Journal of Analytical Toxicology, 2019;43:415–443
doi: 10.1093/jat/bkz048
Advance Access Publication Date: 28 June 2019
Review

OXFORD

Review

Oral Fluid Drug Testing: Analytical Approaches, Issues and Interpretation of Results

Nathalie A. Desrosiers[1,*] and Marilyn A. Huestis[2]

[1]Centre of Forensic Sciences, Toronto, Ontario, Canada and [2]Lambert Center for the Study of Medicinal Cannabis and Hemp, Institute of Emerging Health Professions, Thomas Jefferson University, Philadelphia, PA, USA

*Corresponding Author: Nathalie Desrosiers, F-ABFT 25 Morton Shulman Ave., Toronto, ON M3M 0B1, Canada. Email: Nathalie.desrosiers@ontario.ca

Abstract

With advances in analytical technology and new research informing result interpretation, oral fluid (OF) testing has gained acceptance over the past decades as an alternative biological matrix for detecting drugs in forensic and clinical settings. OF testing offers simple, rapid, non-invasive, observed specimen collection. This article offers a review of the scientific literature covering analytical methods and interpretation published over the past two decades for amphetamines, cannabis, cocaine, opioids, and benzodiazepines. Several analytical methods have been published for individual drug classes and, increasingly, for multiple drug classes. The method of OF collection can have a significant impact on the resultant drug concentration. Drug concentrations for amphetamines, cannabis, cocaine, opioids, and benzodiazepines are reviewed in the context of the dosing condition and the collection method. Time of last detection is evaluated against several agencies' cutoffs, including the proposed Substance Abuse and Mental Health Services Administration, European Workplace Drug Testing Society and Driving Under the Influence of Drugs, Alcohol and Medicines cutoffs. A significant correlation was frequently observed between matrices (i.e., between OF and plasma or blood concentrations); however, high intra-subject and inter-subject variability precludes prediction of blood concentrations from OF concentrations. This article will assist individuals in understanding the relative merits and limitations of various methods of OF collection, analysis and interpretation.

Introduction

With advances in analytical technology and new research informing result interpretation, oral fluid (OF) testing has gained acceptance over the past decades as an alternative biological matrix for detecting drugs in forensic and clinical settings. OF testing offers simple, rapid, non-invasive, observed specimen collection, making sample adulteration more difficult and eliminating the need for specialized bathroom collection facilities or same-sex collectors, as may be the case with urine. Other advantages include potential for on-site collection and screening, lower biohazard risk during collection (compared with collection of blood) and ease of multiple sample collections. Also, as compared with urine, OF is both more likely to contain parent drugs, which may reflect more recent drug use, and more likely to have drug findings that correlate better with blood. OF testing, including on-site testing and laboratory confirmation, utilizes well-validated and accepted analytical methods and instrumentation; and published scientific studies have documented its reliability in drug treatment, workplace, pain management and driving under the influence of drugs (DUID) programs.

Distribution of Drugs into Oral Fluid

Secretions from the salivary glands are termed saliva, while OF consists of saliva and other debris and food products in the oral

© The Author(s) 2019. Published by Oxford University Press. All rights reserved. For permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

cavity. Healthy adults produce ~0.5–1.5 L of saliva per day (1). OF pH ranges from 6.2 to 7.4, becoming more alkaline when saliva secretion is stimulated due to increased bicarbonate excretion and possibly a loss of dissolved carbon dioxide (1–5). OF composition and flow rate are influenced by circadian rhythm, sensory stimuli, hormonal changes, mechanical stimulation, psychological state (e.g., anger, fear and depression), genetics, oral hygiene, sympathomimetic and parasympatholytic (anticholinergic) drugs and systemic diseases (e.g., diabetes, kidney dysfunction, anorexia and cystic fibrosis) (6, 7). In turn, drug transfer from blood into OF is affected by OF composition, flow rate and pH, as well as the drug's pKa, protein binding, lipophilicity, spatial configuration and molecular weight (4, 8). Free, unbound drugs can enter OF by passive diffusion from blood. Glucuronidated metabolites also can be detected, albeit in lower concentrations than free metabolites (9, 10). Following smoking, insufflation, sublingual and/or oral drug administration, the oral mucosa is directly exposed to drug(s) resulting in relatively high OF drug concentrations (9, 11–13). In contrast, capsule ingestion typically does not contaminate the oral mucosa unless the capsule is adulterated or chewed (14, 15).

The process of ion trapping basic drugs in OF from blood produces higher drug concentrations in OF. This phenomenon occurs due to the lower OF pH compared with that of blood (~7.4); once in the blood, free, uncharged bases diffuse across membranes into OF and ionize at the lower pH, reducing diffusion back into blood and yielding higher OF than blood concentrations.

There is no consensus on an appropriate biomarker to normalize OF drug concentrations. OF creatinine concentrations showed large intra-subject and inter-subject variation; coefficient of variation (%CV) over 10 weeks was 141% (range 39–225%) (16, 17). OF immunoglobulin G (IgG) concentrations ≥0.1–1.0 mg/L were suggested to identify undiluted OF, but even after a second rinse of the mouth with tap water, IgG concentrations still exceeded this criterion, indicating that diluted OF samples still had IgG concentrations that would be considered 'undiluted' (4). One suggested approach normalized hydrocodone OF concentrations to dose, body mass index, lean body weight, body surface area and calculated blood volume; however, in workplace, pain management and forensic situations, these data would be unavailable (18). As noted above, variations between drugs, their route of administration and between individuals can influence drug detectability in OF. It is important to recognize and account for these variations when evaluating OF drug findings.

Oral Fluid Collection

OF can be collected by a variety of techniques including passive drool and expectoration (with or without stimulation) and via a wide variety of collection devices. Each collection method has advantages and disadvantages (e.g., passive drool is unpleasant for donors and collectors, and stimulated OF samples are diluted due to increased salivary flow). Common collection techniques are characterized below.

Passive drool

Passive drool requires individuals to drool into a collection tube. This collection method best reflects drug concentrations in excreted saliva, as opposed to expectoration or even putting a collection device in the mouth, which can increase salivation and alter OF composition. However, passive drool OF collection is slow and unpleasant for donors and collectors. Few studies utilize this method, making interpretation of results difficult (19).

Expectoration and salivary stimulation

Expectoration or spitting provides neat OF without buffer dilution, increasing assay sensitivity. Although this collection method is less expensive than other collection methods, it is also unpleasant for donors and collectors. Expectorated OF is viscous and contains mucus, food particles and/or other mouth debris. Samples need to be centrifuged prior to laboratory analysis to remove precipitant material, which may yield lower drug concentrations due to drug loss in the pellet or adsorption to the tube. For example, in Δ^9-tetrahydrocannabinol (THC) fortified expectorated OF centrifuged for 10 min, only 28.8% THC was recovered in the supernate, 51.7% was recovered from the protein pellet and 14.7% from the polypropylene tube after addition of the surfactant Triton® X-100 (20). Mucus in OF, which can be variable in composition, can also interfere with proper interaction with solid-phase extraction sorbent, reducing drug concentrations and increasing imprecision (21). Furthermore, the absence of stabilizing buffer may lead to lower drug stability in expectorated samples, as was demonstrated with cannabinoids (22). Finally, many drugs can produce dry mouth, yielding difficulty in expectorating OF and resulting in low sample volumes.

Earlier research utilized stimulated salivary flow to facilitate OF collection. OF stimulation, for example, occurs when chewing paraffin or sucking on an acidic candy. Although the initial OF pH of specimens collected with citric acid-treated cotton swabs (mean 2.8, range 2.4–3.6) was lower than OF pH collected with a neutral cotton swab (mean 6.0, range 4.2–7.2) (14, 23), salivary flow stimulation increased saliva volume and pH, due to increased bicarbonate excretion (1, 3, 4). Stimulation increased saliva excretion and lowered, rather than increased, drug concentrations, complicating result interpretation. Lower concentrations following citric acid candy-stimulated expectoration was documented for multiple drugs, including methamphetamine and amphetamine (14) and codeine (24).

Via collection device

Commercial OF collection devices generally include a pad or sponge to absorb the OF and a buffer to better stabilize drugs and extract them from the collection pad. Collection time varies by device and amount of OF collected but is generally completed within a few minutes (Table I). The absorbent pad filters the OF, reducing extraneous material collection (e.g., food). Buffers reduce OF viscosity, improving measurement accuracy and increasing stability, but also dilute analyte concentrations. The collection pad must remain in the buffer for the manufacturer-specified period of time (from 4 h to overnight) in order to ensure adequate drug recovery. This is not an issue in most cases, as OF samples are generally shipped overnight or brought to a laboratory for analysis. Buffers and surfactants also interfere with liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis if injected directly or after dilution, producing ion suppression or enhancement (matrix effects); therefore, sample cleanup is suggested (31).

