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Oral Fluid Testing for Drugs of Abuse

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Abstract

BACKGROUND—Oral fluid (OF) is an exciting alternative matrix for monitoring drugs of abuse in workplace, clinical toxicology, criminal justice, and driving under the influence of drugs (DUID) programs. During the last 5 years, scientific and technological advances in OF collection, point-of-collection testing devices, and screening and confirmation methods were achieved. Guidelines were proposed for workplace OF testing by the Substance Abuse and Mental Health Services Administration, DUID testing by the European Union's Driving under the Influence of Drugs, Alcohol and Medicines (DRUID) program, and standardization of DUID research. Although OF testing is now commonplace in many monitoring programs, the greatest current limitation is the scarcity of controlled drug administration studies available to guide interpretation.

CONTENT—This review outlines OF testing advantages and limitations, and the progress in OF that has occurred during the last 5 years in collection, screening, confirmation, and interpretation of cannabinoids, opioids, amphetamines, cocaine, and benzodiazepines. We examine controlled drug administration studies, immunoassay and chromatographic methods, collection devices, point-of-collection testing device performance, and recent applications of OF testing.

SUMMARY—Substance Abuse and Mental Health Services Administration approval of OF testing was delayed because questions about drug OF disposition were not yet resolved, and collection device performance and testing assays required improvement. Here, we document the many advances achieved in the use of OF. Additional research is needed to identify new biomarkers, determine drug detection windows, characterize OF adulteration techniques, and evaluate analyte stability. Nevertheless, there is no doubt that OF offers multiple advantages as an alternative matrix for drug monitoring and has an important role in DUID, treatment, workplace, and criminal justice programs.

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Oral fluid (OF)[3] is a suitable alternative matrix to test drugs of abuse in clinical, workplace, driving under the influence of drugs (DUID), drug treatment, and criminal justice settings. The main advantages of OF are the simplicity and noninvasiveness of sample collection, which can be easily observed (1), obviating the need for special restroom facilities and same-sex collectors and making adulteration more difficult (2). Infection risk is lower than for blood (3), and OF may better reflect recent drug use. The parent drug is frequently prominent in OF and may reflect free plasma concentrations, providing a better correlation with pharmacodynamic effects, such as impaired performance (4). It is difficult to differentiate heroin from morphine or codeine ingestion with urine drug testing, whereas 6-acetylmorphine (6AM) and heroin are frequently present in OF, clearly delineating heroin abuse.

Weak bases are detected in higher concentrations and for longer times in OF than in plasma because of ion trapping. Depending on a drug's p*K*a and lipophilicity, ion trapping occurs because of pH differences in blood (7.4) and OF (4–6). Free uncharged drug is in equilibrium across membranes separating blood and OF. At the lower OF pH, weak bases ionize, increasing total OF drug concentrations.

For clinical toxicology applications, including drug treatment, physician office, and emergency room testing, onsite OF testing offers rapid availability of results for diagnostic purposes and ability to confront treatment patients with immediate indicators of drug relapse. In 2004, Substance Abuse and Mental Health Services Administration (SAMHSA) proposed recommended guidelines (Table 1) for mandated federal workplace OF testing (5). Lack of resolution of important scientific questions delayed final approval, but SAMHSA guidelines, including low cutoff concentrations, are frequently applied in nonregulated settings. The guidelines encompass mandated cutoffs and procedures for sample collection, custody, and control to ensure sample identity and integrity; sample validity tests; testing facility requirements; analytical method result review and reporting; alternative medical explanations; and laboratory certification (6). In the US, OF testing is expanding at a rapid pace in nonregulated workplace testing, treatment, and driving under the influence of drugs (DUID) programs.

One major incentive for improving OF testing is the increasing problem of DUID. Drugs and alcohol were contributing factors in up to 22% of motor vehicle crashes in the US (7). Unfortunately, an even higher prevalence is likely, because additional analyses are usually not conducted for drugs if alcohol is above the legal limit. OF testing offers a new tool to improve traffic safety with rapid, easy roadside drug testing of drivers. ROadSIde Testing Assessment (ROSITA) was the first European Union effort to determine the best means of identifying drugged drivers at the roadside. OF was selected over urine or sweat as the best matrix, although OF collection and testing was then considered inadequate (8). ROSITA-2 in Europe and the US specifically evaluated available OF point-of-collection testing (POCT) devices, concluding that none were yet adequately reliable (9). The latest European initiative, Driving Under the Influence of Drugs, Alcohol, and Medicines (DRUID), promotes research and scientific support to reduce European Union road deaths by 50%. Guidelines were proposed for DUID testing in the DRUID program, as shown in Table 1. In Victoria, Australia, OF DUID testing began in 2003, with public acceptance and reductions

[3]Nonstandard abbreviations: OF, oral fluid; DUID, driving under the influence of drugs; 6AM, 6-acetylmorphine; SAMHSA, Substance Abuse and Mental Health Services Administration; ROSITA, ROadSIde Testing Assessment; POCT, point-of-collection testing; DRUID, DRiving Under the Influence of Drugs, alcohol, and medicines; THC, Δ9-tetrahydrocannabinol; DDS, Drug Detection System; BE, benzoylecgonine; LOQ, limit of quantification; LC-MS/MS, liquid chromatography-tandem mass spectrometry; APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; THCCOOH, 11-nor-9-carboxy-THC; 11-OH-THC, 11-hydroxy-THC; GC-MS/MS, gas chromatography–tandem mass spectrometry; CBD, cannabidiol; CBN, cannabinol; MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; EME, ecgonine methylester.

in impaired driving, despite less than optimal performance of collection devices and assays (10). The Talloires recommendations for OF cutoff concentrations (11) also are included in Table 1. In 2006, international experts met to draft guidelines for conducting research on drugged driving. Currently, it is difficult to compare results across studies owing to the lack of standardization in experimental methods. Recommendations focused on 3 broad areas, i.e., behavior, epidemiology, and toxicology. Included among the 80 recommendations for toxicological contributions were suggested OF drug cutoff concentrations.

OF testing does have disadvantages. Drugs may reduce salivation, limiting sample volume and necessitating sensitive analytical methods to quantify multiple analytes in 1 assay. Drug concentrations are frequently lower in OF than in urine (4). Other issues include contamination from ingested food and beverages, and unknown adulteration techniques (12). Drugs that are smoked, inhaled, insufflated, or taken orally also may contaminate the oral mucosa and OF, increasing detection, but reducing correlation with blood concentrations for 30 – 60 min. We observed that OF concentrations of Δ^9-tetrahydrocannabinol (THC) greatly exceeded plasma concentrations for approximately 30 min after cannabis smoking (13). The magnitude and duration of contamination have not been clearly defined for many drugs, and require additional controlled drug administration studies. Stimulation of salivary flow leads to increased OF pH due to increased bicarbonate excretion and reduced drug concentrations due to dilution (12).

In this review, we focus on advances, primarily those occurring in the last 5 years, in OF measurement and interpretation of OF concentrations of cannabinoids, opioids, amphetamines, cocaine, and benzodiazepines. We also summarize progress in OF testing and unresolved issues. Our aim is to inform laboratories adding OF collection and testing, improve interpretation of OF test results, and promote evidence-based drug policies and relevant traffic safety legislation.

OF Collection

There are 2 main approaches to OF testing. One approach involves OF collection by expectoration or with a specialized device, and transfer of the sample for conventional laboratory-based immunoassay screening and chromatographic confirmation. The other approach is a system for collection and initial screening of OF at the site of collection (POCT), followed by a laboratory-based chromatographic confirmation at a later time. Collecting OF without a specialized device can be achieved by the passive drool technique or by expectoration. The passive drool technique best reflects drug concentrations in excreted saliva, because expectoration increases the rate of salivary excretion to a minor extent. Although samples collected without a specialized device are useful from a scientific perspective, the collection process is distasteful for donors and collectors. Thus OF collection devices are now used for large-scale testing applications. As interest grew in testing this alternative matrix, a wide variety of OF collection devices were developed, with variable success. Major problems are variability of OF collected and deficiency of sample amounts.

Many devices collect <1 mL OF, limiting available sample for multiple drug confirmations. However, as chromatographic methods and instruments improve in analytical sensitivity, smaller sample volumes are being required. In addition, the trend is to develop assays that quantify multiple drug classes simultaneously, reducing the needed sample size. Another factor affecting adequate sample volume is the expected OF drug concentrations. Depending on a drug's potency and physiochemical characteristics, concentrations range from nanograms per liter to micrograms per liter. Therefore, the determination of adequate sample volume must take into consideration the drug being tested.

Some OF collection devices have built-in volume-adequacy indicators, facilitating appropriate collection, whereas other manufacturers report only approximate amounts collected. The Arco Biotech device has a mark on the vial indicating the amount collected (3), and the Immunalysis Quantisal™ and StatSure Saliva Sampler™ contain indicators for collection of 1 mL. With the use of these indicators, the collector can keep the device in the donor's mouth for the time required to collect an adequate volume, despite individual variability in OF excretion. A similar indicator on the Cozart® Drug Detection System (DDS) documents 340 (60) μL OF (14). Another approach is gravimetric determination by weighing the device before and after OF collection (15, 16). With the Greiner device a novel tactic is employed that determines OF amount based on dilution of a dye in the extraction solution (3). The Greiner device differs from other collection devices in that the sample donor rinses the mouth with the extraction solution, which is then expectorated with the OF into a collection beaker. The absorbance of the resulting solution is read in a spectrophotometer to calculate collected OF amount.

