Review

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Recent advances of drugs monitoring in oral fluid and comparison with blood

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Abstract: The use of alternative matrices in toxicological analyses has been on the rise in clinical and forensic settings. Oral fluid (OF), as non-invasive fluid, has attracted attention in the field of drug screening, both for therapeutic and forensic purposes, as well as for medical diagnosis, clinical management, on-site (real time) doping and for monitoring environmental exposure to toxic substances. A good correlation between OF and blood is now established for drug concentrations. Therefore, OF might be a potential substitute of blood, especially for long-term surveillance (e.g., therapeutic drugs) or to screen a large number of patients, as well as for the development of salivary point-of-care technologies. In this review, we aimed to summarize and critically evaluate the current literature that focused on the comparison of drugs detection in OF and blood specimens.

Keywords: alternative matrix; blood; drugs of abuse; drugs testing; saliva.

Introduction

The use of alternative matrices in toxicological analyses has been on the rise in clinical and forensic settings. Drug testing has been traditionally performed in whole blood, plasma, serum and urine specimens, which are considered conventional biological fluids. More recently, oral fluid (OF), as non-invasive fluid, has attracted attention in the field of drug monitoring, both for therapeutic and forensic purposes [1, 2], as well as for medical diagnosis, clinical management [3–6], on-site (real time) doping [7] and environmental exposure to toxic substances [8–10]. The term 'oral fluid' refers to the clear, slightly acidic, hypotonic and mucoserous exocrine biological matrix excreted by the major salivary glands (i.e., submandibular, parotid and sublingual) as well as from the multitudes of minor salivary glands (300–1,000 units) distributed throughout the oral mucosa. The latter can be divided into labial, buccal, palatal, lingual and retromolar glands [11–14], at a rate of 0.5–1.5 L per day. OF consists mainly of water (approximately 99 %), electrolytes, proteins including enzymes and immunoglobulins, DNA, epithelial cells, bacteria, food debris and traces of drugs found in the oral cavity; this composition differs the OF from the *mere* saliva which is the fluid collected from a specific salivary gland and is free from other constituents present in the mouth [15, 16] (Figure 1A).

The OF is considered a direct filtering of blood because the salivary glands are highly vascularized [17]. The main mechanisms by which drugs pass from blood into the OF are the passive diffusion (hydrophobic compounds) and the ultrafiltration (low molecular hydrophilic substances) [18]. Drugs are usually present in their free fraction forms since the bounded drug may not infiltrate through the salivary tissues [16].

Whole blood is the most common biological fluid used for drug confirmation and quantification analysis in driving under the influence of drugs (DUID) due to a good correlation between blood drug concentrations and the pharmacological effects. However, the collection of blood samples is invasive, and it requires qualified medical personnel.

Conversely, OF collection is convenient as it is non-invasive, less intrusive than blood sampling, and does not require trained personnel. Additionally, OF sampling can be done under direct supervision without intrusion of

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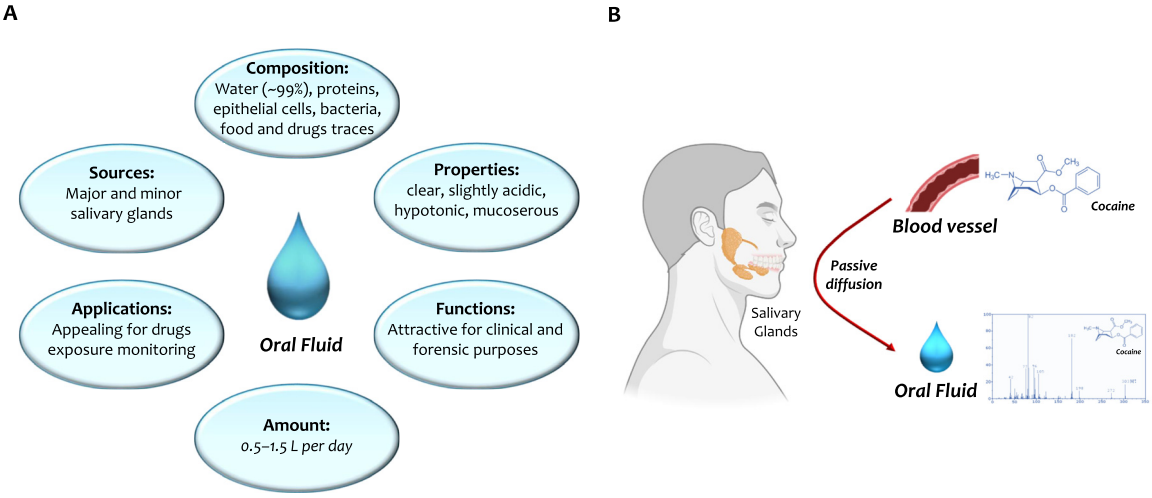

Figure 1: Characteristics of oral fluid and drug diffusion from blood. It is referred to as 'Oral fluid (OF)' the clear, slightly acidic, hypotonic and mucoserous biological fluid, excreted by the major salivary glands (i.e., submandibular, parotid and sublingual) as well as from the multitudes of minor salivary glands (300–1,000 units), at a rate of 0.5–1.5 L per day. OF is constituted mainly of water (approximately 99 %), electrolytes, proteins including enzymes and immunoglobulins, DNA, epithelial cells, bacteria, food debris and traces of drugs found in the oral cavity (A). The OF is a direct filter of blood because the salivary glands are highly vascularized. The main mechanisms by which drugs pass from blood into the OF are the passive diffusion (hydrophobic compounds) and the ultrafiltration (low molecular hydrophilic substances) (B). OF is becoming an attractive alternative matrix in toxicological analyses and drug monitoring.