A serum separator tube or filter separates the OF–buffer mixture from the pad (e.g., Quantisal, StatSure and Oral-Eze) or a specially designed tube for centrifugation that performs separation for other devices (e.g., Intercept and DCD 5000). Alternately, devices may not include a buffer (e.g., Salivette), instead using centrifugation with a tube insert to separate OF from the pad. As the buffer volume can be variable, OF drug concentrations are reported as neat (undiluted, that is, considering the ratio of OF volume compared with the elution

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table I. Volume of Oral Fluid Collected with Various Oral Fluid Collection Devices

Device	Volume Adequacy Indicator?	Collection Volume as Stated by Manufacturer	Collection Method	Mean ± RSD Volume Collected (mL)	Range (mL)	Reference
Quantisal (Immunalysis)	Yes	1 mL ± 10%	In vitro	1.009 ± 0.99%	—	25
			Volunteers	1.086 ± 7.27%	—	25
			Volunteers	0.84 ± 6.7%	—	26
			In vitro	1.15 ± 7.68%	1.04–1.22	27
Saliva Sampler (StatSure Diagnostic Systems)	Yes	1 mL	In vitro	1.176 ± 1.87%	—	25
			Volunteers	0.952 ± 11.97%	—	25
Intercept (OraSure Technologies)	No	1 mL max	In vitro	0.863 ± 1.97%	—	25
			Volunteers	0.790 ± 29.87%	—	25
			Volunteers	0.224	<0.05–0.795	28
			Volunteers	0.89 (median)	0.38–1.54	29
Salivette (Sarstedt AG & Co.)	No	Unknown	In vitro	1.968 ± 1.07%	—	25
			Volunteers	1.905 ± 20.94%	—	25
Cozart (Cozart Bioscience)	Yes	1 mL	In vitro	1.294 ± 12.60%	—	25
			Volunteers	0.967 ± 33.51%	—	25
Certus (Concateno)	Yes	1 mL	Volunteers	0.94 ± 19%	—	26
DCD 5000 (Draeger)	Yes	0.38 mL	In vitro	0.487 ± 1.3%	—	30
			Volunteers	0.467 ± 13.5%	0.387–0.535	30

Data reported in weights were converted to volumes employing a 0.993 g/mL OF density.

buffer volume) OF concentrations, as it allows a direct comparison of drug concentrations from different collection devices.

Early collection devices did not include a volume adequacy indicator, leading to large variability in OF volume collected and resultant measured drug concentrations (Table I). Higher cocaine and cannabinoid concentrations were documented in samples collected with one collection device compared with a second type of collection device, leading authors to postulate that different volumes of OF are collected with the two devices or that recovery may differ between the two devices (32, 33). On the other hand, duplicate OF samples collected with the Quantisal collection devices did not have significant differences in THC and 11-nor-9-carboxy-THC (THCCOOH) concentrations (34). To protect against such variability in collected volume, the European Workplace Drug Testing Society (EWDTS) requires a device to have a volume adequacy indicator, and a precise quantity of collected OF must be determined gravimetrically or spectrophotometrically (35). The Substance Abuse and Mental Health Services Administration (SAMHSA) proposed mandatory guidelines for OF testing that requires collection of a minimum of 1.0 ± 0.1 mL of OF (36).

Oral Fluid Screening

On-site OF testing

An advantage of OF testing is the ease of collection and amenability to rapid on-site (point-of-collection) testing. On-site devices include an OF collector and a built-in detection system (lateral flow immunoassay on a test strip) for screening multiple drugs or drug classes. The presence or absence of a drug or drug class can be determined visually (e.g., by the appearance of a line) or through a more objective reading of drug intensity along the test strip. Several commercial devices were developed before much of the basic science on OF drug disposition was known. Many devices had poor sensitivity and specificity and are no longer available or were substantially modified. Earlier studies compared positivity rates on the on-site devices with confirmatory urine, blood or plasma tests rather than with confirmatory laboratory OF analysis, leading to poor accuracy and agreement between screening and confirmation results due to inherent differences between the matrices. Newer devices demonstrate better sensitivity, specificity and efficiency. For example, as manufacturers lowered detection limits and increased assay run times to achieve better THC sensitivity, detection of toxicologically relevant THC concentrations was achieved. Cannabis is the most common drug, other than ethanol, identified in DUID cases and drivers (37). Therefore, an on-site OF testing device should have high efficiency for detecting cannabis to be considered for inclusion in workplace, pain management and DUID programs.

Several studies assessed the time course of specific drugs in OF and the detection rates of on-site devices, improving result interpretation (32, 38–44). Other studies had low drug positive rates, making it difficult to accurately assess sensitivity, specificity and efficiency of the device (45). Furthermore, several others demonstrated that sensitivity, specificity and efficiency are highly dependent on the analytes and confirmation cutoffs employed and the matrix (OF and blood) used for confirmation (32, 38–40). These factors are important considerations when selecting commercially available on-site OF testing devices. A complete review of on-site OF testing devices is beyond the scope of this review, and comparisons of devices are available (44–47).

Laboratory-based immunoassay screening

In addition to roadside immunoassay screening devices, multiple validated laboratory-based immunoassays are available for screening drugs in OF (48–55). Because parent drugs are present in higher prevalence in OF than metabolites, blood immunoassays should be adapted for OF screening rather than urine immunoassays, as these typically target metabolites. The drug concentration range should be considered when converting immunoassays for OF, as many drugs have higher OF concentrations compared with blood, especially shortly after the time of use after drug smoking, vaporization, insufflation, sublingual or oral intake, when concentrations >1,000 µg/L can occur due to direct exposure of the oral mucosa to drug(s).

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Potential interferences were investigated by studying the effects of food, mouthwash, toothpaste and vinegar on various OF assays; only vinegar produced false positives in one assay (56). On the Immunalysis THC cannabinoid OF enzyme-linked immunosorbent assay, no interferences were produced following mouthwash, orange juice, toothpaste, coffee and soy milk intake (54).

One disadvantage of screening with immunoassays is the poor cross-reactivity with novel psychoactive substances (NPSs). For example, synthetic cathinones have poor cross-reactivity with most amphetamine immunoassays (57, 58), and the synthetic cannabinoid immunoassays tend to rapidly become outdated in their ability to identify the latest synthetic cannabinoids on the market. Chromatographic mass spectrometric screening methods offer improved identification of NPSs.

Oral Fluid Confirmation

Quantitative OF methods utilize similar analytical methods and sample volumes as blood and urine analyses. With instrumentation improvements, there is a trend towards developing chromatographic methods capable of detecting and quantifying multiple classes of drugs in OF in a single assay (Table II). Sample volume required for analysis is dependent upon the number of drugs included in the method, the sensitivity of the analytical method and whether the sample is collected by expectoration or with a collection device that includes a buffer dilution. Several validated methods are available for expectorated OF; however, if these methods are used with OF collected with collection devices, it is important to validate the method with matrix-matched samples. Manufacturers include different components in their proprietary buffers such as surfactants that can produce matrix effects (83, 84). Interferences with the mass spectrometer, such as quadrupole charging, were documented with OF samples collected with some collection devices (85). Furthermore, authentic rather than synthetic OF is recommended for method validation despite the difficulty in obtaining it, unless synthetic OF is validated as equivalent, as potential endogenous interferences can be better evaluated.

Amphetamines

Gas chromatography–mass spectrometry (GC-MS) and LC–MS/MS quantification methods for OF amphetamines typically include amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxyethylamphetamine, with approximate linear ranges of 20–250 μg/L (Table III). Derivatization, required for GC-MS analysis, is generally achieved with heptafluorobutyric anhydride. When enantioselective quantification of D- and L-amphetamines is required, derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) produces diastereomers for LC–MS/MS analysis and that with *S*-heptafluorobutyrylprolyl chloride produces diastereomers for negative-ion chemical ionization analysis by GC-MS (90, 92).

Cannabis

Analytical methods quantifying multiple cannabinoids in OF can improve interpretation of results. THC is the primary and sole analyte included in most published OF cannabinoid methods (Table IV). Published methods include GC-MS, 2D-GC-MS, LC-MS, LC–MS/MS and LC–high-resolution mass spectrometry methods; and analytes included in published methods are THC, 11-hydroxy-THC (11-OH-THC), THCCOOH, cannabidiol (CBD), and cannabinol (CBN). Due to contamination of the oral mucosa during cannabis inhalation or ingestion, a large dynamic range or validated dilution integrity is needed for OF THC (e.g., 0.5–>1,000 μg/L). However, THC is also detected in OF following passive exposure to intense environmental cannabis smoke (114–118). THC's inactive metabolite, THCCOOH, may be monitored to confirm cannabis intake, as it is not present in cannabis smoke (118). Furthermore, THCCOOH in OF extends the detection window of cannabis intake in chronic frequent cannabis smokers and also identifies intake of oral synthetic THC or Marinol, while THC does not (34, 119). Thus, despite its presence in low ng/L concentrations, monitoring THCCOOH in OF may be warranted, as it may provide additional information. Highly sensitive analytical methods achieving the required low ng/L limits of quantification (LOQs) were published for various cannabinoids (Table IV). It should be noted that some cannabis smoke components may create interferences that mask the low ng/L THCCOOH concentrations; authentic samples should be evaluated to ensure that any interferences are adequately resolved (85).

Identifying minor cannabinoids CBN, tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in OF was suggested to document past-day intake in occasional and chronic frequent cannabis smokers, although various cannabis strains were not evaluated (85, 102, 109). These cannabinoids are easily included in OF analytical methods, although their concentrations are generally lower. Inclusion of CBD, CBG, CBN and THCV can improve interpretation of cannabis OF results.

Cocaine

Cocaine and its metabolite benzoylecgonine (BE) are relatively easy to detect in OF due to their high concentrations, frequently by LC separation on a C18 column. Cocaine and BE can also be detected by GC-MS. Several analytical methods are available for quantification of cocaine and metabolites alone, with BE extending cocaine's detection window (Table V), or in combination with other drugs (Table II). Typical concentration ranges for cocaine and BE are from 1 or 10 to 100 or 1,000 μg/L. Other OF assays also include cocaethylene and ecgonine methyl ester. Cocaine and BE concentrations >1,000 μg/L were reported due to ion trapping and contamination of the oral mucosa during smoking or insufflation; therefore, large dynamic ranges are required for quantifying cocaine markers or dilution may be necessary.