Even more problematic than the amount collected is variability in OF volume obtained within and between devices. The Orasure Intercept® Drugs Of Abuse oral sample collection device was reported to collect from 0.38–1.53 g (16). A recent evaluation of 3 collection devices provided within and between collection device variability ranging from 1.045–1.667 g (15). This variability reflects imprecision in elution buffer volume included in the device, and more importantly, inconsistency in donor OF amount. Obviously, drug concentration is dependent upon the degree of dilution. Some manufacturers now provide assurances of the degree of variability in OF collection; within-device variability of <10% for the Quantisal collection device and <5% for the Cozart and StatSure devices were reported (3).

OF drug concentration also is dependent on OF excretion stimulation, which may occur even to a small extent by placement of a collector in the mouth. Thus, it is impossible to prevent an increase in OF excretion during collection, except by employing passive drool. Older methods of increasing OF excretion included chewing on paraffin, and newer approaches embed citric acid or other chemicals onto collection devices. Early studies, including those in our laboratory, used devices that stimulated OF excretion; however, we learned that stimulation ultimately lowered rather than increased drug concentrations and complicated interpretation of results.

One of the most important limitations of OF collection devices is adsorption of drugs to the device, which frequently leads to false-negative test results (2). This effect was unknown early in OF test development, leading to low sensitivity. Manufacturers addressed the problem, with mixed results, by eluting drugs off the device with buffers. Buffers also reduce OF viscosity, improving measurement accuracy, but also dilute analyte concentrations. Many buffers and surfactants also interfere with direct injection techniques for LC-MS methods by increasing matrix effect. Generally, the more lipophilic a drug, the greater the adsorptivity to the collection device, but recoveries must be empirically determined. Device materials and buffers are proprietary, and it is not possible to predict when recovery might be an issue.

As is true for all toxicological analyses, the analysis is only as good as the sample. Specific challenges for OF collection devices include collection of adequate volume with good imprecision; although readily achievable, as demonstrated with some currently available devices, the speed of collection also is important. For DUID testing, one of the major challenges for manufacturers, and the source of complaints by police, is the time required for collection and testing at the roadside. Although elution buffers appear necessary for effective removal of drugs from the collection device, the resulting drug dilution and presence of salts that must be removed before LC-MS analysis are disadvantages. Although

variability is inherent in OF collection and contributes to the difficulty in interpreting OF drug concentrations, is it appropriate to hold OF testing to a higher standard than is achieved with the currently accepted urine testing technology?

Laboratory-Based Drug Screening

The importance of screening OF for parent drugs and metabolites has been clearly documented by controlled drug administration data. With most RIAs for commonly abused drugs removed from the market, new ELISA assays became available for screening drugs in blood; many were adapted for OF testing. Adaptation of blood rather than urine assays is preferable in many cases because of the presence of greater proportions of parent drug in blood and OF. Manufacturers that simply tried to adapt urine assays that primarily target metabolites had difficulty in meeting the required detection requirements, especially for cannabinoids. However, pH differences in blood and OF yield distinct biomarker disposition. For example, benzoylecgonine (BE) concentrations are higher than cocaine in blood, whereas the reverse is true in OF.

Another important consideration in the use of OF is expected drug concentrations. In general, drug concentrations in OF are much lower than in urine, although oral mucosal contamination for 30–45 min after smoking a drug can produce microgram per liter concentrations. Oral or sublingual administration also may contaminate the oral mucosa, although we observed little increase in OF concentrations after sample donors had ingested encapsulated or coated pills. Thus, challenges include achieving low limits of detection to extend the window of drug detection and expanding the linear range or validating dilution procedures to account for high concentrations found after smoked or oral doses.

In addition, it is essential that calibrators and controls be prepared in the same matrix as authentic samples. Matrix effects are important considerations, especially with LC-MS, and require documentation that analyte recovery and assay imprecision are equivalent for calibrator, QC, and authentic samples. Many manufacturers prepare calibrators and QC in synthetic OF; laboratories should validate performance against calibrators prepared in human OF.

Most OF assays are nonhomogeneous, presenting challenges for large-scale automation. To meet the high workplace testing demands in the US, rapid throughput and automation have enabled the achievement of low costs per test for urine analysis. Fully automated procedures, including pipetting, incubation, washing, and absorbance measurement, are available for 96-well ELISA plates. Evaluations of drug immunoassays are included in Table 2.

POCT

The promise of worldwide OF testing spurred commercial research and development of POCT devices, and commercial devices were rushed to market before much of the basic science of drug excretion into OF was known. A POCT device includes OF collection and a built-in system for screening multiple drug classes. A large number of POCT devices have been evaluated (Table 3), although many are no longer available or were substantially modified (9, 12). The major problems with early generation OF POCT included inadequate limits of detection, specificity, and efficiency for identifying cannabinoids, amphetamines, and benzodiazepines; poor performance in bad weather; difficult-to-read results; complicated testing procedures; insufficient sample volume and prevalent device failures (9). Many deficiencies were revealed, leading to additional research and modifications, which led to improvement of available products. The ROSITA-2 project evaluated the performance of multiple POCT devices and set acceptance criteria for diagnostic sensitivity,

diagnostic specificity, positive and negative predictive values of ≥90%, and efficiency of ≥95% (9).

For all evaluations reported since 2004, Table 3 contains POCT devices, drug classes evaluated, confirmation methods employed, diagnostic sensitivities, diagnostic specificities, efficiencies, recoveries, and device failures, when reported. In addition, the types of samples tested are described, including fortified blank OF or authentic samples from drug treatment, DUID, or after experimental controlled drug administration. Critical factors must be taken into account when referencing these data: (*a*) Data are relevant for devices available at the time of testing. (*b*) Many early devices simply failed to operate correctly. (*c*) Only a few devices included adequate volume indicators. Collected OF volume varied substantially within and between devices, limiting ability to quantify OF concentrations. (*d*) Devices may perform differently with fortified authentic or synthetic OF than with authentic OF samples collected after drug ingestion, because the spectrum of analytes present may differ. Knowledge of antibody cross-reactivity data is key for understanding device performance. (*e*) Drug recovery from the device is a major issue, especially for lipophilic drugs like cannabinoids. (*f*) Many evaluations judged device performance only against stated manufacturer cutoff concentrations that may be unreasonably high compared to proposed SAMHSA, DRUID, or DUID research recommendations. Other evaluations assessed performance against low laboratory limits of quantification (LOQs), providing a better idea of false-negative rates. (*g*) Some devices performed poorly at night or in poor weather conditions, or had endpoints that were difficult to discriminate. (*h*) Some investigators compared OF POCT device performance to serum drug concentrations collected up to several hours after OF. (*i*) In some investigations, for later confirmation additional OF samples were collected by expectoration or with the Intercept® device.

Most POCT devices obtained good results for opioids with cutoffs at or below SAMHSA cutoffs, although false-negative results may have been missed if all negative screening tests were not subjected to confirmation (Table 3). Reported efficiencies do not always agree, owing to differences in populations, analytes, and concentrations tested and cutoffs used. Opioids have a wide range of potency, leading to differences in sensitivities for various analytes.

Many POCT devices (Table 3) performed well for amphetamines, with diagnostic sensitivities, diagnostic specificities, and efficiencies in the range of 70%–100%. Notable exceptions occurred, however: diagnostic sensitivity was too low for the Varian OraLab® and Dräger Drug Test® (17) and the Securetec Drug-Wipe® (18). The Biomar Toxiquick® (19) and 4 devices evaluated by Walsh et al. (20) performed poorly, with low diagnostic sensitivities, diagnostic specificities, and efficiencies. Development of a POCT for amphetamines is challenging owing to the large number of over-the-counter and prescription drugs sharing similar sympathomimetic amine structures. The challenge is to detect drugs of abuse without detecting multiple therapeutic drugs.

POCT devices (Table 3) performed variably for cocaine. The Cozart RapiScan had diagnostic sensitivities ranging from 5% (20) to 98.2% (21) and efficiencies of 45.7% (20) to 99% (21). Diagnostic specificity was generally good, with a range from 88.7% for cocaine/BE with the Biomar Toxiquick® (cutoff 50 μg/L) (19) to 100% for 6 different devices (20). Diagnostic specificities were poor for the Sun OraLine® IV substance of abuse test and Ansys OralLab® (20).

Other important OF testing issues have been poorly investigated. For example, adulteration of OF samples and potential interferences with POCT devices have not been systematically investigated. It is clear that considerable effort is expended to produce false-negative urine

test results, with numerous products available. Certainly similar efforts will be directed at falsifying OF tests. The risk of test falsification requires initial characterization and continuous surveillance of potential interfering substances and adulterants. Specified foods, drinks, mouthwash, and cigarettes did not interfere with the Cozart RapiScan and the Cozart Microplate ELISA assays for opiates (22), cocaine (23), and amphetamines (24), nor with the Branan Oratect® collection device (25). Alcohol and hemoglobin did not affect the Cozart assays and the RapiScan collection device (22–24). Toothpaste, lipstick, gum, and 2 commercially available adulterants also had no effect on Branan Oratect results (25). These few reports provide to date the only available data on adulterants and interferences. Additional research is critically needed to characterize potential problems with OF collection devices and immunological and chromatographic assays.

