varying methods, target substances, sample handling, as well as detection windows of each matrix. While OF is useful for detecting substance use, further investigation is still needed due to significant variability. Multiple biological matrices and comprehensive toxicological analyses are essential for accurately assessing drug use.

**Teeth**

Bones and teeth are among the last body parts to disintegrate after death, with teeth being particularly resilient to extreme environmental changes such as high temperatures and decomposition (Higgins & Austin, 2013). Several studies have demonstrated the viability of teeth as a matrix for drug analysis, especially in decomposed (Ichioka et al., 2023) and exhumed (Cippitelli et al., 2018) cases, proving teeth can be a viable alternative matrix for postmortem toxicology when other matrices are unavailable. In a case where only skeletal remains are available, teeth can serve as a crucial resource for toxicological analysis.

Spinner et al. (2014) and Klima et al. (2023) investigated factors affecting drug deposition in dentin using in vitro models. Both studies found that drugs are incorporated into dentin through both the blood supply in the pulp (perfusion) and through contact with oral fluid on enamel and exposed dentin (counter perfusion). Klima et al. (2023) further demonstrated that perfusion causes drug concentrations in dentin to increase with the duration of contact, reaching a plateau after 15 days, while counter perfusion results in lower concentrations compared to regular perfusion. These studies suggest that drug deposition occurs primarily via the blood vessels in the pulp, with the inherent durability of teeth allowing them to withstand numerous physically damaging events, thereby preserving the analyzable blood vessels in the pulp (Saxena et al., 2017).

Teeth have proven to be stable materials over time, making them valuable for long-term drug detection and providing a unique retrospective window (Klima et al., 2016). Ottaviani et al. (2017) developed a method for toxicological analysis on a single tooth from a known drug addict, demonstrating that teeth can retain drugs for extended periods of time. Ichioka et al. (2023) found that carbamazepine (CBZ) in the teeth of decomposed rat models remained stable up to 15 days postmortem, further supporting the utility of teeth in postmortem analysis and in constructing a biological profile of the deceased.

When compared to other samples such as body fluids and hair, dental tissue may still detect drugs but at lower concentrations (Klima et al., 2016). Cippitelli et al. (2018) compared the toxicological analysis of liver, kidney, hair, and teeth from an exhumed body, finding that teeth showed the lowest concentration of morphine but a slightly higher concentration of 6-Monoacetylmorphine than hair. These findings suggest that teeth are suitable for post mortem sampling, while for living individuals, the invasive nature of tooth extraction makes other matrices more practical for toxicological analysis.

*Dental Plaque*

Dental plaque is a biofilm embedded in a polymeric matrix on the tooth surface, containing a complex microbial community (Marsh, 1994). It is primarily composed of water, bacteria and polysaccharides, salivary proteins and glycoproteins (Marsh & Bradshaw, 1995). Plaque can form both supragingivally and subgingivally (Rosan & Lamont, 2000). While mechanical removal through brushing or flossing can help reduce plaque, its formation rate is still influenced by eating patterns and oral hygiene habits (Marsh & Bradshaw, 1995).

Henkel et al. (2018) developed and validated a method for extracting and quantifying ten drugs of abuse from non-mineralized dental biofilm (plaque) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method was tested on three postmortem cases with known drug histories, demonstrating high sensitivity and reliability. Later, Henkel et al. (2022) reused this method to detect methadone, morphine, and its metabolite, comparing opioid replacement therapy (ORT) patients with postmortem cases. Both studies showed that dental plaque is effective for detecting a range of drugs, applicable in both living and postmortem scenarios. Henkel et al. (2022) also found that parent drugs in dental plaque could be detected at concentrations up to two orders of magnitude higher in postmortem cases, though not all metabolites were detected. Normorphine, for example, was only found in cases of severe morphine intoxication.

Dental plaque forms after food consumption, making it readily available for sampling. It is relatively easy and less invasive to collect (Riedel et al., 2023). Henkel et al. (2017) demonstrated that dental plaque could detect more drugs than the femoral blood, and the metabolite/parent drug ratio could indicate the route of administration, with lower ratios suggesting oral intake and higher ratios indicating intravenous use (Henkel et al., 2022).

Variety of drugs can be incorporated into dental plaque directly after oral or intranasal consumption or indirectly via oral fluid (Henkel et al., 2022). However, interindividual variability can affect drug detection results. Riedel et al. (2023) used intraoral splints with demineralized bovine enamel on healthy volunteers in an in-situ study to further investigate this variability. The study showed that the intensity, duration of drug exposure, and concentration significantly impact drug detection. Additionally, the formation and composition of dental plaque depend on individual factors such as oral hygiene and dietary habits (Ten Cate, 2006). The location of sampling might also affect drug composition, as each biofilm can have a different structure and makeup (Marsh & Bradshaw, 1995).

*Dental Calculus*

Archaeological studies have long utilized dental calculus to gain insights into dietary habits, lifestyle, and pathological conditions (Mackie et al., 2017). Dental calculus is a mineralized microbial plaque that forms on the surface of teeth (Hillson, 2008). Its rough surface promotes the development of more plaque (Hardy et al., 2012). During the mineralization process, substances passing through the oral cavity and present in saliva are trapped and fossilized within the calculus layers (Radini et al., 2017). Although dental calculus analysis has become routine in archaeological research, its use in forensic science remains uncommon (MacKenzie et al., 2023).

Sørensen et al. (2021) developed a sensitive method for detecting drugs and their metabolites in dental calculus using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). In a study involving dental calculus and whole blood samples from ten corpses undergoing forensic autopsy, a total of 131 drugs were detected in the dental calculus, compared to 117 in whole blood. Notably, 82 drugs were found at higher concentrations in dental calculus than blood.

This study underscores the value of dental calculus in providing information on past drug intake, which may not be detectable in blood at the time of autopsy. Dental calculus is known for its longevity and stability (Hershkovitz et al., 1997), making it a viable alternative matrix in cases where only skeletal remains are available. Sampling dental calculus is relatively non-invasive and can be performed under close supervision. In forensic settings, this method offers the potential for sampling in living individuals, as dental calculus is considered an ectopic growth that needs to be removed in normal dental care (Mackie et al., 2017).

The accumulation rates of dental calculus can vary significantly among individuals due to factors such as oral hygiene, age, systemic health, diet, and ethnicity (Forshaw, 2022). While dental calculus can provide information on past drug intake, it does not offer the precise timeline that hair analysis can provide (Sørensen et al., 2021). Despite these limitations, dental calculus holds significant potential as an alternative matrix for forensic toxicology, though further research is needed to explore its applicability in various forensic scenarios.

## 4. CONCLUSIONS

Alternative matrices from the oral cavity, such as oral fluid, teeth, dental plaque, and dental calculus, offer valuable options for drug detection in forensic toxicology. These matrices provide non-invasive or minimally invasive collection methods and can detect a wide range of substances, reflecting both recent and historical drug use. Continued research and method development will further enhance the reliability and applicability of these matrices in various forensic scenarios.

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