privacy or can be performed by the patient himself. Compared to other biological fluids (i.e., urine), the likelihood of adulteration is significantly reduced. Detection times of several drugs in OF are similar to blood [19], and, for some of them, positive findings in OF have also been shown to correlate with pharmacological effects [20]. Even though the detection time window of drugs is similar in both matrices, the OF drug concentrations are not just a simple reflection of the total amount of drug in blood. Indeed, the transfer from blood to OF is affected by several physicochemical factors such as pH of the matrices, pKa, lipid-solubility, molecular weight, spatial configuration and protein binding of the drug compound. Blood pH is quite constant at 7.4, while the pH of OF is slightly acidic (pH 5.8–6.8) and it can also significantly fluctuate between individuals. The variations in pH value of OF may affect the final concentration of drugs. This explains why weak basic drugs tend to be ionized and, consequently, present in higher concentrations in OF (referred as ion trapping) [21, 22]. It is important to mention that many psychoactive drugs including cocaine, opiates, and amphetamines have an elevate pKa [22]. In addition to physicochemical factors, the collection method and the route of drug administration can affect the OF drug concentration [23]. Some OF collection techniques may stimulate salivation, which can change the salivary pH and dilute the sample. Drugs that are insufflated, smoked (such as cocaine, nicotine and heroin), or taken orally may contaminate the oral cavity, resulting in increased concentrations and thus justifying their poor correlation between OF and blood during the first half-hour after intake [24]. Therefore, disadvantages of OF testing include the difficulty in collecting proper volumes (i.e., available small sample volume), reduced salivation after intake of drugs with sympathomimetic properties, oral cavity contamination after *per os* or smoked administration and, finally, low concentrations requiring high sensitivity. Indeed, due to inherent characteristics, drug levels in OF may be reduced in comparison to blood or urine. For this reason, only recent improvement in instrumental technologies as well as in extraction and analysis procedures, have made possible its deeper exploration and analysis.

The utility of OF has been explored as a tool to assess compliance, monitor drugs of abuse and evaluate the presence of both therapeutic and illicit drugs in the clinical management of patients receiving a pharmacotherapy, during preanesthetic assessment and also in the emergency room [25, 26]. The drug analysis in OF is a potential substitute for blood testing, especially for long-term monitoring (e.g., therapeutic drugs) or to screen a large number of patients, as well as for the development of salivary point-of-care technologies. Although OF has been studied less extensively rather than conventional matrices, we aimed to summarize and critically evaluate the current literature that focused on the comparison of drugs detection in paired OF and blood specimens especially related to their detection time.

Materials and methods

Eligibility criteria

Only studies written in the English language and published between January 2002 and December 2022 (20 years) were included. To satisfy the primary aim of the present review, the following additional inclusion criteria were applied: (1) studies reporting the detection of drugs in OF and (2) studies reporting the comparison of paired OF and blood specimens for the detection of drugs. All studies which do not satisfy the inclusion criteria were excluded.

Information sources and search strategy

A comprehensive literature research was conducted electronically via PubMed and Scopus bibliographic databases by two independent authors (S.C. and M.B.). A manual evaluation of the reference lists of all selected full-text articles was further conducted to complement the electronic search. The purpose was to identify all the available pertinent information on the detection of drugs of abuse in OF and their correlation with conventional matrices. In all databases, the date of coverage was from January 2002 to December 2022 (20 years). The most recent search was performed on 31/12/2022.

For the electronic search, specific keywords, medical subject headings [MeSH], and other terms not indexed as MeSH were combined to search all relevant studies. As such, publications were screened according to the following search query adapted to each database: (drugs of abuse OR drug abuse OR substance abuse OR abuse* OR psychotropic drugs) AND (detection OR finding OR investigat* OR detect* OR test OR test* OR detect*) AND (saliva OR saliv* OR spit OR spittle saliva OR oral fluid*) as either keywords or MeSH terms. Additional screening of the reference lists of all pertinent articles and of recent literature reviews on the topic was performed to identify further relevant studies.

Selection criteria

Primary screening of the titles and abstracts was performed by adding studies of any level of evidence published in peer-reviewed journals written in the English language. During this step, duplicates, abstracts, conference presentations, editorials, and expert opinions were also removed. Data regarding the type of analyte(s), the type of matrix(ces), the type of sampling, the analytical technique(s) and the timeline of sampling were included.

Recently proposed methods and applications in OF analysis

The general methods for analyzing therapeutic and illicit drugs in different biological fluids are based on a combination of efficient separation procedure with a sensitive detection technique. At present, numerous separation techniques, including high- or ultra-high-performance liquid chromatography (LC or UHPLC) [27–33], gas chromatography (GC) [34] and capillary electrophoresis (CE) [35, 36], have been employed for their analysis. Various detection methods have also been coupled to separation techniques in order to obtain an accurate and sensitive drugs determination and quantification such as: fluorescence [36, 37], diode array [26], UV adsorption [38, 39], and mass spectrometry (MS) [26–34]. Immunochemical methods, mainly enzyme-multiplied immunoassay technique (EMIT) and enzyme-linked immunosorbent assay (ELISA) [40], are generally used as screening tools. Positive samples from the screening test are then confirmed by a more specific technique such as GC-MS or LC-MS. According to internationally accepted criteria in the forensic toxicology field, all the data reported in this review were obtained by confirmatory analysis using chromatography coupled to mass spectrometry (GC-MS or LC-MS).

Results

Initially, 3,634 articles were identified, 2022 in PUBMED and 1612 in SCOPUS. Following the evaluation of titles and