Chromatographic Confirmation and Quantification of Drugs in OF

From 2004 to 2008, reports of 71 OF drug assays were published, documenting increasing interest in OF as an alternative matrix for identifying and quantifying drug exposure. Chromatographic methods, analytes, extraction procedures, and LOQs for quantifying OF drug biomarkers are described in Table 4. Most new methods utilize liquid chromatography-tandem mass spectrometry (LC-MS/MS), which permits simultaneous analysis of multiple, nonvolatile, labile, polar, and/or high molecular weight compounds in a limited OF volume (26–29). Comprehensive methods for multiple drugs also limit optimization for individual drugs, sometimes yielding insufficient detection capabilities (88, 90). The major disadvantage of LC-MS is matrix enhancement or suppression, which is best managed by inclusion of deuterated internal standards for all analytes; however, this technique may not fully compensate for matrix effects (30). OF is less complex than blood, with fewer proteins, characteristics suggesting the possibility of successful measurement with limited sample preparation, such as dilution, protein precipitation, or centrifugation and direct injection. Unfortunately, more extensive sample preparation is frequently required to limit matrix suppression. Our laboratory found atmospheric pressure chemical ionization (APCI) to be less susceptible to matrix effects than electrospray ionization (ESI) (30). Owing to more efficient ionization, however, ESI is preferred for some analytes, i.e., glucuronides. Many laboratories use ESI routinely and do not consider the advantages of APCI in reducing matrix effects. In addition to LC-MS, many GC-MS assays for drugs in OF also are available, but generally require biomarker derivatization, which increases cost and analysis time.

OF Testing of Specific Drug Classes

With various classes of drugs that are measured in OF, including cannabinoids, amphetamines, opiates, cocaine, and benzodiazepines, specific issues arise with regard to collection, screening, POCT, and result confirmation and interpretation. Controlled drug administration provides a scientific database to guide interpretation of OF tests. The disposition of illicit drugs in OF is affected by metabolic, physiological, and chemical processes (31), including biomarkers of interest, minimal detectable doses, expected concentrations, detection windows, and collection device requirements. The major research findings are detailed by drug class. Specific studies are referenced in Table 5.

CANNABINOIDS

THC is the primary analyte for cannabinoids in OF, whereas 11-nor-9-carboxy-THC (THCCOOH) predominates in urine. Most available antibodies from urine immunoassays are directed toward THCCOOH and have poor cross-reactivity with THC, limiting diagnostic sensitivity.

Recovery of the sample from the collection device is a major issue, especially for lipophilic drugs like cannabinoids, although 94.5% THC recovery from the Cozart DDS device (32) and up to 91.4% recovery from the Quantisal device at a concentration of 4 μg/L (33) were recently reported. It is suggested that manufacturers evaluate each lot of OF collection devices and publish the data in the package insert, which would alleviate each laboratory from performing the same function. To our knowledge, only THC recovery from devices has been examined, although THCCOOH recovery may be equally important.

Initially, THC was thought to be present in OF only from oral mucosal contamination from cannabis smoke. No radiolabel was detected in human OF following radiolabeled intravenous THC administration (34). Using GC-MS with 0.5 μg/L LOQs, we detected no THC metabolites, 11-hydroxy-THC (11-OH-THC) or THCCOOH, in OF after 6 participants smoked a 1.75% or 3.55% THC cigarette (35). However, later we observed that after approximately 30–60 min, gross oral mucosal contamination following cannabis smoking was cleared and OF and plasma concentrations were temporally correlated (13). THC concentrations as high as 5800 μg/L were observed 12 min after cannabis smoking, falling to 81 μg/L by 20 min, documenting rapid drug clearance. Mean (SD) THC OF/plasma ratio in simultaneously collected samples was 1.18 (0.62) (range 0.5–2.2) 0.3 to 4 h after cannabis smoking. Similarity in OF and plasma THC concentrations following contamination dissipation likely indicates a physiological link (transmucosal THC absorption into blood) between these matrices.

Other investigators recently reported a strong linear correlation ($r = 0.84$, $P < 0.001$) in OF and serum THC concentrations up to 6 h after sample donors had smoked 250 or 500 μg/kg THC (36). Mean (SD) THC OF concentrations were 900 (589) and 1041 (652) μg/L, respectively, in samples collected 15 min after donors had smoked, decreasing to 18 (12) μg/L during the next 6 h (37). OF/serum ratios were markedly higher than previously reported at 46 (27) (250 μg/kg) and 36 (20) (500 μg/kg), with a longer period of THC OF detection in OF compared to serum samples. It is not yet clear why the OF/plasma (13) and OF/serum (37) ratios are so discrepant between these studies; additional research is underway. We noted that although plasma and OF THC concentrations were temporally correlated, there was too much variability to predict plasma concentrations from single OF results.

Another major issue is the possibility of false-positive OF test results in sample donors exposed to environmental cannabis smoke. With a 0.5 μg/L gas chromatography–tandem mass spectrometry (GC-MS/MS) cutoff, passive cannabis smoke exposure produced positive THC OF test results in 4 nonsmokers after they spent 15–30 min in a sealed room with 5 cannabis smokers (38). Contamination cleared rapidly, with negative results for all later samples. In another passive exposure study in which sample donors were exposed to cannabis smoke while in an unventilated van, OF samples were collected with the Intercept device inside the van 1 h after smoking cessation (study 1) and outside the van after up to 72 h (study 2), with 4 study participants smoking 39.5 mg THC mixed with tobacco (study 1) or 83.2 mg THC (study 2) cigarettes (39). Peak OF THC concentrations in passively exposed sample donors were up to 7.5 μg/L (study 1) and 1.2 μg/L (study 2); THC was not quantifiable by 30–45 min after exposure. Collection devices environmentally exposed in the van contained 3–14 μg/L in study 1. When OF samples were collected outside the van after the 1 h exposure, all OF samples were negative, suggesting that earlier studies may have used environmentally contaminated collection devices. OF results were positive for active smokers for 0–8 h.

More recently, THCCOOH (40, 41) and its glucuronide metabolite (42) were quantified in OF in nanogram per liter concentrations. This finding was important, because THCCOOH is not in cannabis smoke. Demonstrating THCCOOH in OF could clearly differentiate passive

exposure from active smoking. Until recently, no analytical procedures existed for simultaneously extracting and quantifying THC and THCCOOH from the same OF sample. We recently developed and validated an assay for simultaneous identification and quantification of THC, cannabidiol (CBD), cannabinol (CBN), 11-OH-THC, and THCCOOH in OF collected with the Quantisal device (43). Simultaneous analysis was problematic owing to varying physiochemical characteristics and concentration ranges. THC, 11-OH-THC, CBD, and CBN were eluted with hexane/acetone/ethyl acetate (60:30:20 vol/vol/vol), derivatized with *N*, *O*-bis-(trimethylsilyl) tri-fluoroacetamide and quantified by 2-dimensional GC electron-impact MS with cryotrapping. Acidic THC-COOH was separately eluted with hexane/ethyl acetate/acetic acid (75:25:2.5 vol/vol/vol), derivatized with tri-fluoroacetic anhydride and hexafluoroisopropanol, and quantified by the more sensitive 2-dimensional GC-MS–electron capture negative chemical ionization method. Linearity was 0.5–50 μg/L for THC, 11-OH-THC, and CBD and 1–50 μg/L for CBN. The linear dynamic range for THCCOOH was 7.5–500 ng/L. This new analytical method is being applied to OF samples collected in our cannabinoid controlled drug administration studies, and we hope it will provide new insights into cannabinoid disposition in OF.

Acceptance of OF testing for cannabinoids has been difficult for multiple reasons, including short detection times. The mean time for the last positive result for THC in OF, collected with the Intercept OF collection device and analyzed by GC-MS/MS (LOQ 0.5 μg/L), was 34 (11) h (range 1–72 h) after the sample donor had smoked a 20–25 mg THC cigarette (44). However, study participants left the laboratory after 8 h and were instructed not to smoke cannabis, but they were not monitored. Mean OF THC concentrations 1 h after smoking were 27.8 (6.2) and 22.6 (6.2) μg/L from the right and left sides of the mouth, with good concordance throughout 72 h after smoking (44). OF samples were positive up to 72 h after eating 20–25 mg THC-laced brownies; concentrations were <6.9 μg/L THC (44). Currently, few data are available for detection windows after acute cannabis smoking, and no data are available for detection after ingestion of THC-containing pharmacotherapies or after chronic, heavy cannabis smoking. The lack of such data limits our ability to interpret OF test results.

Cannabinoid cutoffs were proposed before THC-COOH was detected in OF. Certainly quantification of THCCOOH requires a highly sensitive assay, but such an assay may prove to be the answer for cannabinoid OF testing. The window of detection and prevalence of THCCOOH in OF is still unknown. Perhaps an immunoassay screen for THC and confirmation for THC-COOH would provide definitive identification of cannabis use, although this must be proven. Because THC and cannabinoids are the most prevalent drugs in clinical, workplace, and DUID testing (45), it is difficult to move to an alternative matrix if cannabinoids are not adequately and specifically identified. These limitations have slowed acceptance of OF for multiple drug testing applications.