Opioids

OF opioid methods are available for GC-MS with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide derivatization and by LC–MS/MS (Table VI); however, due to the structural similarities between opioids, chromatographic separation and characteristic transition selection are imperative (125). Opioids such as codeine, morphine, 6-acetylmorphine (6-AM), heroin, hydrocodone, hydromorphone, oxycodone and oxymorphone are the most common analytes, with 6-AM presence identifying heroin administration. Concentration ranges are quite variable due to the differing potencies of opioids. Typical 6-AM OF concentrations are 2–500 μg/L, although higher 6-AM concentrations are common and may require dilutions following some routes of administration (e.g., smoking).

LC–MS/MS analysis of OF methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) achieved

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table II. Chromatographic Method for the Detection of Drugs in Oral Fluid

Instrumental Method	Sample Volume and Type	Sample Preparation	Derivatization	Column	Run Time (min)	Analytes						
						AMPS	Opioids	Cocaine and Metabolites	Benzodiazepines	Methadone	BUP	Reference
LC-QTOF-MS	200 µL of expectorated oral fluid	SPE	N/A	Hypersil™ BDS phenyl column	28	✓	✓	✓				59
LC–MS/MS	200 µL of expectorated oral fluid	Protein precipitation	N/A	Synergi Polar RP	25.5		✓	✓		✓		60
GC-MS	250 µL of expectorated oral fluid	SPE	MTBSTFA, MSTFA and HFBA	DB-35ms and DB-5ms	9.5, 10.63 and 10.67	✓	✓	✓	✓	✓	✓	61
LC–MS/MS	250 µL of Intercept oral fluid	SPE	N/A	XTerra® MS C18	20	✓	✓	✓				67
LC–MS/MS	1 mL of Omni-Sal® oral fluid	SPE	N/A	Luna C18	36	✓	✓	✓	✓	✓	✓	62
LC–MS/MS	100 µL of expectorated oral fluid	SPE	N/A	Atlantis dC18	17	✓	✓	✓				63
GC-MS	Oral fluid collected with Salivette	SPE	MSTFA	Unspecified methyl-silicone column	13.1	✓	✓	✓	✓			64
LC–MS/MS	1 mL of StatSure oral fluid	SPE	N/A	Atlantis dC18	12	✓	✓	✓	✓	✓		65
LC–MS/MS	500 µL of Quantisal oral fluid	SPE	N/A	Pinnacle II C18 and Allure PFP Propyl column	5 and 6	✓	✓	✓				66
LC–MS/MS	200 mg of StatSure oral fluid	SPE	N/A	Acquity HSS T3 C18	16.2	✓	✓	✓	✓	✓	✓	68
LC–MS/MS	200 µL of Intercept oral fluid	SPE	N/A	Allure PFP Propyl column	12	✓	✓	✓				69
LC–MS/MS	250 µL of expectorated oral fluid	Isolute PPT+ protein precipitation	N/A	Synergi Polar RP	16		✓	✓		✓	✓	70
LC–MS/MS	100 µL of expectorated oral fluid	Dilute and shoot	N/A	Acquity HSS T3 C18	8	✓	✓	✓	✓			71

Continued.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table II. Continued

Instrumental Method	Sample Volume and Type	Sample Preparation	Derivatization	Column	Run Time (min)	Analytes						Reference
						AMPS	Opioids	Cocaine and Metabolites	Benzodiazepines	Methadone	BUP	
GC-MS	1 mL of StatSure oral fluid	Liquid–liquid extraction and SPE	MSTFA and MTBSTFA	DB-5ms and DB-5ht	9.73, 4.5 and 5.75	✓	✓	✓	✓	✓	✓	72
LC–MS/MS	250 µL of RapidSTAT or Concateno DDS	Dilute and shoot	N/A	Kinetex C18	8	✓	✓	✓		✓		73
LC–MS/MS	500 µL of Quantisal oral fluid	Liquid–liquid extraction	N/A	Luna C18	22	✓	✓	✓	✓	✓		74
LC–MS/MS	10 µL of expectorated oral fluid	Direct injection	N/A	Zorbax SB-Aq	18	✓	✓			✓	✓	75
LC–MS/MS	120 µL of passive drool	MEPS	N/A	PFP Kinetex	12.5	✓	✓	✓		✓	✓	76
LC–MS/MS	200 µL of Intercept or Quantisal oral fluid	SLE	N/A	Acquity BEH C18	7.1	✓	✓	✓	✓	✓	✓	77
LC–MS/MS	200 µL of oral fluid/StatSure buffer or 300 µL of Quantisal/Certus buffer	SPE	N/A	Acquity BEH C18	8.5	✓	✓	✓				78
LC–MS/MS	100 µL of Intercept oral fluid	Direct injection with online extraction column	N/A	Ascentis Phenyl	16		✓	✓				79
LC–MS/MS	150 µL of Oral-Eze oral fluid	Dilute and shoot	N/A	Acquity BEH C18	5	✓	✓	✓	✓	✓	✓	80
LC–MS/MS	500 µL of Quantisal oral fluid	SPE	N/A	Acquity BEH C18	8.5 + wash	✓	✓	✓	✓	✓		81
LC–MS/MS	200 µL of expectorated oral fluid	Liquid–liquid extraction	N/A	Kinetex C18	5.5	✓	✓	✓	✓	✓	✓	82

AMPS: amphetamines; BUP: buprenorphine; GC-MS: gas chromatography mass spectrometry; HFBA: heptafluorobutyric anhydride; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LC-QTOF-MS: liquid chromatography–quadrupole time-of-flight mass spectrometry; MEPS: microextraction by packed sorbent; MSTFA: *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide; MTBSTFA: *N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide; SLE: supported liquid extraction.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table III. Chromatographic Methods for the Detection of Amphetamines in Oral Fluid

Instrumental Method	Sample Volume and Type	Sample Preparation	Derivatization	Column	Run Time (min)	Analytes, LOD, LOQ and Linear Range (µg/L)						Reference
						AMP	MAMP	MDMA	MDA	MDEA	Other Analytes	
LC–MS/MS	10 µL of expectorated oral fluid	Protein precipitation and centrifugation	N/A	Hypersil™ BDS C18	~ 4	0.5, 0.5 0.5–500	0.2 0.5 0.5–500	0.2, 0.5 0.5–500	0.5, 1.0 1–500	0.15, 0.5 0.5–500	N/A	86
GC-MS	100 µL of expectorated oral fluid	Alkaline liquid extraction	HFBA	DB-5ms	18	5, 20 20–5,000	5, 20 20–5,000	5, 20 20–5,000	5, 20 20–5,000	5, 20 20–5,000	Pseudoephedrine Norephedrine Ephedrine BDB, MBDB	87
GC-MS	400 µL of expectorated oral fluid	SPE	HFBA	HP-5ms	19	2.5, 5 5–250	1, 5 5–250	2.5, 5.0 5–250	2.5, 5 5–500	2.5, 5.0 5–250	HMA HMMA	88
GC-MS	250 µL of Quantisal sample	SPE	HFBA	DB-5	8	25 25–200	25 25–200	25 25–200	25 25–200	25 25–200	N/A	89
GC-MS-NICI	50 µL of expectorated oral fluid	Liquid extraction	*S*-HFBPCl	HP-5ms	16	25 25–1,250	25 25–1,250	25 25–1,250	5 5–250	25 25–1,250	N/A	90
GC-MS	1.5 mL Quantisal sample	SPME	Propylchloroformate	HP-5ms	20	2, 2 2–256	0.5, 2 2–256	N/A	N/A	N/A	Fenproporex Diethylpropion methylphenidate	91
LC–MS/MS	250 µL of Oral-Eze or Quantisal sample	SPE	Marfey's reagent N(α)-(2,4-dinitro-5-fluorophenyl)-1-alaninamide	Kinetex® C18	20	0.5, 1 1–500	0.5, 1 1–500	N/A	N/A	N/A	N/A	92
LC–MS/MS	100 µL of Quantisal sample	SPE	Marfey's reagent	Zorbax Eclipse Plus C18	3.4	N/A	25–10,000	N/A	N/A	N/A	N/A	93

AMP: amphetamine; BDB: 1,3-benzodioxolylbutanamine; GC-MS: gas chromatography mass spectrometry; GC-MS-NICI: gas chromatography mass spectrometry negative-ion chemical ionization; HFBA: heptafluorobutyric anhydride; HMA: 4-hydroxy-3-methoxyamphetamine; HMMA: 4-hydroxy-3-methoxymethamphetamine; LC–MS/MS: liquid chromatography–tandem mass spectrometry; MAMP: methamphetamine; MBDB: 1,3-benzodioxolyl-*N*-methylbutanamine; MDA: 3,4-methylenedioxyamphetamine; MDEA: 3,4-methylenedioxyethylamphetamine; MDMA: 3,4-methylenedioxymethamphetamine; *S*-HFBPCl: *S*-heptafluorobutyrylprolyl chloride; SPE: solid-phase extraction; SPME: solid-phase microextraction.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table IV. Chromatographic Methods for the Detection of Cannabinoids in Oral Fluid