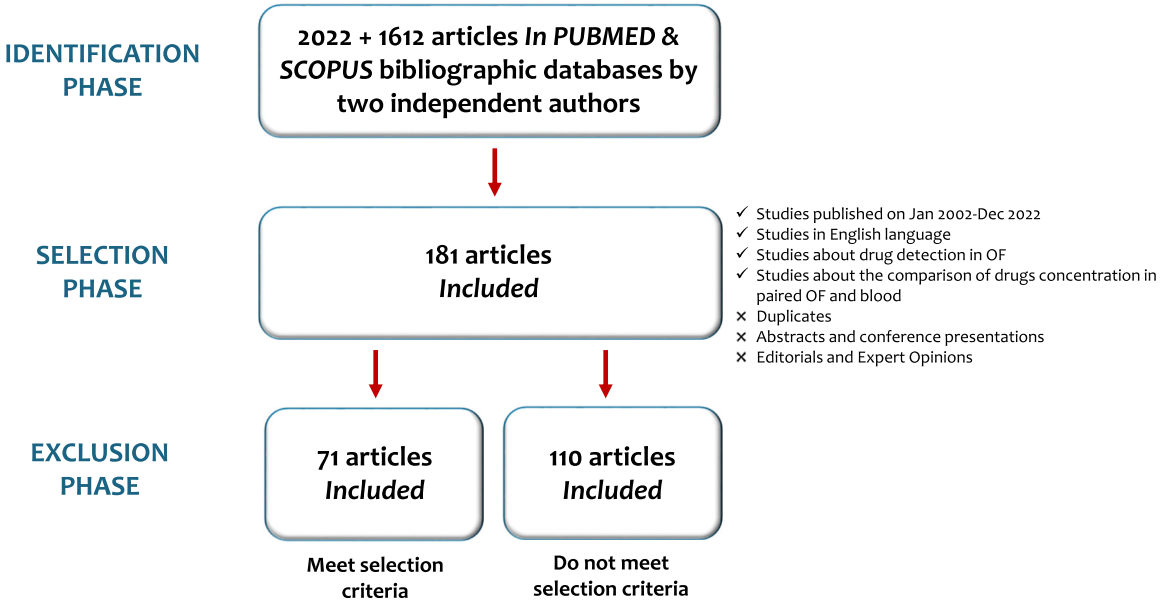

Figure 2: Information sources and search strategy: Identification, selection and exclusion phases. We have considered studies published on Jan 2002–Dec 2022 and in English language that investigate the possibility to detect drugs in OF and compare their concentration in paired OF and blood. Duplicates, abstracts, conference presentations, editorials and expert opinions have been excluded.

abstracts, 181 publications were included. Overall, 110 articles were excluded after reading the full text because not meeting the selection criteria. Finally, 71 studies focused on the comparison between OF and blood were included after the review process (Figure 2).

The analysis of articles showed that the main classes of drug tested in this comparison were: cannabinoids, opiates, cocaine, amphetamines, benzodiazepines and barbiturates.

In detail:

- 11 articles evaluated the pharmacokinetic and biodistribution of THC and its metabolites,
- 8 articles focused on opiates and their main metabolites detection,
- 4 studies assessed the opportunity to measure cocaine and its metabolites,
- 7 articles have faced the dosage of amphetamines after ingestion or inhalation,
- 1 study has compared the concentrations of benzodiazepine,
- 1 article described the assessment of barbiturates.

Discussion

In the last years, drug testing with saliva or OF has gained interest for the screening of individuals accessing to emergency room with different purposes. The aim of this screening is the identification of substances of abuse or drugs potentially related to patients' status, to prevent diseases by population control and epidemiological studies. Moreover, OF testing may be useful for pharmacotherapy and routine traffic control with the purpose to reduce serious accidents and consequently the social costs for the national health systems. The moving forward in technological processes has enabled the introduction of sensitive quantitative methods for the drug monitoring, raising the possibility to use OF in routinely drug testing programs [15, 20, 41]. The progress in analytical methods may improve the predictive value of oral tests, reducing the number of false positive (i.e., discriminating the different enantiomers as in the case of methamphetamine) and may ameliorate the correlation between oral and blood concentrations, although changes in salivary composition or protein binding led to higher variability compared to blood matrix. Indeed, several biological factors may possibly affect drug concentrations in OF, such as the route of administration, salivary pH, lipophilicity and pKa of the drug [33]. Nonetheless, several studies reported a deep correlation between OF and systemic concentrations, to the point that drug availability in OF run parallel to those in blood, mirroring their timing profile (Figure 3).

Notably, OF represents a valid non-invasive and self-collected route for drug screening, but it harbors possible disadvantages, including difficulty in collecting adequate sample volume and addition of preservative buffers that dilute specimens [42]. Thus, it is imperative to accurately assess the pharmacokinetic properties of the different substances of abuse in both OF and blood, with the purpose to better outline whether OF mimics systemic availability. Of note, oral exposure to illicit analytes is frequent, but differently from other routes, it requires a slower absorption and the bioavailability may be lower due to first-pass metabolism appearing in serum after hours [43]. Overall, in the screening of some specific populations the OF is preferred to other biological fluids [44]. An important question that remains open is the correct evaluation of the window of detectability in OF, which is strictly dependent on the intrinsic molecular properties of the drug [22].

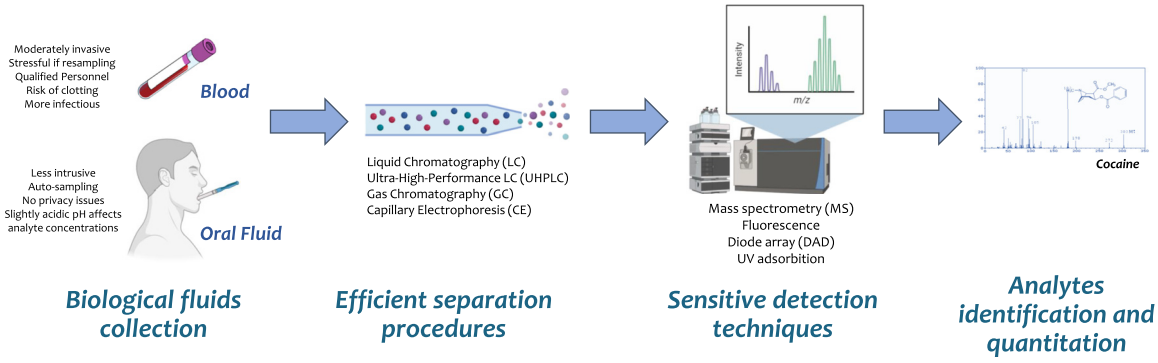

Figure 3: Workflow for drug monitoring starting from OF and blood and their Pros and Cons. Schematic illustration of the analytical workflow for drugs monitoring starting from collection of salivary or blood samples by patients; analytes separation through chromatography techniques or capillary electrophoresis, coupled to sensitive detectors for their identification and quantification. The advantages and disadvantages to exploit OF or blood as biological fluids to assess the presence and the quantity of drugs were reported. Compared to OF, blood sampling is moderately invasive and required trained personnel. The resampling is more stressful and dangerous for the operators. Concerning the OF, a possible disadvantage is due to its intrinsic properties that may affect the analyte concentration.

Cannabinoids

Among the substances of abuse, cannabinoids are the first for consumption both in Europe and United States, according to World Health Organization (WHO). This finding is ever increasing with the legalization in several states and after its introduction in clinics for medical purposes.

With regard to cannabinoids, the main analytes that have been researched in the cited works are: Δ9-Tetrahydrocannabinol (THC), 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), 11-Hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC), Tetrahydrocannabivarin (THCV), Cannabidiol (CBD), Cannabinol (CBN), Cannabigerol (CBG). In all manuscripts, a known dose of THC was administered via different routes such as: smoked, vaporized or eaten through brownies.