OPIOIDS

Opioids have many therapeutic applications, but these drugs also are abused. Heroin has a short half-life and is rapidly metabolized to 6AM and then morphine. Although 6AM can be detected in urine for a short time after exposure, one of the main advantages of OF over urine testing for opiates is that 6AM and sometimes heroin are frequently detected in OF. SAMHSA increased the mandated urine testing cutoff from 300 to 2000 μg/L because Medical Review Officers acted on so few opiate-positive tests; most were attributed to codeine use or poppy seed ingestion rather than heroin abuse.

Recoveries of opioids from collection devices were approximately 80% for most devices, except the Sarstedt Salivette® (35.2% for morphine and 39.0% for codeine) (3). Existing immunoassay antibodies adequately target morphine, codeine, and 6AM in OF. A problem

for opioid OF and urine testing is the wide range of analytes that are abused, including heroin, codeine, oxycodone, buprenorphine, hydromorphone, and hydrocodone. Additional specific assays are available that target oxycodone (46) and buprenorphine (47). Multiple screening assays must be conducted to adequately cover this large class of abused drugs.

There are few controlled opiate administration studies to guide interpretation of OF tests. Our laboratory administered placebo, low (60 mg/70 kg) and high (120 mg/70 kg) oral codeine sulfate to 19 participants and then collected OF for up to 72 h by citric acid–stimulated expectoration and by stimulated and un-stimulated Salivette. Samples were analyzed by GC-MS with LOQs of 2.5 μg/L for codeine, norcodeine, morphine, and normorphine (48, 49). At 0.08–1.0 h after administration, codeine was detected with all collection methods after both doses. Mean peak OF codeine concentrations did not differ significantly between collection methods and were 638.4 (64.4) μg/L at 1.7 (0.23) h (low) and 1599.3 (241.0) μg/L at 1.6 (0.14) h (high). During the 1–12-h period after administration, the OF/plasma ratio was approximately 4. Codeine is a basic drug and is ion trapped in OF, increasing its detection. OF and plasma codeine concentrations were significantly correlated ($r = 0.22$, $P < 0.0001$), but variability precluded predicting plasma concentrations from OF concentrations. Mean codeine half-lives were 2.5 (0.21) h and 1.8 (0.19) h, similar to plasma half-lives. Mean codeine OF detection time was 21 h (using the LOQ), but only 7 h at proposed SAMHSA cutoffs. Norcodeine/codeine ratios increased over time from 0.5–8 h, ranging from 0.3%–31%. Norcodeine OF/plasma ratios were lower than those of codeine owing to increased polarity and a lower p*K*a, conditions that decrease ion trapping. Morphine and normorphine have not been detected in OF or plasma after codeine administration, whereas late in the time course of urinary excretion of codeine, low morphine concentrations may be present without measurable codeine, making interpretation difficult. This does not occur in OF after codeine ingestion, another advantage of OF compared to urine testing for opiates.

These data document similar opiate detection windows for OF and plasma, making OF a good matrix for DUID, because the OF detection window better reflects drug intoxication than urinary detection windows. OF also is a good alternative matrix for drug treatment, owing to the shorter opiate half-lives in OF than urine. For the same reason, consecutive OF samples are less likely to be positive after a single opiate exposure than consecutive urine samples. A difficult problem in drug treatment programs is differentiating new opiate use from residual opiate excretion in urine. Our controlled drug administration data support that such differentiation would be much less problematic with OF than with urine testing.

Another problem with opiate urine testing is that positive results may occur after sample donors have ingested poppy seed foodstuffs. A recent study showed that ingestion of poppy seed bagels and poppy seeds (9.8–20.8 g) also can produce positive OF tests, albeit only for up to 1 h after ingestion, when a morphine OF cutoff of 40 μg/L is applied (50).

Thus, OF opiate testing offers multiple advantages to urine testing, including observed, easy collection, even at the roadside; enhanced identification of heroin abuse; better differentiation of new opiate exposure from residual drug excretion; and reduced possibility of positive tests following ingestion of opiate-containing food. Urine testing for opiates offers the advantage of higher drug concentrations and greater availability of data on controlled administration.

AMPHETAMINES

Amphetamine is the primary abused sympathomimetic amine in Europe, whereas methamphetamine is more prominent in the Americas, Asia, and Australia. Cross-reactivity with methamphetamine, amphetamine, and 3,4-methylenedioxymethamphetamine (MDMA)

(ecstasy) is considered an advantage because only one screen adequately covers the three most commonly abused drugs, while cross-reactivity with phenylpropanolamine, ephedrines and other common cold medication ingredients is a disadvantage, due to the need for expensive and time-consuming confirmatory procedures to rule out these drugs. Analytes of interest are the same in urine and OF, thus, cross-reactivity is a major testing issue for both. Also, drug potencies and doses are similar for the analytes of interest, making sensitivity less of a problem.

One of the problems encountered in OF testing after stimulant abuse is dry mouth. During routine OF testing and controlled dosing research, mouth dryness in sample donors may lead to low sample volumes and/or long collection times. Recovery from collection devices was rarely problematic for amphetamines, with percentages generally above 75%. However, the Sarstedt Salivette had low recoveries of 51.8%/26.5% for amphetamine/MDMA, as did the Malvern Medical OraCol (69.1%/52.0%) for the same analytes (3).

Amphetamines are weak bases and are subject to ion trapping in OF, providing the advantage of higher concentrations in OF than plasma. We first detected methamphetamine in expectorated OF with citric acid stimulation within 0.08–2 h after sample donors had ingested 10 or 20 mg sustained-release oral S-(+)-methamphetamine hydrochloride (GC-MS LOQs 2.5 μg/L) (51). We found that samples collected with the use of citric acid stimulation had amphetamine concentrations 1.5-fold lower than samples collected without stimulation. The decrease in OF pH that occurs with citric acid should increase basic amphetamine concentrations, but stimulation had a greater effect on salivary volume. For samples collected by expectoration, mean peak OF concentrations after single 10- or 20-mg doses were 106.1 (24.7) μg/L (range 25–312 μg/L) during the period of 4–8 h after dosing and 192.2 (120.8) μg/L (range 75–322) 2–12 h after dosing. The linear correlation between OF and plasma concentrations, although significant, was only 0.222. The median OF/plasma methamphetamine ratio was 2.0 and was highly variable within and between participants. Methamphetamine was detected in the OF of all study participants for 24 h; amphetamine was detected in only 62.5% (low) and 100% (high) of samples after administration at 8.6 (6.5) μg/L (range 4–21 μg/L) and 14.3 (6.1) μg/L (range 3–20 μg/L). Interestingly, OF was not contaminated by oral administration of this preparation, as noted by a lag time before first drug detection. It was thought that swallowing the methamphetamine pill would lead to contamination of the oral mucosa, but such contamination did not occur. If methamphetamine was smoked, extensive contamination of the oral cavity would be expected. The OF detection rate at 50 μg/L (proposed SAMHSA cutoffs) was only 4% after 10 mg and 28.6% after 20 mg methamphetamine, which are therapeutic doses and quite low compared to abuse doses (52). Mean detection times until last positive OF after the last of 4 daily 10- or 20-mg methamphetamine doses (using the LOQ) were 24.6 (9.9) h and 74.1 (17.0) h, respectively, and with a 50-μg/L methamphetamine and 2.5-μg/L amphetamine cutoff were 4.0 (1.8) h and 20.6 (3.2) h. Detection times for OF were much shorter than those for urine.

In contrast to the controlled dosing data available for methamphetamine, MDMA doses spanning the recreational range have been administered. Median MDMA and 3,4-methylenedioxyamphetamine (MDA) OF concentrations collected with the OraSure Intercept® device 1.5 h after sample donors had received 75 or 100 mg oral MDMA were 447.6 μg/L (range 44.1–3993.1 μg/L) and 7.7 μg/L (range 1–42.4 μg/L), respectively (53, 54). By 5.5 h, median MDMA concentrations were 315.7 μg/L (range 42.4–3078.9 μg/L) and MDA concentrations were 16.5 μg/L (range 1.5–102.6 μg/L). Unfortunately, no later samples were collected, negating the determination of MDMA OF detection windows.

Determination of MDMA detection windows also was not possible in a study in which racemic MDMA (75 mg) was administered to 9 participants and OF was collected by expectoration from 1–5 h after administration (55). Interestingly, however, MDMA and MDA enantiomers were quantified by GC-MS. Mean peak OF concentrations 1–4 h after ingestion were approximately 600 μg/L, with R-(−)-MDMA significantly ($P < 0.05$) exceeding S-(+)-MDMA (approximately 400 μg/L) concentrations in all samples. Enantiomer ratios (R/S) increased over time and ranged from 1.04–1.92. R-(−)-MDA concentrations were <5 μg/L in 78% of samples and never >21.4 μg/L, whereas S-(+)-MDA concentrations were quantified up to 74.7 μg/L in 72% of samples.

As weak bases, amphetamines are ion trapped in OF, leading to increased concentrations and windows of drug detection. OF testing appears to be a good alternative matrix to urine, although data informing interpretation of methamphetamine, amphetamine, and MDMA OF concentrations, including windows of drug detection, are limited.

COCAINE

Cocaine is a highly abused drug in the US and South America, and prevalence of abuse has increased in Europe in recent years (56). Because cocaine is a weak base and stimulant, ion trapping in OF is expected, as is the incidence of dry mouth and restricted sample volume. In simultaneously collected plasma and OF samples, we found higher concentrations of cocaine in plasma than OF, and the reverse for the primary, more polar, BE metabolite (57, 58). Recovery of cocaine from collection devices was reported as good (85.6%–100%), except for the Malvern Medical OraCol device (3).