Instrumental Method	Sample Volume and Type	Sample Prep	Derivatization	Column	Run Time (min)	Analytes, LOD, LOQ and Linear Range (μg/L)						Reference
						THC	11-OH-THC	THC-COOH (pg/L)	CBD	CBN	Other Analytes	
LC-MS	200 μL of expectorated oral fluid	Liquid–liquid extraction	N/A	XTerra® MS C18	5	2 2–250	N/A	N/A	N/A	N/A	N/A	94
LC-MS	500 μL of Salivette oral fluid	SPE	N/A	XTerra® MS C18	6	2 2–100	N/A	N/A	N/A	N/A	N/A	95
LC–MS/MS	100 μL of or 500 μL of Intercept oral fluid	Liquid–liquid extraction	N/A	XTerra® MS C18	8	0.5–100 (with 100 μL of sample) 0.1–10 (with 500 μL of sample)	N/A	N/A	N/A	N/A	N/A	96
GC-MS/MS	100 μL of Intercept oral fluid	SPE	HFIP and PFAA	DB-5	6	N/A	N/A	10 10–240	N/A	N/A	N/A	97
2D-GC-MS	1 mL of Quantisal oral fluid	SPE	HFIP and TFAA	DB-35ms and DB-1	12.5	N/A	N/A	2 2–160	N/A	N/A	N/A	98
GC-MS	Unspecified volume of Quantisal oral fluid	SPE	BSTFA	DB-5ms	6	0.5 1–16	N/A	N/A	0.5 1–16	1 1–16	THCAA	99
LC-QTOF-MS	500 μL of synthetic oral fluid	Liquid–liquid extraction	N/A	XTerra MS C18	19	0.05, 0.1 0.1–100	N/A	0.2, 0.1 0.1–100	N/A	N/A	N/A	100
LC-MS	500 μL of expectorated oral fluid	SPE	N/A	Symmetry® C18	15	2, 5 5–2,000	N/A	N/A	N/A	N/A	N/A	101
2D-GC-MS (NICI for THCCCOOH)	1 mL of Quantisal oral fluid	SPE	BSTFA TFAA (for THCCOOH)	ZB-50 and DB-1ms DB-1ms and ZB-50 (for THCCOOH)	21 and 13	0.5, 0.5 0.5–50	0.4, 0.5 0.5–50	6, 7.5 7.5–500	0.5, 0.5 0.5–50	1, 1 1–50	N/A	102
LC–MS/MS	400 μL of Intercept oral fluid	SPE	N/A	XTerra MS C18	12	0.2 0.25–8	N/A	0.2 0.25–8	N/A	N/A	N/A	103
LC–MS/MS	1 mL of Quantisal oral fluid	SPE	Triphenylphosphine, 2-picolylamine and 2,2′-dipyridyl disulfide	Stable Bond-C18	8.5	0.6, 1 1–100	N/A	6, 10 10–1,000	N/A	N/A	N/A	104

Continued.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table IV. Continued

Instrumental Method	Sample Volume and Type	Sample Prep	Derivatization	Column	Run Time (min)	Analytes, LOD, LOQ and Linear Range (µg/L)						
						THC	11-OH-THC	THC-COOH (pg/L)	CBD	CBN	Other Analytes	Reference
LC–MS/MS (quadrupole/Orbitrap)	400 µL of oral fluid in preservation buffer	Liquid–liquid extraction and SPE	N/A	Hypersil GOLD aQ	12.5	2 (one-point calibration)	N/A	7.5 7.5–300	N/A	N/A	N/A	105
LC–MS/MS	200 µL of expectorated oral fluid	Liquid–liquid extraction	N/A	Zorbax® Eclipse XDB C18	3.5	1 1–500	N/A	N/A	N/A	N/A	N/A	106
LC–MS/MS	250 µL of expectorated oral fluid	Dilute and shoot	Dansyl chloride	Gemini C18	4	0.005, 0.025 0.2–20	N/A	2, 5 5–500	N/A	N/A	N/A	107
LC–MS/MS	250 µL of expectorated oral fluid	SPE	N/A	Kinetex® C18	12.5	0.1 0.1–50	0.1 0.1–50	0.1 0.1–50	0.1 0.1–50	0.1 0.1–50	N/A	108
LC-HRMS	250 µL of Oral-Eze and 500 µL of Quantisal oral fluid	SPE	N/A	Hypersil GOLD aQ	10	0.5 0.5–50	N/A	15 15–500	0.5 0.5–50	0.5 0.5–50	N/A	109
LC–MS/MS	1 mL of Quantisal oral fluid	SPE	N/A	Kinetex C18	9	N/A	N/A	9, 12 12–1,020	N/A	N/A	N/A	110
LC–MS/MS	225 µL of expectorated oral fluid	MEPS	N/A	Kinetex C18-XB	10	0.08, 0.25 0.25–250	0.12, 0.4 0.4–250	8, 20 20–1,000	0.1, 0.3 0.3–250	0.12, 0.3 0.3–250	N/A	76
LC–MS/MS	100 µL of StatSure, 200 µL of Quantisal or Certus oral fluid	Liquid–liquid extraction	N/A	Acquity UPLC BEH C18	5	5 5–320	N/A	N/A	N/A	N/A	N/A	111
LC–MS/MS	1.5 µL of combined Quantisal sample/methanol extract	SPE	N/A	Kinetex C18	11	0.3, 0.5 0.5–75	0.2, 0.5 0.5–75	50, 80 50–500	0.3, 0.5 0.5–75	0.3, 0.5 0.5–75	THCAA	112
GC-MS/MS	1 mL of Quantisal or 750 µL of Oral-Eze oral fluid	SPE	HFIP and TFAA	2 HP-5ms	5.2	N/A	N/A	7.5, 10 10–1,000	N/A	N/A	N/A	113
LC–MS/MS	1 mL of Quantisal oral fluid	SPE	N/A	Selectra PFPP	14	0.1, 0.2 0.2–100	0.1, 0.2 0.2–50	15, 15 15–3,750	0.1, 0.2 0.2–50	N/A	THCV CBG	85

11-OH-THC: 11-hydroxy-THC; 2D-GC-MS: 2D GC-MS; BSTFA: *N*,O-bis(trimethylsilyl)trifluoroacetamide; CBD: cannabidiol; CBG: cannabigerol; CBN: cannabinol; GC-MS/MS: gas chromatography–tandem mass spectrometry; GC-MS: gas chromatography–mass spectrometry; HFIP: 1,1,1,3,3,3-hexafluoro-2-propanol; LC-HRMS: liquid chromatography–high-resolution mass spectrometry; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LC-MS: liquid chromatography–mass spectrometry; LC-QTOF-MS: liquid chromatography–quadrupole time-of-flight mass spectrometry; MEPS: microextraction by packed sorbent; PFAA: pentafluoropropionic anhydride; SPE: solid-phase extraction; TFAA: trifluoroacetic anhydride; THC: Δ^9-tetrahydrocannabinol; THCAA: Δ^9-tetrahydrocannabinolic acid A; THCCOOH: 11-nor-9-carboxy-THC; THCV: tetrahydrocannabivarin.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table V. Chromatographic Methods for the Detection of Cocaine in Oral Fluid

Instrumental Method	Sample Volume and Type	Sample Prep	Derivatization	Column	Run Time (min)	Analytes, LOD, LOQ and Linear Range (μg/L)					Reference
						Cocaine	Benzoylecgonine	Ecgonine Methyl Ester	Cocaethylene	Other Analytes	
GC-MS	1 mL of expectorated oral fluid	SPE	BSTFA	HP-1	N/A	1 1.1–500	1 1.1–500	1 1.1–500	N/A	N/A	13
LC-QTOF-MS	100 μL of oral fluid	SPE	N/A	Hypersil™ BDS C18	33	1, 10 10–1,000	1, 10 10–1,000	N/A	1, 10 10–1,000	N/A	120
2D-GC-MS	750 μL of Oral-Eze or 500 μL of StatSure oral fluid	SPE	MTBSTFA	DB-1ms and ZB-50	18	1, 1 1–100	0.5, 1 1–100	N/A	N/A	N/A	33
LC-MS	100 μL of Multi-Drugs Multi-Line Twist Screen Test Devices oral fluid	Filtration	N/A	Kinetex® HILIC	~12	4.25–544	4.25–544	N/A	4.25–544	AEME AEC	121

2D-GC-MS: 2D GC-MS; AEC: anhydroecgonine; AEMA: anhydroecgonine methyl ester; BSTFA: *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; GC-MS: gas chromatography–mass spectrometry; LC-QTOF-MS: liquid chromatography–quadrupole time-of-flight mass spectrometry; MTBSTFA: *N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide; SPE: solid-phase extraction.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table VI. Chromatographic Methods for the Detection of Opioids in Oral Fluid

Instrumental Method	Sample Volume and Type	Sample Prep	Derivatization	Column	Run Time (min)	Analytes, LOD, LOQ and Linear Range (µg/L)						
						Morphine	Codeine	6-AM	Hydrocodone/ Hydromorphone	Oxycodone/ Oxymorphone	Other Analytes	Reference
GC-MS	1 mL of unspecified oral fluid	SPE	BSTFA	DB-5	15	2, 2 2–50	2, 2 2–50	2, 3 3–50	3, 10/2, 3 10–50/3–50	2, 3 3–50 (oxycodone only)	N/A	122
GC-MS-PICI	1 mL of Salivette oral fluid	Toxitubes A®	BSTFA	Unspecified 5% phenyl-methyl-siloxane column	13.5	2, 6.7 30–500	0.7, 2.3 30–500	0.6 30–500	N/A	N/A	N/A	123
GC-MS	1 mL of Quantisal oral fluid	SPE	BSTFA or MSTFA	DB-5ms	7, 8 and 17.5	N/A	N/A	N/A	N/A	10 10–80 (oxycodone only)	Meperidine Tramadol	124
LC–MS/MS	1 mL of Quantisal oral fluid	SPE	N/A	Zorbax® Eclipse XDB C18	8	1 1–160	1 1–160	1 1–160	1 1–160	1 1–160	6-Acetylcodeine Dihydrocodeine Norhydrocodone Norcodeine Noroxycodone	125
LC–MS/MS	100 µL of Salivette oral fluid	Protein Precipitation	N/A	Linearly bridged dual columns of Xbridge HILIC and Poroshell 120 EC-C18	10	N/A	N/A	N/A	N/A	N/A	Tramadol and metabolites	126
LC–MS/MS	500 µL of Quantisal oral fluid	SPE	N/A	Zorbax Eclipse Plus C18	5.5	2.5–1,000	N/A	N/A	2.5–1,000	2.5–1,000	Fentanyl Tramadol	127

BSTFA: *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; GC-MS: gas chromatography–mass spectrometry; GC-MS-PICI: gas chromatography–mass spectrometry–positive-ion chemical ionization; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LC-QTOF-MS: liquid chromatography–quadrupole time-of-flight mass spectrometry; MSTFA: *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide; SPE: solid-phase extraction.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

linear ranges of 1–500 μg/L (128–132), with some separating methadone enantiomers (128, 130, 131). In addition, methadone and EDDP are frequently included in methods with multiple analytes (Table II). Although methadone is basic, with expected high OF/blood ratios, it is highly protein-bound; thus, OF/blood ratios were found to vary from 0.13 to 1.97 (median 0.76) depending upon time after dosing (133). Delays in sample analysis may affect methadone results; in one study, which used the Cozart RapiScan, although methadone OF concentrations were stable for 2 months at 4°C, EDDP concentrations decreased (134).