In particular, Spindle et al. [40] studied how concentrations of THC and THCCOOH vary, within biological matrices, depending on the method of administration (smoking, vaping) in 17 healthy adults who were infrequent users of cannabis. Cannabinoids were quantified by enzyme-linked immunosorbent assay (ELISA) and LC–MS/MS and OF was collected via expectoration. The highest concentrations of cannabinoids were detected both in OF and blood ten minutes after drug administration. THC was measurable in OF for a longer time compared to blood, whereas THC-COOH was fluctuating in OF. The higher detection sensitivity is obtained in vaporized settings for blood, and in smoked sessions for OF.

The same route of OF collection (expectoration) was used by Vandrey et al. [45]. They decided to administer three different THC doses (10, 25 and 50 mg) through brownies containing. Compared to other studies in which THC was inhalated, quantitative levels of cannabinoids in whole blood and OF were lower.

In two studies, Fabritius et al. [46] and Newmeyer et al. [47] administered the same dose of THC (about 10 mg in the first study and about 20 mg in the second one) to heavy and occasional smokers by inhalation and per os (cookies), respectively. Both studies aimed to observe how the concentrations of THC and its metabolites varied between the two groups and between OF and blood. Overall, these authors demonstrated the presence of a peak of concentrations in OF, followed by a rapid deceasing. THC detected in OF was higher (>300 μg/L) compared to blood (<100 μg/L) mainly due to oral exposure. Moreover, the studies highlighted discrepancies between regular and occasional smokers, especially for THCCOOH levels in both blood and OF. Differences between OF and blood THC and THC-COOH concentrations could be related to the used OF collection device, which was different in the two cited papers, in particular Newmeyer [47] evaluated the performance of Draeger Drug Test 5,000 (5 μg/L cutoff) and Alere DDS-2 (25 μg/L THC cutoff), while Fabritius [46] chose a Quantisal device for heavy smokers and Salivette for occasional ones. Unfortunately, similarly to other stimulants a possible limitation in cannabinoids testing in OF may be due to dry mouth after use, thus making difficult to collect sufficient samples for the evaluation.

The Quantisal device for OF collection was also used by Hubbard et al. [48], Odell [49] and Wille [50]. In the paper by Odell [49], twenty-one dependent cannabis users were recruited, allowing to provide once-daily blood, urine and OF samples for seven consecutive days after admission, involving abstinence from all cannabis use. In some subjects THC was detectable in blood for seven days, OF specimens were positive up to 78 h whereas in urine the THC metabolite (THC-COOH) exceeded 129 h. Hubbard [48] and Wille [50] administered different THC doses by smoking (inhalation), finding higher THC levels in OF than in blood possibly due to the OF collection device. Roadside testing was performed by Rohrich [51] and Laloup [52] as well, and OF was collected by two different devices, RapidStat and Intercept, respectively. Rohrich [51] pointed out that THC concentrations in OF were higher by using RapidSTat compared to those obtained through expectoration, underlying the crucial role of the collection procedure.

Opiates/opioids

Opiates and opioids that have been considered within the cited works are the following: oxycodone, noroxycodone, hydrocodone, norhydrocodone, codeine, norcodeine, morphine, tramadol, O-desmethyltramadol (ODMT), buprenorphine and methadone. Mostly, a known dose of exogenous was administered (oral or sublingual administration); then OF and blood samples were taken after different time intervals in order to study their pharmacokinetics and compared the two matrices. Codeine is currently used in cough relief and mild-moderate pain medication. Given several issues emerged in the compliance with the analgesic prescription, the monitoring of its concentrations is essential in some cases. Therefore, researchers have focused their attention on the identification of reproducible quantitative analysis useful to leverage various matrices. After the administration of 19.5 mg codeine phosphate in 12 healthy volunteers, Coucke and colleagues [53] examined its OF/plasma ratio and the variability in drug concentrations in OF, by using two different approaches, Saliva Collection-System (SCS) and Quantisal. Next, codeine levels were measured by GC-MS in the two different matrices. At 1 h, by using the two different types of collection system, the amount of codeine identified was higher in OF than blood

(ratio OF/plasma ~2), carrying a significant Pearson's correlation coefficient between the two matrices. Both sampling procedures resulted efficient in codeine determination, however SCS may mirror better plasmatic fluctuations. The correlation elucidated between OF and plasma codeine levels is better than that identified in less controlled studies, in which the determinations are performed during epidemiological roadside surveys or in subjects who undergo treatments for addiction [50, 54]. For instance, Guinan et al. [54] tested the presence of methadone in OF and plasma of 13 adult subjects, under treatment for opioid dependence. Methadone is an opioid substance greatly diffused as replacement in the medication of heroine abusers, for its low excretion properties. The authors compared LC-MS and pSi SALDI-MS, establishing a good correlation between these two analytical techniques, testing either water or biological fluids and an excellent agreement among the two matrices.

Cocaine

Cocaine, a psychoactive drug obtained from coca leaf, is one of the most consumed illicit substances, after cannabis, according to United Nations Office on Drug and Crime. Only few studies have investigated the impact of cocaine-controlled administration in humans, and the majority of researches has been conducted in polydrug users [55–57]. Among them, Scheidweiler and colleagues studied the pharmacokinetics of cocaine and its metabolites in OF and plasma, after drug subcutaneous administration in 19 participants [58]. Levels of cocaine, and its metabolites produced by liver (benzoylecgonine (BE), and ecgonine methyl-ester (EME)) were assessed by GC-MS, demonstrating that cocaine was detectable in OF between 0.08 and 0.32 h, but it disappeared rapidly (1.1–3.8 h). Conversely, its metabolites have shown a longer half-life. These authors demonstrated for the first time that all analytic species assessed in OF displayed a good correlation profile with plasma levels. Other studies confirmed this data, showing that EME predominates the OF after repeated oral cocaine doses [58]. Hence, measuring metabolites in OF offers the possibility to extend the detectability window of cocaine.

Chantada-Vazquez et al. [57] compared cocaine quantification in polydrug users within OF and serum obtained by two different analytical techniques, LC-MS/MS and molecularly imprinted polymer – Mn-doped ZnS quantum dot (MIP-QD). The latter exploited the changes in luminescent properties of quantum dots (QD) nanoparticles to sense and quantify cocaine in OF. Indeed, when the analyte (cocaine) is present, the luminescence of QD on the surface of MIPs is quenched, to the point that these luminescence alterations are directly ascribable to analyte concentration. These authors reported that the alternative assessment of cocaine in both types of clinical samples by using the MIP-QD gained in versatility and reduced the costs of laboratory instrumentation, compared to conventional LC-MS/MS.