Although cocaine is an important analyte in emergency toxicology, drug treatment, criminal justice, and workplace drug testing programs, there are only 2 reports of cocaine OF concentrations after controlled drug administration. In the first, cocaine and metabolite disposition in OF after single 25-mg intravenous, 32-mg intranasal, and 42-mg smoked doses of cocaine was determined in OF collected by stimulated expectoration before and up to 12 h after dosing (59). Mean last detection times for cocaine were short, 4.7 (0.6), 6.3 (1.2), and 4.1 (1.0) h with an 8 μg/L proposed SAMHSA cutoff. However, these administered doses are low compared to recreational doses and doses self-administered by tolerant users. BE mean last detection times were 6.7 (1.9), 8.7 (1.5), and 5.0 (2.1) h at the same cutoff. Cocaine and BE OF disposition also were evaluated following up to 5 daily escalating doses of oral cocaine, beginning with 100 mg and increasing by 25 mg to a maximum single dose of 400 mg and a maximum total dose of 2000 mg/day for up to 16 days (59). OF detection times with stimulated expectoration after repeated dosing were 4-fold and 7-fold longer for cocaine and BE compared to single doses. The authors suggested that detection times based on single doses underestimate the utility of OF for identifying cocaine use because high, repeated doses are frequently self-administered.

In the other investigation of controlled cocaine administration, participants each received placebo or 75 and 150 mg/70 kg cocaine sulfate on different days, administered subcutaneously for safety purposes (58). OF collection occurred for 48 h after each dose and samples were obtained by expectoration with citric acid candy stimulation. OF was analyzed by GC-MS with 2.5 μg/L LOQs for cocaine, BE, and ecgonine methylester (EME). OF was positive for cocaine within 5 min in 65% of low and 100% of high-dose samples. Mean onset times of EME and BE were similar, approximately 0.1 h in plasma and 0.3 h in OF. OF cocaine maximum concentrations were 1322.3 (848) and 3130.7 (2228.2) μg/L after the 75 and 150 mg/70 kg doses. Cocaine and EME maximum concentrations were significantly higher in OF than plasma, whereas BE maximum concentrations were higher in plasma. Mean BE OF maximum concentrations were 154.7 (100.3) and 308.0 (163.8) μg/L. Cocaine maximum concentrations was slightly lower in plasma than in OF. Although cocaine

concentrations exceeded those of BE in OF, detection times were generally longer for BE in this matrix. Cocaine, EME, and BE were ≥LOQ in 93% of participant samples at 8 h. After study participants received the high dose, cocaine OF/plasma ratios tended to be 1–2 up to 0.25 h and >3 at 0.5–8 h; about half remained >3 up to 48 h.

Cocaine is a weak base and thereby is subject to OF ion trapping, which leads to increased concentrations and windows of drug detection. Reduced salivary volume follows the use of this stimulant. Abuse of smoked crack cocaine, insufflation of cocaine hydrochloride, and oral cocaine abuse will contaminate the oral cavity, with resulting high initial OF concentrations compared to concentration that occur after intravenous cocaine abuse. There are no data describing how long cocaine concentrations remain increased after mucosal contamination. OF appears to be a good alternative matrix to urine, with reasonable detection windows of 1–2 days, even after recreational single 100-mg doses.

BENZODIAZEPINES

Benzodiazepines encompass a large variety of drugs with differing pharmacodynamic effects and pharmacokinetic profiles. SAMHSA does not include benzodiazepines in workplace drug testing, but DRUID recommends cutoffs (Table 1). Benzodiazepines have a high prevalence in DUID cases (4) and appear to be especially impairing during the first few weeks of therapy (60). Monitoring benzodiazepine exposure with OF is especially challenging owing to the wide range of available benzodiazepines with highly variable potencies, low concentrations, legal prescription status, high lipophilicity, analyte instability in nonpreserved OF, and variable cross-reactivities with antibodies. Many jurisdictions, especially in Europe, strongly advocate inclusion of benzodiazepines in testing strategies because of their contribution to DUID. However, few data are available for OF benzodiazepines.

Research on collection devices for benzodiazepines is limited and results are more variable (Table 3). Langel et al. reported 97.1% and 96.9% recoveries for diazepam and alprazolam from OF collected by expectoration, and 95.7% and 95.8% with the Arco Biotech Salicule™ device (3). The Varian OraTube, Sarstedt Salivette, and Malvern Medical OraCol were not suitable for benzodiazepine collection, with recoveries <49% (3). Recoveries for the Cozart device were 66.0% for alprazolam but 91.6% for diazepam, results that highlight the variability within this class (3). Speedy et al. found a recovery of 92.1% for temazepam with Cozart DDS (32) and Quintela et al. 101.3% for oxazepam with Immunalysis Quantisal (33).

Flunitrazepam was detected only up to 6 h in expectorated OF after oral dosing of 1 mg. Concentrations were <0.6 μg/L if OF was preserved with 2% sodium fluoride. Concentrations of flunitrazepam's metabolite 7-aminoflunitrazepam were <3.1 μg/L(61). OF was positive for tetrazepam from 0.25–10 h after a 50-mg dose, and no diazepam or nordiazepam metabolites were measurable in OF, but these metabolites were positive in urine (62). Diazepam was found as a pill contaminant, but also may have derived from metabolism of tetrazepam. Three study participants receiving 10 mg diazepam in the same study had positive OF samples for >10 h. Other investigators reported that after study participants received 15 or 30 mg oxazepam, oxazepam and oxazepam glucuronide were detected in expectorated OF for >8.5 h, in much lower concentrations than in simultaneously collected blood (63). OF/blood ratios ranged from 0.04–0.07 for oxazepam and from 0.002–0.006 for oxazepam glucuronide.

These data indicate that OF testing for benzodiazepines will be challenging indeed. Low OF concentrations, a multitude of drugs within the class, instability of some parent drugs, and potential recovery issues from collection devices must be addressed. Although a single study has addressed glucuronide-benzodiazepine conjugates, the available data suggest that

glucuronide concentrations may be an order of magnitude lower than free drug in OF, and hydrolysis of OF samples may not be necessary. Alternatively, LC-MS/MS may permit direct measurement of glucuronide conjugates; however, matrix suppression and coelution with endogenous matrix components are adverse events that frequently occur with early elution of conjugates on the most common reversed-phase columns.

Applications

Applications of OF testing in DUID, treatment, and workplace settings reported in the last 5 years are listed in Table 6. The number of such reports is increasing, providing excellent data on the usefulness of this new technology, the prevalence of drugs in various populations, and the performance of various onsite tests. It is hoped that recommendations proposed at the Talloires meeting of experts for standardizing research DUID methods will be followed for future studies, allowing better comparison of data across studies than can be currently accomplished.

DUID studies have tested impaired or suspected drivers (18, 19, 64–67), random drivers at road-blocks (10, 68), and drivers near discotheques (17) and high-risk accident areas (69), and have evaluated injured hospitalized drivers (70). Percentages of positive tests were highly variable (range 0.1%–90.5%) owing to differences in study populations, collection and analytical methods, LOQs, times between stop and OF collection, and DUID laws (69).

In 46.7% of reports, cannabinoids were the most prevalent drug. THC concentrations ranged from 0.7 (71) to 6484 μg/L (10). The higher concentrations appear to be attributable to contamination of the oral mucosa from cannabis smoke immediately after use; median concentration was 81 μg/L (10). Concheiro et al. evaluated the Varian OraLab® and Dräger Drug Test®, but also collected OF for confirmation by expectoration, eliminating the variable of cannabinoid recovery from the collection device (17).

Opioids are one of the least commonly identified drug classes in DUID. Detected concentrations have ranged from 4 μg/L for codeine to 7600 μg/L for 6AM, the latter possibly due to oral contamination, because high-purity heroin may be smoked, inhaled, or snorted (69). Opioids are one of the most investigated drug classes in treatment settings (54.5% of studies).

Amphetamine prevalence was as high as 90.5% (67) and 87.7% (66) in 2 Finnish studies of DUID suspected drivers. In a study by Engblom et al. OF concentrations up to 131 000 μg/L for amphetamine were observed, most likely due to oral contamination, because the median concentration was 7440 μg/L (66).

Cocaine was one of the least commonly reported drugs in DUID, having the lowest prevalence rate in 50% of worldwide studies. In 2 populations, however, Spain 18.5% (17) and Utah 14.6% (18), it was the most commonly found drug.

Benzodiazepines are an important drug class to monitor because these drugs are frequently reported in DUID cases (72), yet they are some of the most commonly prescribed medications (73). Another difficulty is that an individual may be highly impaired when first taking a benzodiazepine, but rapidly develop tolerance to the drug's impairing effects (60). Thus, determining an appropriate cutoff concentration that reflects impairment is difficult for this and most other drug classes. Reported concentrations ranged from 4 μg/L for temazepam, nordiazepam, or oxazepam to 221 μg/L for nordiazepam in DUID (69). In treatment and workplace settings, concentrations ranged from 0.5 μg/L for diazepam, nordiazepam, oxazepam, and temazepam to 14 301 μg/L for diazepam, with a median of 2.8 μg/L (74). In this interesting study, which compared the sensitivity of drug detection in 635

000 treatment and workplace urine and OF samples, investigators concluded that OF was as efficient as urine for identifying drug use.