Few methods were published for the detection of buprenorphine and metabolites in OF, possibly due to low prevalence of buprenorphine relative to other opioids at the time earlier methods were published. Nevertheless, newer methods include quantification of buprenorphine and its metabolites by LC–MS/MS with reported linear ranges of 0.1–500 μg/L (9). Buprenorphine markers are typically included in more comprehensive LC–MS/MS methods, especially for pain management testing (Table II).

Benzodiazepines

Benzodiazepines are highly protein-bound in blood, leading to relatively low OF (0.1–50 μg/L) concentrations (Table VII) (135–140). Published methods involve LC–MS/MS analysis and typically include alprazolam, diazepam, lorazepam, midazolam and nordiazepam, with the possibility of including zolpidem, zopiclone and zaleplon in the same method. Concentration ranges of ~0.5–50 μg/L are achieved. In the few multi-analyte methods that include benzodiazepines, a two-step elution is frequently necessary for their recovery.

Novel psychoactive substances

OF analysis offers advantages over testing urine for the presence of NPSs because of the presence of parent drugs. When an NPS is first introduced onto the market, urinary metabolites are typically unknown, making it difficult for urine analysis to identify the drug producing the toxicity. It is critical from a public health perspective to know which drug is responsible for overdoses and deaths, so officials can notify first responders, emergency department personnel and most importantly the public about the new drug. OF may be advantageous in prevalence testing, as parent drugs can be identified, while urine testing may not be feasible until urinary metabolites are identified. Analytical methods require constant updating as NPSs emerge. Several methods, including GC-MS and LC–MS/MS methods, were published for the detection or quantification of NPSs in OF, including synthetic cannabinoids (73, 141–143), synthetic cathinones (143–146), and piperazines (143, 144, 146).

Other Considerations

Recovery of drugs from collection devices

Another consideration in confirmation analysis is that, in addition to variability in collected OF volume and buffer volume, drug recovery differs by collection device. Drugs can adsorb onto the collection device, and good recovery of the drugs from the pad is key to obtaining accurate results. Cannabinoids are particularly susceptible to drug adsorption and, as a result, exhibited poor recovery from first-generation OF collection devices. Early efforts revealed that some low drug recoveries were caused by insufficient time in the buffer to allow drugs to elute off the pad (25). Therefore, it is imperative to allow sufficient time (4–24 h depending upon the manufacturer's recommendation) for the pad to interact with the buffer and allow maximal drug recovery. In general, OF–buffer mixes are shipped overnight to laboratories for analysis, providing sufficient time for maximal recovery from the pad. Table VIII identifies drug recoveries with common OF collection devices. Recoverable volume of OF–buffer mixture may be lower than the total volume (OF volume plus buffer volume), leaving ~1–3 mL of OF–buffer mixture for analysis.

Agency cutoff concentrations

Various agencies proposed or standardized cutoffs for various drug-testing programs, including impaired driving drug testing and workplace drug testing. In 2004, the SAMHSA proposed the Mandatory Guidelines for Federal Workplace Drug Testing Programs (Table IX) (152). However, lack of resolution of important scientific questions delayed final approval. In 2015, revised guidelines were proposed (Table IX) (36). As of April 2019, the final SAMHSA guidelines are still not approved. The guidelines indicate that for initial immunoassay tests that have multiple target analytes (e.g., cocaine/BE), the assay should be calibrated with one of the targets and demonstrate >80% cross-reactivity with the other analyte(s). The revised SAMHSA guidelines also include new required analytes (hydrocodone, hydromorphone, oxycodone and oxymorphone) and lowered cutoffs for some analytes.

In Europe, the EWDTS published the 'European Guidelines for Workplace in Oral Fluid' recommending maximum cutoffs for screening and confirmation tests (Table IX) (35). Other EWDTS screening and confirmation cutoffs are proposed for barbiturates (screen only), ketamine, lysergic acid diethylamide or metabolites, other opioids (oxycodone, hydromorphone, tramadol, tilidine, and fentanyl), phencyclidine, pregabalin, synthetic cannabinoids, synthetic cathinones (3,4-methylenedioxypyrovalerone (MDPV), etc.) and Z-drugs (zopiclone, zolpidem and zaleplon) (35). Separate guidelines exist for OF testing for DUID programs, such as Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID), Talloires, Australian Standards and Canada's Drugs and Driving Committee (Table IX) (153–156).

Authentic vs. synthetic oral fluid for calibrators and controls

Another important consideration when analyzing OF is the matrix for calibrators and controls. Synthetic blank OF is available for calibrator and control preparation. However, due to potential differences in extraction efficiency and matrix effects, authentic OF obtained by passive drool or expectoration, centrifugation and dilution with buffer is often the preferred matrix for calibrator and control preparation. If it is not possible to prepare calibrators and controls in this manner, laboratories should validate performance of synthetic OF compared with human OF to ensure that calibrators and controls prepared in synthetic OF do not differ significantly from human OF, as differences were previously noted (68). As human OF can be variable, several sources of OF should be compared.

Interpretation

Drug OF disposition data are improving test interpretation by toxicologists and medical review officers. The prevalence of drugs in different populations was reported for workplace testing (157), pain management monitoring (158–160), substance abuse treatment (148, 161, 162) and drugged drivers (37, 150, 163–166). Good agreement

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table VII. Chromatographic Methods for the Detection of Benzodiazepines in Oral Fluid

Instrumental Method	Sample Volume and Type	Sample Prep	Column	Run Time (min)	Analytes, LOD, LOQ and Linear Range (µg/L)							
					Alprazolam	Diazepam	Lorazepam	Midazolam	Nordiazepam	Triazolam	Other Analytes	Reference
LC–MS/MS	500 µL of Intercept	Liquid extraction	XTerra® MS C18	10	0.1 0.1–20	0.1 0.1–20	0.1 0.1–20	0.1 0.1–20	0.1 0.1–20	0.1 0.1–20	7-Aminoclonazepam 7-Aminoflunitrazepam Bromazepam Clobazam Lormetazepam Oxazepam Temazepam Tetrazepam Zaleplon Zopiclone Zolpidem	135
LC–MS/MS	500 µL of Salivette oral fluid	Liquid Extraction	XTerra® RP18	16	0.2, 0.5 0.5–50	0.1, 0.2 0.2–50	0.2, 0.5 0.5–50	0.1, 0.2 0.2–25	N/A	0.1, 0.2 0.2–50	Bromazepam Flunitrazepam Lormetazepam Tetrazepam	136
LC–MS/MS	1 mL of expectorated oral fluid	Liquid extraction	Luna® C18	8	N/A	N/A	N/A	0.025, 0.05 0.05–20	N/A	N/A	1′-Hydroxymidazolam 4-Hydroxymidazolam	137
LC–MS/MS	1 mL of Quantisal oral fluid	SPE	Zorbax® Eclipse XDB C18	14.5	0.5 0.5–40	1 1–40	5 5–40	0.5 0.5–40	0.5 0.5–40	0.5 0.5–40	Bromazepam Chlordiazepoxide Clonazepam Flunitrazepam Flurazepam Nitrazepam Oxazepam Temazepam	138

Continued.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table VII. Continued

Instrumental Method	Sample Volume and Type	Sample Prep	Column	Run Time (min)	Analytes, LOD, LOQ and Linear Range (µg/L)							
					Alprazolam	Diazepam	Lorazepam	Midazolam	Nordiazepam	Triazolam	Other Analytes	Reference
LC–MS/MS	400 µL of Intercept oral fluid	SPE	Zorbax® Bonus-RP	8	0.02, 0.1 0.1–20	0.05, 0.1 0.1–20	0.05, 0.1 0.1–20	N/A	0.05, 0.1 0.1–20	N/A	7-Aminoclonazepam 7-Aminoflunitrazepam α-Hydroxyalprazolam Chlordiazepoxide Clonazepam Desalkylflurazepam Flunitrazepam Hydroxyethylflurazepam Oxazepam Temazepam	139
LC–MS/MS	1 mL of Quantisal sample	Liquid extraction	Zorbax® Eclipse XDB C18	15	0.05, 0.1 0.1–5	0.1, 0.1 0.1–5	0.25, 0.5 0.5–5	0.05, 0.1 0.1–5	0.25, 0.25 0.25–5	0.1, 0.1 0.1–5	7-Aminoclonazepam 7-Aminoflunitrazepam 7-Aminonitrazepam Bromazeam Chlordiazepoxide Clobazam Clonazepam Desalkylflurazepam Estazolam Flunitrazepam Flurazepam Hydroxyalprazolam Hydroxymidazolam Hydroxytriazolam Lormetazepam Nitrazepam Oxazepam Prazepam Temazepam Zolpidem	140