Amphetamines

Several studies analyze amphetamine, d,l,-methamphetamine, l-methamphetamine, 3,4-methylene-dioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 4-fluoroamphetamine (4-FA), mainly by GC-MS and LC-MS/MS methods. In almost all articles, a known dose of exogenous was administered (ingestion or inhalation) and subsequently quantified within the biological matrices at different times after administration.

Newmeyer and collaborators focused on the OF as alternative matrix for Methamphetamine testing, given its high abuse potential. Methamphetamine exists in two enantiomeric forms (destro-d and levo-l) and the d-methamphetamine isomer is the most powerful in stimulating the central nervous system, while the l-methamphetamine is contained in nasal decongestants. Thus, for more efficacious tests, the two enantiomers should be distinguished, to do not overinterpret the results. In this study, the authors administered 7 doses of the l-methamphetamine, through Vicks® VapoInhaler™ (2 inhalations every 2 h) to healthy adults (n=16). Then, OF and plasma specimens were collected with two different devices (Quantisal™ and Oral-Eze®), before and up to 32 h after the first dose. D and l-methamphetamine, as well as d and l-amphetamine were quantified by LC-MS/MS, applying a chiral derivatization. Positive OF samples to l-methamphetamine were produced by all participants after multiple doses, while no d-methamphetamine or d-amphetamine was detected. Conversely, only two patients were positive for l-methamphetamine in plasma samples, suggesting a huge inter and intra-individual variability in OF/plasma ratios. Notably, the use of the correct methodological approach which includes chiral analysis is essential to differentiate l-methamphetamine from the illicit one. Other studies addressed the assessment of amphetamine, d or l-methamphetamine, MDMA, MDA and 4-FA mainly by using mass spectrometry [43, 44, 51, 59, 60], with the pursuit to parallel OF and blood pharmaco-distribution. However, the contamination of the oral cavity during drug consume should be taken into account, explaining the great discrepancies between the two matrixes in the first time points of observation. Indeed, while in OF the substances can be detected in high concentrations during the first 3 h, probably because of oral contamination, in serum the maximal levels appeared after few hours.

Benzodiazepines

Among the Benzodiazepines, oxazepam is the most frequently identified in blood of drivers [61]. Since the sampling of the OF represents a valid tool for roadside testing, Smink and colleagues [62] performed pharmacokinetic studies to examine the correlation between the concentrations of oxazepam and its metabolite (oxazepam glucuronide), in OF and blood (whole blood and serum), after the oral administration of a single known dose (of 15 or 30 mg) in eight male healthy subjects. Samples were collected until 8.5 h after oral administration and quantifications were obtained by LC-MS/MS. As expected, similar concentration–time profiles have been identified in whole blood and serum. Accordingly, in OF both analytes have been detected, albeit at low levels, at least for 8.5 h. In details, oxazepam is detectable in the OF in dose-dependent manner and its concentration reflects its systemic availability. Thus, OF may represent a good methodological approach to detect recent ingestion of the drug in drivers, although further studies are required to better understand whether the saliva composition may impact on analyte concentrations and to estimate the real predictive value of OF.

Barbiturates

Currently, the abuse of barbiturates has been largely replaced with that of benzodiazepines and only few barbiturates remain in clinical setting as anesthetic, analgesics or anticonvulsant agents. However, given their low safety profile and the high risk of dependence, they remain a health concern in clinical and non-clinical settings.

In this context, the use of OF in monitoring barbiturates consumption is gained in popularity, since this means guarantee the possibility to exploit simple, non-invasive and supervised collection procedures. In a single center clinical study, Fritch and colleagues [33] have investigated the clinical effectiveness and the bioavailability of different barbiturates, among which Butalbital, Phenobarbital and Secobarbital, both in the OF and plasma. After the oral administration of a low dose of one of these three barbiturates (n=15 healthy individuals/group), OF and blood samples have been collected for the three consecutive days after the assumption at different timepoints and the quantitative analysis has been performed by LC-MS/MS and GC- MS/MS in OF and blood, respectively. Their intestinal absorption was fast and their effects on central nervous system appear quickly with only mild side effects recorded.

Butalbital and Phenobarbital were the most rapidly detected in both OF and plasma in the first 15 min and remained detectable until 52 h, while Secobarbital was dosable within 30–60 min after the administration. The mean concentrations measured in OF and plasma, are schematically listed in Table 1. Overall, the OF concentrations of the three barbiturates were higher in plasma compared to OF, although the timing of bioavailability was similar between these two biological fluids and their Cohen's kappa coefficient of agreement was 0.876. Therefore, the authors claimed that the OF may be useful to predict barbiturates plasma concentrations, since their similar pattern of detection.

Table 1: Summary of studies aiming to compare drugs monitoring in OF and blood in forensic and clinical settings from 2002 to 2022. In detail the following data are summarized: drug family, analyte, analytical technique, sampling time after administration of the drug, dose of the administered drug, samples size, drug quantification into OF and blood, OF/blood (OF/B) ratio and reference.

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
Cannabis	THC[b]	Almost 6 collections for day	100 mg/d (3 day)	0–8	3.3–44.4	–	GC-MS	[63]
	THCCOOH[b,o]			0–0.8	53.2–366			
	THC[b]		120 mg/d (3 day)	0–1.1	4.2–67.6			
	THCCOOH[b,o]			0–1.08	85.5–411			
	THC[b]		40–120 mg/d (37 doses)	ND	1.2–31.3			
	THCCOOH[b,o]			0.025–1.05	38.4–427			
	THC[a]	4 OF and 8 blood samples were collected within 6 h	41.3	0.6–9,628	0–231	–	LC-MS/MS	[48]
	11-OH-THC[a,o]			0–0.8	0–38.8			
	THCV			0.4–79.9	0			
	CBD[a]			0.5–43.1	0–0.5			
	CBN[a]			0.4–1,263	0–18.8			
	CBG[a]			0–978	0–10			
	THC[a]		93.8	0.4–18.126	0–128			
	11-OH-THC[a,o]			0–3	0–22.6			
	THCV[a]			0.4–201	0–0.5			

Table 1: (continued)