In conclusion, the technology of OF collection devices, laboratory-based assays, on-site testing devices, and chromatographic confirmation methods has greatly advanced in the last 5 years. POCT devices are improving, with better recoveries, especially for THC, and more accurate performance. However, successful development of a POCT device that performs acceptably for all drug classes is a challenge. Currently available chromatographic methods quantify multiple analytes at increasingly lower concentrations, and important new analytes (e.g., THCCOOH) have been identified. Perhaps the greatest current limitation for OF testing is the small number of controlled drug administration studies available to inform interpretation of OF tests. Additional research is needed to identify new biomarkers, determine drug detection windows, characterize OF adulteration techniques, and evaluate analyte stability in OF. There is no doubt that OF offers multiple advantages as an alternative matrix for monitoring licit and illicit drug use, and that OF testing has an important role in DUID, treatment, workplace, and criminal justice programs.

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Table 1

SAMHSA, DRUID, and Talloires recommended oral fluid cutoffs.[a]

Drug/analyte	SAMHSA screen, μg/L	SAMHSA confirmation, μg/L	DRUID confirmation, μg/L	Talloires confirmation, μg/L
Cannabinoids	4	2[b]	1[b]	2
Opiates	40			
Morphine		40	20	20
Codeine		40	20	20
6AM	4	4	5	5
Methadone		—	20	20
Phencyclidine	10	10	—	—
Amphetamines	50			
Amphetamine		50	25	20
Methamphetamine		50[c]	25	20
MDMA	50	50	25	20
MDA		50	25	20
MDEA		50	25	20
Cocaine or benzoylecgonine	20	8	10	10
Benzodiazepines	—			
Flunitrazepam		—	1	—
Diazepam		—	5	—
Alprazolam		—	1	—
Oxazepam		—	5	—
Nordiazepam		—	1	—
Lorazepam		—	1	—
Clonazepam		—	1	—

[a] See references: SAMHSA [Department of Health and Human Services, SAMHSA (5)]; DRUID [Pil et al. (75)]; Talloires [Walsh et al. (11)].

[b] THC.

[c] Specimen must also contain amphetamine ≥ method limit of detection.

Table 2

Evaluation of immunological methods for analysis of drugs in OF, including sample type, confirmation method, screening and confirmation cutoffs, diagnostic sensitivity (SN), diagnostic specificity (SP), and efficiency (EFF).

			Cutoffs, μg/L													
			Cannabinoids		Opiates		AMP[a]		COC		Benzodiazepines					
Method	Type	Confirm	Screen	Confirm	Screen	Confirm	Screen	Confirm	Screen	Confirm	Screen	Confirm	SN, %	SP, %	EFF, %	Reference
OraSure Intercept ELISA	—	GC-MS/MS LC-MS/MS	THC 1	—	Methadone 5 Opiates 10	—	MAMP 40; AMP 100	—	BE 5	—	nDZP 1	—	50–100	99–100	—	Clarke and Wilson (76)
Roche KIMS	—	—			Opiates 40	—	MAMP & AMP 50	—					—	—	—	Ananias et al. (77)
Cozart Microplate ELISA	E	GC-MS			Opiates 2.5, 10, 20, 30, 40	COD, nCOD, MOR, nMOR 2.5, 20, 30, 40							73.4–91.5	88.6–99.2	89.3–95.6	Barnes et al. (78)
Cozart RapiScan	E	GC-MS			Opiates 30	COD, nCOD, MOR, nMOR 2.5, 15, 30, 40							56.1–99.0	96.2–99.9	85.7–98.2	Kacinko et al. (49)
Cozart Microplate ELISA	T	GC-MS			Opiates 30	Opiates 30							99.1	94.4	96.8	Cooper et al. (22)
Cozart Microplate ELISA	T	GC-MS			Methadone 20 & 30	Methadone 30							91.3–92.2	97.9–100	94.9–95.5	Cooper et al. (79)
IDS BUP One-step ELISA	T	GC-MS			BUP 0.5	—							100	100	100	De Giovanni et al. (47)
Cozart Microplate ELISA	E	LC-MS/MS					MDMA & MDA 51	MDMA & MDA 10					98.6	98.6	98.6	Laloup et al. (53)
Cozart Microplate ELISA	F	GC-MS					AMP 45	AMP 30					87.2	97.8	93.5	Cooper et al. (24)
Bio-Quant Direct ELISA	F						MAMP & AMP ≤ 6 (1 in PBS)	—					—	—	—	Apollonio et al. (80)
Cozart Microplate ELISA	E	GC-MS							BE 10, 20, 30	COC, BE, EME 2.5, 8, 10, 15			89.8–99.0	58.8–89.6	80.0–89.7	Kim et al. (81)

			Cutoffs, μg/L													
			Cannabinoids		Opiates		AMP[a]		COC		Benzodiazepines					
Method	Type	Confirm	Screen	Confirm	Screen	Confirm	Screen	Confirm	Screen	Confirm	Screen	Confirm	SN, %	SP, %	EFF, %	Reference
Cozart RapiScan	E	GC-MS							BE 20, 30	COC, BE, EME 2.5, 8, 15, 30			66.5–95.5	82.7–98.1	74.1–92.0	Kolbrich et al. (82)
Cozart Microplate ELISA	T	GC-MS							BE 20	COC, BE, CE 30			99.0	100	99.5	Cooper et al. (23)
EMIT II Plus	D	LC-MS/MS									LMP 10	NZP 0.1; APZ, LZP 1.2; LMP 1.3; MZL 2.3; DZP 6.9; nDZP 7.6; TZP 8.1; OZP 13.1	75	96.9	96.0	Smink et al. (83)

[a] AMP, amphetamine; COC, cocaine; MAMP, methamphetamine; nDZP, nordiazepam; E, experimental; COD, codeine; nCOD, norcodeine; MOR, morphine; nMOR, normorphine; T, treatment; BUP, buprenorphine; F, fortified; CE, cocaethylene; LMP, lormetazepam; NZP, nitrazepam; APZ, alprazolam; LZP, lorazepam; MZL, midazolam; DZP, diazepam; TZP, temazepam; OZP, oxazepam.

Table 3

Evaluation of collection and POCT devices for drugs in OF including types of samples tested, drug classes evaluated, diagnostic sensitivity (SN), diagnostic specificity (SP), efficiency (EFF), recovery of drug from the device, % device failures, and references.

	Type	THC	OP[a]	AMP	COC	BENZO	Confirmation	SN (%)	SP (%)	EFF (%)	Recovery (%)	Failures (%)	Reference
POCT devices													
American Bio Medica OralStat	F	x	x	x	x	x	HPLC GC-MS	0–100	100	71.4–100	—		Walsh et al. (20)
Biomar Toxiquick	D	x	x	x	x		GC-MS[b] HPLC-DAD	42.1–100	63.9–88.7	64.3–86.1	—		Biermann et al. (19)
Branan Oratect	F	x	x	x	x		GC-MS or LC-MS/MS	0–100	33.3–100	71.4–100	—		Crouch et al. (84)
Cozart DDSV	T	x					GC-MS	44.4	100	60	—	0	Kintz et al. (14)
Cozart RapiScan	T		x		x		GC-MS	Opiates 96.7 COC 98.2	100	Opiates 98.0 COC 99.0	—		Cooper et al. (21)
Cozart RapiScan	E		x				ELISA & GC-MS	ELISA 98.6 GC-MS 91.3	ELISA/GC-MS: 98.1/98.9	ELISA/GC-MS: 98.2/97.5	—		Kacinko et al. (49)
Cozart RapiScan	F	x	x	x	x	x	GC-MS	5.0–100	40.0–100	45.7–100	—		Walsh et al. (20)
Cozart RapiScan	—			x			GC-MS	98.3	96.9	97.3	—		Wilson et al. (85)
Dräger Drug Test	D	x	x	x	x		LC-MS	22.2–90.1	89.8–100	77.7–97.3	—	10.2	Concheiro et al. (17)
Dräger Drug Test	D	x					LC-MS/MS	49.5	100	55.0	—		Laloup et al. (86)
Envitec SmartClip	F		x	x	x		HPLC or GC-MS	65–100	33.3–100	65.7–97.1	—		Walsh et al. (20)
LifePoint Impact	F	x	x	x	x		HPLC or GC-MS	70.0–100	33.3–100	71.4–100	—		Walsh et al. (20)
OraSure Uplink	F	x	x	x	x		MS	10.0–100	33.3–100	74.3–100	—		Crouch et al. (84)
Securetec DrugWipe	D	x	x	x	x		ELISA/MS[b]	7.7–42.9	97.4–99.6	86.1–98.1	—	0.7	Crouch et al. (18)
Securetec DrugWipe	D	x	x	x	x	x	GC-MS	50.0–100	84.2–99.3	79.2–98.6	—	5.3	Pehrsson et al. (67)
Securetec DrugWipe	F	x	x	x	x		GC-MS	50.0–100	100	71.4–100	—		Walsh et al. (20)
Sun OraLine IV s.a.t.	T	x					GC-MS	69.2	91.7	80.0	—	10.3	Cirimele et al. (87)
Sun OraLine IV s.a.t.	F	x	x	x	x		HPLC or GC-MS	100	0–100	54.3–100	—		Walsh et al. (20)
Ulti-Med SalivaScreen	F	x	x	x	x		GC-MS	65.0–100	33.3–100	71.4–94.3	—		Walsh et al. (20)
Varian OraLab	D	x	x	x	x		LC-MS	50.0–100	97.9–100	71.4–100	—	24.5	Concheiro et al. (17)
Ansys OralLab	F	x	x	x	x		GC-MS	40.0–100	33.3–100	71.4–100	—		Walsh et al. (20)
Collection devices													
Acro Biotech Salicule	F	x	x	x	x	x	GC-MS				>90 except THC 45.9		Langel et al. (3)