LC–MS/MS: liquid chromatography–tandem mass spectrometry; SPE: solid-phase extraction.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table VIII. Recovery of Drugs from Various Oral Fluid Collection Devices

Analyte	Device	Concentration (μg/L)	Extraction Technique	Recovery (%)	Reference
Codeine	Quantisal	20, 40, 80	Buffer–oral fluid mixture separated with serum separator tube	95.6–104.1	147
		454	Pad placed in buffer for 24 h at 4°C	98	26
		1,000	N/A	99.7	25
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad with filter	81.3	25
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	116.0	25
	Salivette	10, 25, 100, 200	Centrifugation with tube insert	91.7	24
		1,000	Centrifugation with tube insert	39.0	25
	Cozart	1,000	N/A	87.1	25
	Certus	454	Pad placed in buffer for 24 h at 4°C	118	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	92.5, 93.7	30
	Finger Collector	10, 25, 100, 200	Milking	53.3	24
Morphine	Quantisal	20, 40, 80	Buffer–oral fluid mixture separated with serum separator tube	91.9–98.7	147
		454	Pad placed in buffer for 24 h at 4°C	98	26
		1,000	N/A	82.7	25
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad with filter	88.5	25
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	92.4	25
	Salivette	10, 25, 100, 200	Centrifugation with tube insert	93.2	24
		50, 250, 400	Centrifugation with tube insert	73.9–78.3	148
		1,000	Centrifugation with tube insert	35.2	25
	Cozart	1,000	N/A	80.8	25
	Certus	454	Pad placed in buffer for 24 h at 4°C	113	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	98.4–98.5	30
	Finger Collector	10, 25, 100, 200	Milking	60.9	24
Heroin	Salivette	50, 250, 400	Centrifugation with tube insert	79.2–85.2	148
6-Acetylmorphine	Quantisal	2, 4, 8	Buffer–oral fluid mixture separated with serum separator tube	92.2–99.5	147
		454	Pad placed in buffer for 24 h at 4°C	98	26
	Salivette	50, 250, 400	Centrifugation with tube insert	86.9–92.6	148
	Certus	454	Pad placed in buffer for 24 h at 4°C	112	26
	DCD 5000	20, 50	Placed in isopropanol for 1 h and centrifuged	92.3–96.3	30
Oxycodone	Quantisal	10	Buffer–oral fluid mixture extracted from pad the next day with serum separator	96	149
Cocaine	Quantisal	10, 20, 40	Buffer–oral fluid mixture separated with serum separator tube	91.2–95.7	147
		454	Pad placed in buffer for 24 h at 4°C	97	26
		1,000	N/A	81.7	25
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad using filter	85.6	25
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	96.9	25
	Salivette	50, 250, 400	Centrifugation with tube insert	81.7–91.4	148
		1,000	Centrifugation with tube insert	33.3	25
	Cozart	1,000	N/A	76.3	25
	Certus	454	Pad placed in buffer for 24 h at 4°C	106	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	95.2–95.6	30
Benzoylecgonine	Quantisal	10, 20, 40	Buffer–oral fluid mixture separated with serum separator tube	82.7–91.2	147
		454	Pad placed in buffer for 24 h at 4°C	88	26
	Salivette	50, 250, 400	Centrifugation with tube insert	90.7–98.2	148
	Certus	454	Pad placed in buffer for 24 h at 4°C	107	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	86.8–92.2	30

(>85–90%) was frequently shown between matrices, especially correlations between OF and plasma or blood concentrations. Although a significant correlation between OF and blood was frequently demonstrated, high intra-subject and inter-subject variability precludes prediction of simultaneously collected blood concentrations from those in OF.

When interpreting and comparing OF drug concentrations, it is crucial to consider collection method. Earlier published concentrations often utilized citric acid-stimulated collection methods that yielded lower concentrations than newer non-stimulated collection methods. Therefore, concentrations reported in a non-stimulated study may not be in the same range as another study following

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table VIII. Continued

Analyte	Device	Concentration (μg/L)	Extraction Technique	Recovery (%)	Reference
Amphetamine	Quantisal	25, 50, 100	Buffer–oral fluid mixture separated with serum separator tube	94.2–96.9	147
		454	Pad placed in buffer for 24 h at 4°C	98	26
		1,000	N/A	89.7	25
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad with filter	88.7	25
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	103.1	25
	Salivette	1,000	Centrifugation with tube insert	51.8	25
	Cozart	1,000	N/A	75.4	25
	Certus	454	Pad placed in buffer for 24 h at 4°C	78	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	92.1–92.3	30
MDMA	Quantisal	1,000	N/A	82.3	25
		454	Pad placed in buffer for 24 h at 4°C	98	26
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad with filter	86.3	25
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	101.1	25
	Salivette	1,000	Centrifugation with tube insert	26.5	25
	Cozart	1,000	N/A	76.0	25
	Certus	454	Pad placed in buffer for 24 h at 4°C	102	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	89.9–93.9	30
Methamphetamine	Quantisal	25, 50, 100	Buffer–oral fluid mixture separated with serum separator tube	93.1–103.8	147
		454	Pad placed in buffer for 24 h at 4°C	100	26
	Cozart	1,5,10	Eluted with proprietary buffer	96	150
	Certus	454	Pad placed in buffer for 24 h at 4°C	102	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	91.1–92.5	30
THC	Quantisal	2, 4, 8	Buffer–oral fluid mixture separated with serum separator tube	81.3–91.4	147
		454	Pad placed in buffer for 24 h at 4°C	94	26
		1,000	N/A	55.8	25
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad with filter	85.4	25
		1.5, 7.5, 30	Buffer–oral fluid mixture extracted from pad using a filter	65.5–68.1	32
	Oral-Eze	1.5, 7.5, 30	Buffer–oral fluid mixture extracted from pad using a filter	42.5–48.8	151
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	37.6	25
		10, 100	Centrifuged to recover buffer–oral fluid mixture	31.2–57.2	29
		10, 100	Centrifuge, add 2 mL methanol to stabilization buffer and pad, incubate and shake 15 min, centrifuge	Additional 19.2–34.4	29
	Salivette	1,000	Centrifugation with tube insert	<12.5	25
	Cozart	200, 500, 1,000	Elute with proprietary buffer	96	150
		1,000	N/A	75.9	25
	Certus	454	Pad placed in buffer for 24 h at 4°C	54	26
	DCD 5000	20, 50	Placed in isopropanol for 1 h and centrifuged	89.8–93.9	30
Diazepam	Quantisal	1,000	N/A	81.1	25
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad with filter	87.4	25
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	88.9	25
	Salivette	1,000	Centrifugation with tube insert	15.9	25
	Cozart	1,000	N/A	91.6	25
Alprazolam	Quantisal	1,000	N/A	111.0	25
	StatSure	1,000	Buffer–oral fluid mixture extracted from pad with filter	91.1	25
	Intercept	1,000	Centrifuged to recover buffer–oral fluid mixture	91.2	25
	Salivette	1,000	Centrifugation with tube insert	27.3	25
	Cozart	1,000	N/A	66.0	25
Oxazepam	Quantisal	10, 20, 50	Buffer–oral fluid mixture separated with serum separator tube	97.9–109.4	147
Methadone	Quantisal	25, 50, 100	Buffer–oral fluid mixture separated with serum separator tube	99.7–106.5	147
		454	Pad placed in buffer for 24 h at 4°C	98	26
	Certus	454	Pad placed in buffer for 24 h at 4°C	84	26
	DCD 5000	25, 50	Placed in isopropanol for 1 h and centrifuged	96.1–98.1	30
Buprenorphine	Quantisal	454	Pad placed in buffer for 24 h at 4°C	97	26
	Certus	454	Pad placed in buffer for 24 h at 4°C	84	26
	DCD 5000	10, 20	Placed in isopropanol for 1 h and centrifuged	94.7–103	30

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table IX. Screening and Confirmation Cutoffs for Various Agencies

Initial (Screening) Test Analyte	Initial (Screening) Test Cutoff (μg/L					Confirmatory Test Analyte	Confirmatory Test Cutoff Concentration (μg/L)				
	2004 SAMHSA Guidelines	2015 SAMHSA Guidelines	EWDTS Guidelines	Australian Standard	Canadian Drug Testing Committee Standards		2004 SAMHSA Guidelines	2015 SAMHSA Guidelines	EWDTS Guidelines	DRUID Recommendations	Talloires Recommendations
Cannabis (THC)	4	4	10	25	25	THC	2	2	2	1	2
Cocaine/ benzoylecgonine	20	15	30	50	50 (cocaine)	Cocaine	8	8	8	10	10
						Benzoylecgonine	8	8	8	10	10
Codeine/ morphine	40	30	40 (morphine)	50 (opiates)		Codeine	40	15	15	20	20
						Morphine	40	15	15	20	20
						Norcodeine			2		
						6-Acetylcodeine			2		
						Dihydrocodeine			15		
Hydrocodone/ hydromorphone		30				Hydrocodone	N/A	15			
						Hydromorphone	N/A	15			
Oxycodone/ oxymorphone		30				Oxycodone	N/A	15			
						Oxymorphone	N/A	15			
6-Acetylmorphine		3	4			6-Acetylmorphine	N/A	2	2	5	5
L-Methadone			50			Methadone or metabolites (d + l) EDDP			20	20	20
Buprenorphine			5			Buprenorphine or metabolites			1		
Propoxyphene or metabolites			40			Propoxyphene or metabolites			5		
						Tramadol				50	