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
	CBD[a]			0.5–66.5	0–7.5			
	CBN[a]			0–3,234	0–13.9			
	CBG[a]			0–780	0–2.6			
	THC[c]	–	–	0–76	0–33.6	–	GC-MS	[51]
	THC[a,f]	7 collections within	43	0–3,170	0.8–192	–	LC-MS/MS	[46]
	THCCOOH[a,o]	3.5 h		0–2.4	2.5–106			
	THC[a,g]			0–3,110	0–168			
	THCCOOH[a,o]			–	0–38			
	THC[b]	–	–	0.5–1,462	0.6–51.3	–	LC-MS/MS	[52]
	THC[a,d,f]	15 collections within	50.6	39.3–2,111	8–36.1	–	LC-MS/MS	[47]
	11-OH-THC[a,d,o]	48 h		0.2–1.2	4.7–11.4			
	THCCOOH[a,d,o]			123–1,009	27.8–152			
	THC[a,d,g]			115–696	3.2–14.3			
	11-OH-THC[a,d,o]			0.3–0.6	4.1–8.6			
	THCCOOH[a,d,o]			27.9–1,281	26.5–61.2			
	THC[a]	1.2–83	–	1–327	1–15		LC-MS/MS	[49]
	THC[a]	0.17	10–50	47–1,128	0	–	LC-MS/MS	[45]
		0.5		3–851	0–2			
		1		0–196	0–4			
		1.5		0–80	0–5			
		2		0–29	0–5			
		3		0–168	0–4			
		4		0–24	0–4			
		5		0–24	0–3			
		6		0–7	0–3			
		8		0–2	0–1			
		12		0–2	0–2			
		22		0–2	0–1			
	THCCOOH[a,o]	0.17		0–0.122	0			
		0.5		0–0.089	0–7			
		1		0–0.0129	0–21			
		1.5		0–0.067	0–33			
		2		0–0.451	0–30			
		3		0–0.251	0–40			
		4		0–0.367	0–37			
		5		0–0.822	0–31			
		6		0–0.269	0–44			
		8		0–0.160	0–25			
		12		0–0.642	0–18			
		22		0–0.204	0–12			
		26		0–0.114	0–9			
		30		0–0.106	0–14			
		34		0–0.264	0–8			
		50		0–0.137	0–9			
		54		0–0.045	0–5			
		58		0–0.096	0–4			
		70		0–0.028	0–4			
		74		0–0.058	0–3			
		78		0–0.042	0–3			
		82		0–0.032	0–2			
		94		0–0.021	0–3			
	THC[c]	0	0.300 mg/kg	0–68.5	0–5.9	0–171	UHPLC-MS/	[50]
		5′		166–6,328	27.1–57.1	5–234	MS	
		1.25	0.450 mg/kg	77.7–12.360	14.1–48.0	3–404		

Table 1: (continued)

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
		2.40		34–281	4.7–14.7	7–41		
	THC[a,h]	0.17	10–25	23–2,368	1–38	–	LC-MS/MS	[40]
		0.5		11–517	1–14			
		1		7–97	0–8			
		1.5		0–104	0–5			
		2		1–68	0–3			
		3		0–66	0–1			
		4		0–38	0–1			
		5		0–51	0			
		6		0–19				
		8		0–17				
	THCCOOH[a,o]	0.17		0	0–23			
		0.5		0–1.023	0–21			
		1		0–0.081	0–17			
		1.5		0–0.101	0–19			
		2		0–1.037	0–15			
		3		0–0.148	0–13			
		4		0–1.095	0–12			
		5		0–0.131	0–10			
		6		0–0.131	0–10			
		8		0–0.175	0–8			
	THC[a,i]	0.17	10–25	36–1,646	0–29			
		0.5		18–899	0–8			
		1		3–122	0–4			
		1.5		1–88	0–3			
		2		1–60	0–3			
		3		1–36	0–1			
		4		0–19	0			
		5		0–10				
		6		1–7				
		8		0–5				
	THCCOOH[a,o]	0.17		0	0–17			
		0.5		0–0.128	0–18			
		1		0–0.039	0–16			
		1.5		0–0.098	0–12			
		2		0–0.081	0–11			
		3		0–0.072	0–10			
		4		0–0.07	0–8			
		5		0–0.069	0–6			
		6		0–0.084	0–5			
		8		0–0.06	0–5			
	THC[a,d]	10 collections within	10	11–414	0–4	–	LC-MS/MS	[64]
	THCCOOH[a,d,o]	8 h		0–0.5	6–27			
	THC[a,d]		25	9–667	0–9			
	THCCOOH[a,d,o]			0–0.5	17–62			
	THC[a,d]		50	322–1,196	0–18			
	THCCOOH[a,d,o]			0–1.2	17–148			
Opiates/opioids	Oxycodone[a]	0	20	0	0	–	LC-MS/MS	[65]
		0.25		1.1–84.7	–	–		
		0.5		2.1–34.6	6.4	–		
		0.75		8.8–85.7	6.6–16.5	2.37–8.89		
		1		16.4–162.4	5.1–17.3	1.62–9.41		
		1.5		17.2–192.8	5–15.7			

Table 1: (continued)

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
						2.18–12.60		
		2		29.9–218.7	7.2–21.8	1.68–19.40		
		2.5		27.5–139.1	7.5–22.1	2.07–12.73		
		3		32.7–201.6	7.4–22.7	3.31–10.85		
		4		28.5–208.4	9.7–25.3	2.95–12.23		
		5		31.5–130.8	11.8–24.2	1.69–5.98		
		6		21.2–206.8	13.1–24.4	2.82–9.57		
		8		36.9–170.3	10.2–23	1.03–7.71		
		10		27.6–155.5	7.7–17.6	2.16–8.83		
		12		23.2–114.5	5.1–12.6	2.66–9.12		
		14		13.7–84.7	5.7–11.6	2.34–8.91		
		24		3.8–51.1	–	–		
		28		1.1–26.3				
		32		1.1–6.5				
		36		1.1–5.7				
	Noroxycodone[a]	0		0	0			
		0.25		2.2	–			
		0.5		1–2.3				
		0.75		1.7–5.6	5.3–8.6	0.26–0.75		
		1		2.4–11	5.4–11.1	0.28–1		
		1.5		1.6–12.6	6–15.4	0.38–1.70		
		2		2.4–18.1	5.2–14.2	0.28–1.54		
		2.5		6.1–14.8	5.7–18	0.50–2.41		
		3		8.2–18.7	8.6–21.7	0.55–1.24		
		4		7.9–21.7	9.5–22	0.39–1.97		
		5		5.5–19.2	9.9–20.8	0.55–1.46		
		6		5.8–31.8	7.1–18.1	0.52–2.67		
		8		5.5–20.1	7.2–18.9	0.50–1.51		
		10		4–24.5	5.7–18.2	0.51–1.97		
		12		2.5–21.3	6–15.5	0.66–1.92		
		14		2.9–18.4	8.2–13.8	0.62–1.68		
		24		2.1–14	5.1–8.2	0.99–1.20		