	Type	THC	OP[a]	AMP	COC	BENZO	Confirmation	SN (%)	SP (%)	EFF (%)	Recovery (%)	Failures (%)	Reference
Cozart	F	x	x	x	x	x	GC-MS	—	—	—	66.0–91.6	—	Langel et al. (3)
Cozart DDS	T	x	x	x	x	x	GC-MS or LC-MS	—	—	—	91.1–100	—	Speedy et al. (32)
Dräger DCD 5000	F					x	LC-MS/MS	—	—	—	>90	—	Kempf et al. (88)
Greiner Bio-One Saliva Collection System	F	x	x	x	x	x	GC-MS	—	—	—	73.6–98.5; >80, except THC	—	Langel et al. (3)
Immunalysis Quantisal	F	x	x	x	x	x	GC-MS	—	—	—	>80, except THC 55.8	—	Langel et al. (3)
Immunalysis Quantisal	F		x				GC-MS	—	—	—	86.7–96.6	—	Moore et al. (46)
Immunalysis Quantisal	Fs	x	x	x	x	x	LC-MS/MS	—	—	—	81.3–109.4	—	Quintela et al. (33)
Malvern Medical OraCol	F	x	x	x	x	x	GC-MS	—	—	—	<12.5–81.5	—	Langel et al. (3)
Orasure Intercept	F	x					GC-MS	—	—	—	53.0–83.4	—	Kauert et al. (16)
Orasure Intercept	F	x	x	x	x	x	GC-MS	—	—	—	>80, except THC 37.6	—	Langel et al. (3)
Sarstedt Salivette	T		x		x		LC-MS/MS	—	—	—	OP 73.9–95.3 except PV 30.8–35.2, NC 56.4–63.4 COC 74.3–114.9 except *p*OHBE 47.4–49.6	—	Dams et al. (89)
Sarstedt Salivette	F	x	x	x	x	x	GC-MS	—	—	—	<12.5–51.8	—	Langel et al. (3)
StatSure Saliva Sampler	F	x	x	x	x	x	GC-MS	—	—	—	81.3–91.1	—	Langel et al. (3)
Varian OraTube	F	x	x	x	x	x	GC-MS	—	—	—	39.8–86.7	—	Langel et al. (3)

[a]OP, opioids; AMP, amphetamines; COC, cocaine; BENZO, benzodiazepines; F, fortified; T, treatment; D, DUID; DAD, diode array detection; E, experimental; s, synthetic OF; PV, papaverine; NC, noscapine; *p*OHBE, *p*-hydroxybenzoylecgonine.

[b]Confirmation in blood.

Table 4

Chromatographic methods for measurement of drugs in OF.

		LOQ, μg/L					
Method	**Extraction**	**Cannabinoids**	**Opiates**	**Amphetamines**	**Cocaine**	**Benzodiazepines**	**Reference**
GC-MS	SPE[a]	THC 2	6AM, FEN 2; BUP, nBUP 4; Pholcodine 5; Methadone, MOR, COD, EtMOR 20	AMP, MAMP, MDMA, MDA, MDEA 25	COC, BE 8	DZP, nDZP, LZP, MZL, OZP, PZP, TZP 2, APZ, CZP, NZP 5	Gunnar et al. (90)
LC-MS/MS (ESI)	SPE		MOR, 6AM, COD 2	AMP, MAMP, MDMA, MDA 2	COC, BE 2		Wood et al. (54)
LC-MS/MS (ESI)	SPE		Methadone 2.2; EDDP 0.8; 6AM 1.0; nBUP 1.6; MOR 1.7; COD, BUP 1.8	AMP1.2; MAMP 1.3; MDMA 2.5; MDA 3.3; MDEA 4	EME 0.9; COC 3.1; CE 3.4; BE 11.4	DZP 0.9; nDZP 1.2; CLP, TZP 1.6; OZP 2.5	Wylie et al. (91)
LC-MS/MS (APCI)	PP		nMOR, MOR, nCOD, COD, 6AM, Heroin, AC 1		COC, EME, BE, CE 1		Dams et al. (89)
LC-MS/MS (ESI)	LLE	THC <0.16	6AM 0.82; BUP 1.2 Methadone 4.0; COD 6.5; MOR 7.1	MAMP 3.0; MDA <3.6 MDMA <3.9; MDEA <4.1 AMP < 6.8	COC <0.78 BE 7.2	APZ 0.093; OZP <1.4; FTP 0.15 NZP 0.23; CZP 0.26; LZP 0.32 DZP 0.41; FZP 0.75; BZP 7.9	Øiestad et al. (92)
GC-MS	SPE	CBD 0.9; THC 1.9; THCCOOH 4.8; CBN 5.6; 11-OH-THC 12.7	6AM 2.9; MOR 6.5; COD 6.6	MDMA 8.9; MDA 14.4; MDEA 15.0 A 20.6; MAMP 20.9	COC 4.1; BE 8; CE 7.2; EME 12.7	FLP 10.9; DZP 16.3	Pujadas et al. (93)
LC-MS/MS (ESI)	SPE	THC 1	6AM 1; MOR, COD 5	AMP, MAMP, MDMA, MDA, MDEA 5	COC, BE 5	APZ, CZP, DZP, nDZP, FZP, LZP, OZP 1	Concheiro et al. (94)
LC-DAD	LLE/MAE		MOR, 6AM, Methadone, EDDP 50		COC, BE, CE 50		Fernandez et al. (95)
LC-MS/MS (ESI)	LLE/SPE		COD, MOR, HCOD, HMOR, OCOD, OMOR 10	AMP, MAMP, MDMA, MDA 10	COC, BE 2		Kala et al. (96)
LC-MS/MS (ESI)	PP	THCCOOH 3.5; THC 3.7	MOR 1.2; 6AM 1.9	MAMP 0.4; MDE 0.5; AMP, MDMA, MDA 1.5	COC, BE 0.5		Sergi et al. (97)
LC-MS (ESI)	LLE	THC 2					Concheiro et al. (98)
GC-MS/MS	SPE	THC 0.75					Niedbala et al. (38)
LC-MS	SPE	THC 2					Teixeira et al. (99)
LC-MS/MS (ESI)	LLE	THC 0.1 (500 μL), 0.5 (100 μL)					Laloup et al. (100)

Method	Extraction	LOQ, μg/L					Reference
		Cannabinoids	Opiates	Amphetamines	Cocaine	Benzodiazepines	
LC-MS (ESI)	SPE	THC 2					Teixeira et al. (101)
GC-MS/MS (NCI)	SPE	THCCOOH 0.01					Day et al. (102)
GC-MS	SPE	THC 2.4					Kauert et al. (16)
GC-GC-MS (ECCI)	SPE	THCCOOH 0.02					Moore et al. (40)
GC-MS	SPE	11-OH-THC 0.3; THC 0.5; THCCOOH 6.2					Kauert et al. (37)
GC-MS	SPE	Conjugated THCCOOH 0.5					Moore et al. (42)
GC-MS	SPE	THC, CBN 0.5; CBD, 2-carboxyTHC 1					Moore et al. (103)
LC-MS/MS (ESI) (synthetic OF)	LLE	THC 0.1; THCCOOH 0.5					Quintela et al. (104)
LC-MS (ESI)	SPE	THC 5					Teixeira et al. (105)
GC-MS (PCI)	LLE		6AM 2; COD 2.3; MOR 6.7				Campora et al. (106)
LC-MS/MS (ESI)	None		6AM, COD, AC 2; MOR 6; Heroin 10				Phillips and Allen (107)
GC-MS	SPE		Propoxyphene 5				Rana et al. (108)
GC-MS	SPE		MEP, TRA, OCOD 10				Moore et al. (46)
CEP	LLE		EDDP 7.6; Methadone 8.1				Martins et al. (109)
GC-MS	LLE			AMP, MAMP, MDMA, MDA, MDEA 20			Kankaanpää et al. (110)
LC-FD	LLE			MDMA, MDA, MDEA 10			Concheiro et al. (111)
GC-MS	SPE			MDMA, MDA 5; HMMA, HMA 25			Scheidweiler and Huestis (112)
GC-MS	SPE			AMP, MAMP, MDMA, MDA, MDEA 5; HMA, HMMA 25			Scheidweiler and Huestis (113)
GC-MS	SPE			MAMP 2.5			Huestis and Cone (52)
GC-MS	SPE			AMP, MAMP, MDMA, MDA, MDEA 25			Moore et al. (114)