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table IX. Continued

Initial (Screening) Test Analyte	Initial (Screening) Test Cutoff (µg/L					Confirmatory Test Analyte	Confirmatory Test Cutoff Concentration (µg/L)				
	2004 SAMHSA Guidelines	2015 SAMHSA Guidelines	EWDTS Guidelines	Australian Standard	Canadian Drug Testing Committee Standards		2004 SAMHSA Guidelines	2015 SAMHSA Guidelines	EWDTS Guidelines	DRUID Recommendations	Talloires Recommendations
Phencyclidine	10	3				Phencyclidine	10	2			
Amphetamine/ methamphetamine	50	25	40	50	50 (meth)	Amphetamine	50	15	15 (d + l)	25	20
						Methamphetamine	50	15	15	25	20
MDMA/MDA/ MDEA	50	25				MDMA	50	15	15	25	20
						MDA	50	15	15	25	20
						MDEA	50	15		25	20
Benzodiazepines			10			7-Aminoflunitrazepam			3	1	
						7-Aminoclonazepam			3	1	
						7-Aminonitrazepam			3		
						Alprazolam			3	1	
						Bromazepam			3		
						Clonazepam			3	1	
						Desmethyldiazepam/nordiazepam			3	1	
						Diazepam			3	5	
						Flunitrazepam			3	2	
						Flurazepam			3		
						Lorazepam			3	1	
						Lormetazepam			3		
						Midazolam			3		
						Nitrazepam			3		
						Oxazepam			3	5	
						Phenazepam			3		
						Temazepam			10		
						Zolpidem				10	
						Zopiclone				10	

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

Table X. Disparate Results from Testing Two Different Biological Matrices and Possible Explanations

Scenario	Matrix			Possible Explanations for Disparate Results
	Blood	Urine	Oral Fluid	
1	Positive		Negative	Highly protein-bound drugs may be poorly distributed to oral fluid, e.g., benzodiazepines; highly polar compounds and low lipophilicity reducing transfer from blood to oral fluid
2		Positive	Negative	Long interval after dosing; concentration effect by kidney; highly protein-bound drug; sampling time outside oral fluid detection 'window'
3	Negative		Positive	Insufficient time for drug absorption; 'depot' effect, ion trapping
4		Negative	Positive	Insufficient time for drug absorption, metabolism and excretion; 'depot' effect

the same dose but with a different collection method that had a stimulated OF collection.

Comparison with other biological matrices

When test results from different matrices are available, there may be legitimate reasons that drug test results differ. Each biological specimen has unique physiological and chemical properties that may alter drug disposition. Renal excretion favors water-soluble metabolite elimination, whereas OF excretion favors parent drugs capable of rapid passive diffusion across membranes. OF's acidic nature favors ion trapping of drugs containing basic nitrogen moieties (14, 15). Residence times in each matrix also differ substantially, yielding wide variability in detection windows. Disparate specimen results and possible explanations are included in Table X.

Amphetamines

Amphetamines are weak bases and ion trap in OF, leading to higher OF concentrations compared with blood (14, 15). Nevertheless, dry mouth is common following amphetamine use, leading to low sample volumes and/or longer collection times (167). Stability issues are not generally a concern for amphetamines (25, 26, 168).

Methamphetamine

Methamphetamine generally has higher OF concentrations as compared with blood. Oral contamination may occur following smoking, and concentrations of up to ~60 mg/L were documented in a population of Norwegian drivers (165). Both methamphetamine and its metabolite amphetamine can be detected in OF. Following oral administration of four 10-mg doses of methamphetamine HCl sustained-release tablets within 1 week, methamphetamine was detected as early as 0.08–2 h after dosing, and mean (range) C_{max} was 106 μg/L (24.7–312) in samples collected by expectoration after citric acid candy stimulation; after four 20-mg doses, mean C_{max} was 192 μg/L (75.3–322) (14). Amphetamine was also detected but at lower concentrations than methamphetamine: mean C_{max} was 8.6 (3.8–21.3) and 14.2 (2.8–20.2) μg/L after low and high doses, respectively. Maximal OF concentrations occurred 2–12 h for methamphetamine and amphetamine. At the 2004 SAMHSA 50 μg/L methamphetamine cutoff, 60% and 20% of OF samples were still positive at 11.5 and 24 h after the high dose, respectively. The revised 15 μg/L SAMHSA cutoff was not evaluated. Smoked methamphetamine would invariably be expected to contaminate the oral mucosa, leading to higher OF concentrations and possibly extending the window of detection; however, it is unclear how high these concentrations would be or how long methamphetamine would be positive, as no controlled administration studies have been published.

Methamphetamine and amphetamine detection windows following long-term drug use were determined with a positive result of >8 μg/L for methamphetamine and >7 μg/L for amphetamine: positive and negative OF samples were interspersed with negative samples, with the last positive methamphetamine sample occurring after 8 days for users residing on a secure unit (169).

A recent study evaluated D- and L-methamphetamine and amphetamine disposition following Vicks VapoInhaler® (L-methamphetamine) administration according to manufacturers' instructions (two inhalations in each nostril every 2 h over 10 h) (42). No D-methamphetamine or D-amphetamine was detected. L-Methamphetamine concentrations were low (median C_{max} was < 20 μg/L, and only one participant was positive for L-amphetamine. At a 50 μg/L methamphetamine cutoff, 3 of 16 participants had positive methamphetamine samples, although only in 5–6% of their samples. Therefore, chiral analysis may be necessary to rule out intake of D-methamphetamine.

3,4-Methylenedioxymethamphetamine

Similar to methamphetamine, MDMA is also more readily detected in OF than blood due to ion trapping. MDMA OF disposition (unstimulated expectoration) in eight participants following 1.0 and 1.6 mg/kg of oral doses yielded 1,643 (1,160–3,382) and 4,760 (2,881–11,985) μg/L median C_{max}, respectively (167). Its metabolite, MDA, was never present in OF without MDMA; including MDA above the SAMHSA cutoff did not identify additional positive samples. The metabolites 4-hydroxy-3-methoxymethamphetamine and 4-hydroxy-3-methoxyamphetamine were not detected in OF.

Generally, MDMA can be detected in OF for 12–48 h after a single dose. At the 2004 SAMHSA cutoff (50 μg/L of MDMA), 94.8% of positives occurred within the first 23 h. Initial MDMA-positive samples occur as early as 0.25 h, but more often at 0.5 to 0.75 h after dosing. At the 50 μg/L cutoff, median t_{last} occurred at 23.0 (13.0–29.0) and 29 (23.0–47.0) h after low and high doses, respectively. Although the 15 μg/L confirmation (EWDTS and updated SAMHSA) cutoff was not evaluated, the DRUID 20 μg/L was; t_{last} was 12.0 (3.0–29.0) and 23 (23.0–47.0) h after the low and high MDMA doses, respectively. Although OF and plasma concentrations were weakly but significantly correlated, it was not possible to predict one concentration from the other due to high variability (15).

Cannabis

OF cannabinoids and their metabolites' disposition following various controlled cannabis administration were investigated, although most of the scientific literature focuses on smoked cannabis (21, 34, 38, 170–179). THC in cannabis smoke or vapor rapidly contaminates

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

the oral mucosa, leading to high OF concentrations. Oral mucosa contamination also occurs following oral consumption of edible products, but to a much lower degree than after smoking or vaporization (171). There is rapid initial drug clearance from the oral cavity, as >10,000 µg/L of THC OF concentrations 15 min after cannabis smoking fell to ~1,000–2,000 µg/L by 1 h (171, 178, 179). Rinsing the mouth with water significantly reduced OF THC concentrations (180). THC concentration after consuming a cannabis-containing brownie reached up to 938 µg/L in frequent users and 380 µg/L in occasional users (171). It is unclear why there was a difference in the two groups. OF from daily cannabis smokers abstaining from cannabis smoking but who received multiple oral Marinol® (dronabinol, synthetic THC capsules) generally had decreasing OF THC concentrations despite doses of up to 120 mg/day but increasing THCCOOH concentrations (34, 119). This suggests that THC in OF is predominately present from oral mucosa contamination rather than transfer from the blood.

THCCOOH, the primary inactive THC metabolite, is present in low ng/L concentrations in OF, with increased detectability if OF samples are hydrolyzed prior to analysis (171). THCCOOH is not present in cannabis smoke, indicating that it can be utilized to differentiate active use from acute passive cannabis exposure (118). The source of THCCOOH in OF may be from the blood and/or from THC metabolism in the oral mucosa. THCCOOH concentrations are generally higher and more detectable in chronic frequent smokers than occasional smokers (151, 171, 172).

At the SAMHSA and EWDTS 2 µg/L THC OF confirmation cutoffs, detection times as long as 26 h in occasional smokers and >72 h in frequent smokers were documented after smoked, vaporized and oral cannabis (171). Therefore, low THC concentrations can be detected in OF for several days, similar to blood and urine. t_{last} was not significantly different between the three routes of administration. Although not detected in all individuals, minor cannabinoids such as CBG, CBN and THCV in OF were suggested as potential markers of use within 26 h following oral, smoked and vaporized administration in occasional and frequent cannabis users (171). In another study of chronic daily cannabis smokers during sustained monitored abstinence, THC was generally quantifiable for 48 h, although two individuals still had intermittent positive THC samples up to 28 days after initiation of abstinence, with THC concentrations of 1.1 and 2.3 µg/L on Day 28 (181). There is some concern that some individuals may have had access to cannabis from other participants in another study of ad lib cannabis smoking.