Table 1: (continued)

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
		28		1–4.7	6.2	0.76		
		32		1–3.4	5.9	0.55		
		36		1.5–3	–	–		
	Hydrocodone[a]	0	12.1	0	0	–	LC-MS/MS	[66]
		0.25		19.2–198	74.4	1.4		
		0.5		9.9–215.3	6.6–74.4	0.6–5.5		
		0.75		18.8–270.5	7.6–94.2	0.8–5.2		
		1		34–625.6	5.6–61.3	0.9–14.5		
		1.5		15.5–413.2	13.5–43.3	0.4–10		
		2		16.9–253.3	14.4–39.7	0.5–6.8		
		2.5		32.6–169.3	11.9–39.4	1.1–5.5		
		3		39.6–141.5	5.2–37.2	1.2–7		
		4		29.5–163	10.7–32.8	1–5		
		5		13.2–110.1	8.7–29.1	0.6–5.2		
		6		18.6–132.9	8.5–25.1	1–6.2		
		8		17.1–142.3	8.3–18.1	1.2–8.3		
		10		7.1–50.2	5.7–15.3	0.8–5.6		
		12		7.2–74.9	5.1–10.3	0.9–9.6		
		14		3.2–32.7	5.2–8	0.5–6.2		
		24		1–5.8	–	–		
		28		1.2–4.3				
		32		1.1–3.2				
		36		1.1–1.5				
	Norhydrocodone[a]	0		0	0			
		0.25		1.9–4.3	19.6	0.1		
		0.5		1.1–9.1	7.3–31.4	0.2–0.3		
		0.75		1.4–12.5	8.4–32.4	0.1–0.4		
		1		1.4–27	6.5–33.6	0.1–1.8		
		1.5		1.6–22.4	8–24.6	0.1–1.3		
		2		1.2–17.6	7.3–19.6	0.1–1.9		
		2.5		1.4–15	6.7–24.1	0.2–1.2		
		3		1–19.2	5.9–18.5	0.2–2.1		
		4		1.6–18.5	6–17.2	0.2–1.3		
		5		1.2–12.5	5.8–28.8	0.2–1.3		
		6		1.4–16.2	5.3–25.1	0.2–1.4		
		8		1.2–20.7	5.1–10.5	0.2–2.4		
		10		1.7–7.3	5.1–8	0.3–1.4		
		12		1.5–12.4	5.3–6.1	0.6–2		
		14		1.1–5.7	5–5.2	0.4–0.5		
		24		1.1–3.6	–	–		
		28		1.1				
	Codeine[b,d]	10 collections within	60 mg/70 kg	184–1,288.8	66.1–413.2	1.1–17.2	GC-MS	[67]
	Norcodeine[b,d]	48 h		3.9–58	4.2–26.1	1.7		
	Codeine[b,d]		120 mg/70 kg	619.6–3,350.2	184–1,158.1	0.6–16.4		
	Norcodeine[b,d]			10.3–191.2	9.2–63	1		
	Codeine[b,e,l]	1	15.2	85.3	29.8	2.69	GC-MS	[53]
	Codeine[b,e,m]			72.8		2.30		
	Morphine[b,d]	18 collections within	15.7	3.6–76.5	2.8–8.4	0.3–10.2	LC-MS/MS	[42]
	Codeine[b,d]	24 h	3.1	2.1–23.8	1.2–2	1.1–18.9		
	Morphine[b,d]		31.4	4.6–110	3.8–9.3	0.3–4.7		
	Codeine[b,d]		6.2	3.3–22.4	1.1–1.7	1.1–18.9		
	Tramadol[b,d]	11 collections within	50	459–3,905	73–268	–	LC-MS/MS	[68]
	ODMT[b,d,o]	48 h		2.4–158	5–59			
	Buprenorphine[c]	24	1–16 mg/d	0.5–3.8	1.1–8.5		GC-MS	[69]

Table 1: (continued)

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
	Methadone[b]	–	–	320–2,440 271.5–3,248.5	260–1,170 218.7–1,234.6	–	LC-MS pSi SALDI-MS	[54]
Cocaine	Cocaine[b,d]	13 speciments collections in within 48 h	75 mg/70 kg	406–3,006	108.6–434.1	–	GC-MS	[58]
	BE[b,d,o]			81.8–440.6	180.1–411.2			
	EME[b,d,o]			48.5–1,329.7	29.8–67.3			
	Cocaine[b,d]		150 mg/70 kg	1,193–8,495	253.5–1,153.9			
	BE[b,d,o]			132.6–757	336.3–832			
	EME[b,d,o]			141.6–949.6	70.1–338.9			
	COC+BE+EME[c]		–	152–585	597–1,270	–	LC-MS/MS MIP-QD	[57]
	COC+BE+EME[c]			148–618	623–1,280			
	Cocaine[b,e]	–	–	38.8	8.2	–	LC-MS	[55]
	BE[b,e,o]			41.8	181.7			
	AEME[b,e,o]			15.3	0			
	AEC[b,e,o]			12.1	27.4			
	CE[b,e,o]			6.9	4.76			
	Cocaine[b]	–	–	1.8–450.1	1.2–107.2	0.68	LC-MS	[56]
	BE[b,o]			1.7–578.1	4.4–1,652.4	0.004		
	AEC[b,o]			2.5–34.5	6.6–115.7	0.009		
Amphetamines	MDMA[a]	–	100–500	N/A	7–270		LC-MS/MS	[44]
	4-FA[a]		150–1,000	281–378	71	5.32		
	d.l-Methamphetamine[a,e]	2	0.42 mg/kg	343	90	–	GC-MS	[59]
		2.8		475	95			
		4		568	105			
	Amphetamine[c]	–	–	0–863	0–279	–	GC-MS	[51]
	MDMA[c]			0–52	0–50			
	l-Methamphetamine[b,d,l]	7.5	3	5.2–380	10	–	LC-MS/MS	[70]
	l-Methamphetamine[b,d,n]			4–182				
	l-Methamphetamine[b,d,l]	11	4.2	1–18.1	3.8			
	l-Methamphetamine[b,d,n]			1.3–55.8				
	MDMA[b,d]	31 collections within 143 h	1 mg/kg	1.16–3.38	132–218	0.1–40.4	GC-MS	[60]
	MDA[b,d]			23.1–151.3	5.6–14.2	0.7–17.1		
	MDMA[b,d]		1.6 mg/kg	2.88–11.99	250–387	0.4–52.3		
	MDA[b,d]			50.5–403.2	11.4–23.3	0.9–24.3		
	4-Fluoroamphetamine[c,d]	12	100	164–1,458	59–197	1.9–93	LC-MS/MS	[71]
	Amphetamine[c,d]			1.99–23.9	0.54–1.91	1.6–134		
	4-Fluoroamphetamine[c,d]		150	0–2,338	103–138	2.1–37.7		
	Amphetamine[c,d]			0–27.3	1.15–1.74	2.2–39.4		
NPS[a]	Methylone	–	150–1,000	40–10.027	7–375	12.79	LC-MS/MS	[44]
	Alpha -PVP			86–1,301	8–87	10.84		
	Ethylone			41–4,105	210–212	3.10		
	Dimethylone			611	10–157	3.33		
Benzodiazepines	Oxazepam[c,d]	13 collections within 8.5 h	15	8–24	217–391	0.04–0.07	LC-MS/MS	[62]
	Oxazepam glucuronide[c,d,o]			15–45	423–662	0.002–0.006		
	Oxazepam[c,d]		30	0–1	101–236	0.04–0.07		
	Oxazepam glucuronide[c,d,o]			1–2	203–457	0.002–0.006		
	Oxazepam[c,d]		15	8–24	229–441	0.03–0.07		