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Method	Extraction	LOQ, μg/L					Reference
		Cannabinoids	Opiates	Amphetamines	Cocaine	Benzodiazepines	
GC-MS (NCI)	LLE			MDA 5; AMP, MAMP, MDMA, MDEA 25			Peters et al. (55)
LC-MS/MS (ESI)	SPE				COC, BE, CE 10		Clauwaert et al. (115)
GC-MS	SPE				BE 0.75		Robarge et al. (116)
GC-MS/MS (PCI)	SPE				COC, CE 2; EME 5		Cognard et al. (117)
LC-MS (ESI)	LLE					MZL 0.2	Quintela et al. (118)
LC-MS/MS (ESI)	LLE					APZ, BZP, DZP, nDZP, LZP, LMP, MZL, OZP, TZP, TRZ, TZL 0.1; CBZ 0.2	Kintz et al. (119)
LC-MS (ESI)	LLE					DZP, FTP, MZL, TRZ, TZL 0.2; APZ, BZP, LZP 0.5	Quintela et al. (120)
LC-MS/MS (APCI)	LLE					NZP 0.1; APZ, LZP 1.2; LMP 1.3; MZL 2.3; DZP 6.9; nDZP 7.6; TZP 8.1; OZP 13.1	Smink et al. (83)
LC-MS/MS (ESI)	LLE					DZP, nDZP, OZP, 0.05; TZP, TRZ 0.2	Laloup et al. (62)
LC-MS/MS (ESI)	LLE					MZL 0.05	Link et al. (121)
LC-MS/MS (ESI)	SPE					APZ, nDZP, FTP, FLP, MZL, TZP, TZL 0.5; BZP, CZP, DZP, NZP 1; LZP, OZP 5	Moore et al. (122)
LC-MS/MS (APCI)	SPE					APZ, CZP, DZP, nDZP, FTP, LZP, OZP, TZP 0.1	Ngwa et al. (123)
LC-MS/MS	PP					OZP glucuronide 0.25; OZP 0.5	Smink et al. (63)
LC-DAD	SPE					LZP 130; DZP 290; BZP 370; CZP, FTP 390; APZ 450	Uddin et al. (124)

[a]SPE, solid phase extraction; FEN, fentanyl; BUP, buprenorphine; nBUP, norbuprenorphine; MOR, morphine; COD, codeine; EtMOR, ethylmorphine; AMP, amphetamine; MAMP, methamphetamine; MDEA, 3,4-methylenedioxy-ethylamphetamine; COC, cocaine; DZP, diazepam; nDZP, nordiazepam; LZP, lorazepam; MZL, midazolam; OZP, oxazepam; PZP, phenazepam; TZP, temazepam; APZ, alprazolam; CZP, clonazepam; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; CE, cocaethylene; CLP, citalopram; PP, protein precipitation; nMOR, normorphine; nCOD, norcodeine; AC, acetylcodeine; LLE, liquid liquid extraction; FLP, flurazepam; FZP, fenazepam; BZP, bromazepam; DAD, diode array detection; MAE, microwave-assisted extraction; HCOD, hydrocodone; HMOR, hydromorphone; OCOD, oxycodone; OMOR, oxymorphone; MDE, methylenedioxy-N-ethylamphetamine; NCI, negative chemical ionization; ECCI, electron capture chemical ionization; PCI, positive

chemical ionization; MEP, meperidine; TRA, tramadol; CEP, capillary electrophoresis; HMMA, 3-hydroxy-4-methoxymethamphetamine; HMA, 3-hydroxy-4-methoxyamphetamine; FD, fluorescence detection; LMP, lormetazepam; TRZ, tetrazepam; TZL, triazolam; CBZ, clobazam; FTP, flunitrazepam; NZP, nitrazepam.

Table 5

Controlled drug administration studies with oral fluid collection.

THC (cannabinoids)	Opioids	Amphetamines	Cocaine	BENZO[a]	Doses	Study participants, N	Reference
x					1 Cannabis joint, potency unknown	3	Jehanli et al. (125)
x					20–25 mg Smoked THC 20–25 mg THC (brownies)	15 3	Niedbala et al. (44)
x					0, 1.75, and 3.55% Smoked THC	6	Huestis and Cone (13)
x					Passive cannabis smoke exposure	4	Niedbala et al. (38)
x					Passive cannabis smoke exposure	4	Niedbala et al. (39)
x					0, 250, and 500 μg/kg Smoked THC	10	Ramaekers et al. (36); Kauert et al. (37)
x					1 Cannabis joint, potency unknown	1	Moore et al. (40)
	x				30 mg Oral codeine	3	Sharp et al. (126)
	x				60 and 120 mg Intramuscular codeine	1	Cone (127)
	x				Oral codeine	8	Chen et al. (128)
	x				30 mg Oral liquid codeine phosphate	17	O'Neal et al. (129)
	x				30 mg Oral liquid codeine phosphate	37	O'Neal et al. (130)
	x				16 mg Oral codeine	5	Jehanli et al. (125)
	x				Single and multiple 60 and 120 mg/70 kg codeine	19	Kim et al. (48); Kacinko et al. (49)
	x				Poppy seed bagels and poppy seeds	7	Rohrig and Moore (50)
		x			10 or 20 mg MAMP	8	Schepers et al. (51)
		x			0, 75, and 100 mg Oral MDMA	18	Laloup et al. (53); Wood et al. (54)
		x			Single and multiple 10 and 20 mg MAMP	8	Huestis and Cone (52)
		x			75 mg MDMA	12	Peters et al. (55)
			x		25 mg Smoked, 32 mg intravenous, 42 mg insufflated cocaine, multiple oral cocaine up to 2000 mg/day	66	Jufer et al. (59)
				x	1 mg Oral flunitrazepam	4	Samyn et al. (61)
				x	50 mg Oral TZP or 10 mg oral DZP or repeated 25 mg oral TZP	7	Laloup et al. (62)
				x	15 and 30 mg Oxazepam	8	Smink et al. (63)

[a]BENZO, benzodiazepines; MAMP, methamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; DZP, diazepam; TZP, tetrazepam.

Table 6

Application studies with oral fluid testing.

Type	No. of samples	Screening	Confirmation	Prevalence, %					Reference
				THC	Opiates	Amphetamines	Cocaine	Benzodiazepines	
D: I/S[a]	302	Toxiquick	GC-MS[b]	56.8	8.0	48.3	5.7		Biermann et al. (19)
D: Hos	300	Cozart Microplate ELISA	GC-MS & LC-MS/MS	≥1.7	≥1.4	≥1.4	≥0.4	≥6.7	Bernhoft et al. (70)
D	61	DrugWipe & Intercept	GC-MS	29.5					Kintz et al. (71)
D: I/S	131	Dräger DrugTest	GC-MS	54.2	17.6	32.8	14.5		Toennes et al. (64)
D: I/S	177	Dräger DrugTest	GC-MS	54.2	19.8	44.1		16.4	Toennes et al. (65)
D	55	—	LC-MS/MS		3.6	27.3	20.0		Wood et al. (54)
D: Ra	1396	Cozart Microplate ELISA	GC-MS	3.7	≥3.2	≥4.6	1.6	2.0	Wylie et al. (69)
D: Rd	468	Dräger DrugTest	LC-MS	≥38.7	≥3.5	≥7.0	≥35.5		Concheiro et al. (17)
D: Rd	468	OraLab	LC-MS	≥9.5	≥3.5	≥1.2	≥18.5		Concheiro et al. (17)
D: R	13 176	DrugWipe & RapiScan	GC-MS	≥0.7		≥2.0			Drummer et al. (10)
D: I/S	114	DrugWipe	GC-MS			87.7			Engblom et al. (66)
D: I/S	40	DrugWipe	ELISA & MS[b]	37.5	12.5	5.0	20.0		Crouch et al. (18)
D: I/S	267	DrugWipe	ELISA & MS[b]	14.6	2.6	4.1	14.6		Crouch et al. (18)
D: R	10 816	—	LC-MS/MS	0.6	1.1	0.3	0.1	1.4	Gjerde et al. (68)
D: I/S	148	DrugWipe	GC-MS	15.5	2.7	90.5	1.4	50.6	Pehrsson et al. (67)
T	217	Cozart Microplate ELISA	GC-MS				53.5		Cooper et al. (23)
T	358	Cozart RapiScan	GC-MS		25.4		31.6		Cooper et al. (21)
T	198	Cozart Microplate ELISA	GC-MS		57.1				Cooper et al. (79)
T	216	Cozart Microplate ELISA	GC-MS		50.5				Cooper et al. (22)
T	27	Sun OraLine IV s.a.t.	GC-MS	52.0					Cirimele et al. (87)
T	135	Cozart Microplate ELISA	GC-MS			57.8			Cooper et al. (24)
T	513	Orasure Intercept	LC-MS/MS		57.9				Phillips and Allen (107)
T&W	635 000	Orasure Intercept	GC-MS/MS	≥0.1	≥1.1	≥0.1	≥0.2	≥0.1	Cone et al. (74)
T	403	Sarstedt Salivette	LC-MS/MS		15.4		32.3		Dams et al. (89)
T	134	Cozart Microplate ELISA	GC-MS & LC-MS	73.1		23.9			Speedy et al. (32)

Type	No. of samples	Screening	Confirmation	Prevalence, %					Reference
				THC	Opiates	Amphetamines	Cocaine	Benzodiazepines	
T	20	Cozart DDSV	GC-MS	90.0					Kintz et al. (14)

[a] D, DUID; Hos, hospitalized drivers; I/S, impaired or suspected drivers; R, random; Ra, random at high-risk accident area; Rd, random near discotheques; T, treatment; W, workplace testing;

[b] Screening with OF, but confirmation with blood.