Table 1: (continued)

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
	Oxazepam glucuronide[c,d,o]			15–45	193–420	0.001–0.004		
	Oxazepam[c,d]		30	0–1	497–967	0.03–0.07		
	Oxazepam glucuronide[c,d,o]			1–2	387–787	0.001–0.004		
Barbiturates	Butalbital[b]	0	50	0	0	–	GC-MS	[33]
		0.25		11–264	0–914			
		0.5		44–336	138–2,400			
		1		86–291	554–1,500			
		1.5		107–244	802–1,484			
		2		68–229	678–1,128			
		3		45–220	612–1,300			
		4		96–226	534–1,070			
		6		47–215	516–1,246			
		8		62–201	524–954			
		10		82–192	492–944			
		12		81–176	448–964			
		14		48–192	532–934			
		24		52–149	462–758			
		28		59–144	–			
		32		39–138	378–682			
		36		66–131	404–620			
		48		25–105	380–528			
		49		26–100	–			
		50		19–98				
		52		16–93				
	Phenobarbital[b]	0	30	0	0			
		0.25		0–108	0–382			
		0.5		18–139	0–940			
		1		33–142	216–896			
		1.5		26–134	368–838			
		2		49–161	464–1,022			
		3		65–150	572–1,044			
		4		71–133	544–896			
		6		46–134	520–816			
		8		31–109	488–814			
		10		43–121	502–786			
		12		29–128	526–786			
		14		65–129	498–846			
		24		47–115	452–712			
		28		52–121	–			
		32		38–98	374–738			
		36		40–115	446–794			
		48		32–103	369–678			
		49		28–112	–			
		50		38–99				
		52		39–101				
	Secobarbital sodium[b]	0	100	0	0			
		0.25		0–127				
		0.5		0–331	0–3,620			
		1		0–252	89–2,256			
		1.5		65–197	0–2,278			
		2		50–182	972–2028			

Table 1: (continued)

Drugs	Analytes	T after administration, h	Dose, mg	OF concentration range, ng/mL	Blood concentration range, ng/mL	OF/B median	Technique	Ref.
		3		26–159	770–1,690			
		4		43–168	702–1,632			
		6		22–124	584–1,052			
		8		13–106	516–1,014			
		10		25–113	488–918			
		12		12–101	440–876			
		14		0–92	302–814			
		24		0–71	212–650			
		28		0–78	–			
		32		0–55	0–572			
		36		0–53	0–488			
		48		0–40	0–411			
		49		0–43	–			
		50		0–39				
		52		0–45				

[a]Whole blood analysis. [b]Plasma analysis. [c]Serum analysis. [d]Range of maximum concentration obtained from analysis of both OF and blood (whole blood, plasma and serum). [e]Average concentration obtained from analysis of both OF and blood (whole blood, plasma and serum). [f]THC was administered to heavy smoker. [g]THC was administered to occasional smoker. [h]THC taken by smoking. [i]THC taken by vaporized. [l]OF was collected by Quantisal™ device. [m]OF was collected by Saliva Collection System (SCS) device. [n]OF was collected by Oral-Eze® device. [o]Metabolites. THC, Δ9-Tetrahydrocannabinol; THC-COOH, 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol; 11-OH-THC, 11-Hydroxy-Δ9-tetrahydrocannabinol; THCV, Tetrahydrocannabivarin; CBD, Cannabidiol; CBN, Cannabinol; CBG, Cannabigerol; ODMT, O-desmethyltramadol; BE, benzoylecgonine; CE, cocaethylene; AEME, anhydroecgonine methyl ester; AEC, anhydroecgonine; EME, alecgonine methyl-ester; MIP-QD, molecularly imprinted polymer – Mn-doped ZnS quantum dot; QD, quantum dots; MDMA, 3,4-methylene-dioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; 4-FA, 4-fluoroamphetamine; alfa-PVP, α-pirolidinopentiofenone.

Conclusions

In summary, the possibility to use OF as alternative matrix to blood represents an intriguing opportunity for the screening and the monitoring of licit or illicit substances, paving the way to address technological advancement in this field (Figure 3). Here, we reported a great number of studies in which the authors have pinpointed a good correlation between the two biological fluids, thus corroborating the notion that this methodological approach could be widespread applicable to a plethora of areas of studies, from criminal justice, DUID programs until clinical toxicology. It is reasonable that similar findings could be obtained by testing therapeutic drugs, with the goal to precisely follow the therapy and adjust the dosages, thus deeply unraveling the pharmaco-distribution and the bioavailability window of the latter in OF. Simple OF-based devices could be created to rapidly monitor the adherence and the effectiveness of the therapy, adjusting the doses to the needs.

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