Positive THC OF samples may occur following passive exposure to cannabis. An early cannabis exposure study documented THC-positive OF samples after passive exposure; it was ultimately determined that collection devices themselves became contaminated by the smoking environment, as OF samples collected outside of the smoking environment were negative (114). More recent investigations indicated that THC-positive samples following passive exposure to cannabis smoke can occur but are generally <5 µg/L and typically dissipate within 3 h (114–118). Higher OF THC concentrations were reported in non-ventilated environments: concentrations of up to 308 µg/L were documented 15 min after exposure to cannabis smoking in a non-ventilated environment (117). In the non-ventilated environment, median t_{last} at a 2 µg/L cutoff was 2 h (1–12), whereas t_{last} in ventilated environments was 0.25 h (0–2).

Cannabinoids may be particularly susceptible to concentration differences between OF collection devices or between collection and analytical methods. Expectorated OF samples can have variable concentrations due to cannabinoids adsorption to proteins, especially if samples are centrifuged. Significantly higher concentrations were measured in expectorated OF from Dutch ‘coffee shop’ patrons compared with samples consecutively collected from the same individuals using a collection device (182). Furthermore, OF THC concentrations in consecutively collected duplicate expectorated samples were much more variable than duplicate samples collected with collection devices. Indeed, studies involving two different OF collection devices did not detect any significant differences between simultaneously collected OF samples (34, 183). Commercial OF devices offer better stability and more reproducible results than do expectorated samples, as buffers help stabilize drugs, improve drug recovery from the collection pad, reduce OF viscosity and reduce analyte adsorption to container surfaces (22).

Cannabinoid stability depends on collection method, buffer composition in commercial collection devices, the analytes, storage containers and storage temperature and duration. Cannabinoids are more stable in samples collected with OF devices than expectorated OF (22). Acceptable stability was documented with various devices (e.g., Quantisal, Intercept and Cozart) with some showing better stability at 4°C compared with −20°C (22, 25, 176, 184). On the other hand, reduced cannabinoid stability was demonstrated with other collection devices (e.g., Salivette), possibly due to adsorption to the collection pad in those devices (25).

Cocaine

Cocaine can easily be detected in OF, with cocaine and BE concentrations of up to 881 and 2,074 µg/L, respectively, measured in a group of chronic pain patients and ~70,000 µg/L in a population of Norwegian drivers (159, 165). Cocaine and BE OF disposition was also evaluated following various routes of administration (11, 13, 33, 148, 185, 186). Cocaine, a weak base, is ion trapped in OF yielding higher cocaine concentrations in OF compared with blood. In addition, cocaine can contaminate the oral mucosa following insufflation and inhalation. In a study of six participants receiving 25 mg IV, 32 mg intranasal and 42 mg smoked cocaine, OF C_{max} in citric acid-stimulated expectorated samples were 258–1,303, 75–1,255,380 and 94–12,582 µg/L, respectively (13). Interestingly, oral mucosal contamination following intranasal and smoked cocaine did not occur in all individuals, but when it did, contamination dissipated within 0.5–1 h. Another study reported concentrations of up to 504,880 µg/L after smoking 40 mg of cocaine base (citric acid-stimulated expectorated samples) (11). Cocaine and BE disposition was evaluated in a recent study following 25 mg of IV cocaine (33). Median cocaine C_{max} was 732 µg/L (83.3–1,892) with the StatSure collection device and 932 µg/L (394–1,574) with the Oral-Eze device. BE C_{max} was 360 µg/L (77.2–836) with the StatSure device and 248 µg/L (96.9–1,892) with the Oral-Eze device. Therefore, cocaine and BE concentrations can be quite variable depending on the route of administration, with the highest concentrations typically observed after smoked cocaine.

At SAMHSA’s proposed and EWDTS’s 8 µg/L confirmation cutoffs, t_{last} was reported as 6 to ≥12 h after intranasal cocaine and 0.5–6 h after smoking administration (13). Not surprisingly, detection times were extended following repeated cocaine dosing (186). Monitoring OF BE extended detection windows, but BE’s C_{max} was lower than cocaine’s (33, 185). In citric acid-stimulated expectorated samples collected from participants receiving 75 mg/70 kg or 150 mg/70 kg subcutaneous cocaine, C_{max} was 1,092 (406–3,006) and 2,600 (1,193–8,495) µg/L for cocaine and 134 (81.8–441) and 280 (133–757) µg/L for BE after the low and high doses, respectively

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

(185). Median detection times with an 8 μg/L cutoff were 8.0 (4.0–24.1) h for cocaine and 28.0 (4.1–71.6) h for BE after the low dose and 9.8 (7.9–28.5) h and 32 (24.0–72.0) h after the high dose. In the study evaluating 25 mg of IV cocaine, cocaine t_{last} was 2–4 and 2–12.5 h with the StatSure and Oral-Eze collection devices, respectively, at an 8 μg/L cutoff; BE t_{last} was 6.5–21 and 9.5–28 h (33).

Stimulation did not appear to affect cocaine concentrations: there were no significant differences between citric acid-stimulated expectoration, citric acid-treated Salivette and neutral Salivette OF cocaine concentrations (185). In addition, in two studies evaluating 25 mg of IV cocaine, similar C_{max} was observed in citric acid-stimulated expectorated samples and OF samples collected with OF devices (13, 33).

Stability of OF cocaine and BE collected with different devices was evaluated at room, refrigerated and frozen temperatures. Stability was acceptable for most devices, although an increase in BE concentration was documented after 1 week at room temperature with the StatSure device (25, 26, 168).

Opioids

As weak bases, opioids also have higher OF concentrations relative to blood. A growing number of reports documented opioid distribution in pain management patients, improving OF result interpretation. Opioids in OF are relatively stable, although 6-AM losses occurred in some OF samples collected with the Intercept device (25, 26, 168).

Codeine

In 19 volunteers administered 60 and 120 mg/70 kg codeine doses in capsules, citric acid-stimulated expectorated OF had mean codeine C_{max} of 639 (184–1,289) after the low dose and 1,599 (620–3,350) μg/L after the high dose (187). Mean norcodeine C_{max} was 17 (3.9–58) after the low dose and 47 (10–191) μg/L after the high dose. Morphine and normorphine were not detected. Codeine was initially detected between 0.8 and 1.0 h.

t_{last} was not evaluated at the 15 μg/L codeine cutoff (current SAMHSA and EWDTS cutoffs), but mean t_{last} was 21 and 22 h after low and high codeine doses at a 2.5 μg/L cutoff and 7 h for both doses at the 40 μg/L cutoff (187). At a 2.5 μg/L cutoff for norcodeine (EWDTS cutoff is 2 μg/L), t_{last} was 6 and 9 h after the low and high doses, respectively.

Citric acid-stimulated expectoration, Salivette with citric acid-treated cotton swab and Salivette with neutral cotton swab collection methods were compared (187). Codeine concentrations tended to be higher in samples collected after citric acid stimulated expectoration, but this difference was not significant.

Two recent studies evaluated codeine disposition following poppy seed ingestion and demonstrated that codeine and morphine can be detected in OF following poppy seed ingestion, but only for a short time (41, 188). After two 45 g raw poppy seeds doses each containing 3.1 mg of codeine, C_{max} was 8.6 (3.8–31.8) after the first dose and 9.5 (1.1–32.6) μg/L after the second dose in OF samples collected with the Oral-Eze collection device (41). Codeine remained above the 15 μg/L cutoff for a median of 2 (0.5–2) h and 1 (0.5–2.5) h after the first and second doses, respectively. In the second study, codeine C_{max} was 18 (9–28) and 49 (16–112) μg/L after consuming a poppy seed roll containing 16 g of cooked poppy seeds (0.6 mg of codeine) and 15 g of raw poppy seeds (0.6 mg of codeine), respectively (188). In that research, t_{last} at the 15 μg/L cutoff was 0–0.25 h and 0.5–1.5 h, respectively, after these two dosing scenarios.

Morphine

To our knowledge, no clinical studies documented morphine OF disposition following therapeutic morphine administration; however, the prevalence of licit and illicit drugs in chronic pain patients' OF documented a median (range) morphine concentration of 18.1 (1.0–130,570) μg/L (158).

Morphine may be detected in OF following poppy seed ingestion. After consumption of two 45 g raw poppy seed doses containing 15.7 mg of morphine, morphine C_{max} was 34 (11.9–99.9) after the first dose and 9.5 (1.1–32.6) μg/L after the second dose in OF collected with the Oral-Eze collection device (41). OF remained above the 15 μg/L cutoff for a median of 1 h (0.5–2.5) after the first and second doses. Morphine C_{max} of 35 (7–143) and 158 (47–284) μg/L was noted after consuming a poppy seed roll containing 16 g of cooked poppy seeds (3.2 mg of morphine) and 15 g of raw poppy seeds (3.3 mg of morphine), respectively (188). In that study, t_{last} at the 15 μg/L cutoff was 0.5–1.5 h for morphine after the roll and 0.5–3 h after each dosing scenario.

Heroin

One advantage with OF is that heroin and 6-AM are readily detected, providing better identification of heroin use than does blood or urine (11, 148, 189, 190). In a study evaluating three escalating smoked and IV heroin doses in two individuals, OF 6-AM concentrations in samples collected following citric acid-stimulated expectoration reached as high as 20,580 μg/L after smoking and 30 μg/L after IV administration, indicating that 6-AM likely contaminates the oral mucosa (11). In this study, at the SAMHSA and EWDTS 2 μg/L 6-AM cutoff, t_{last} was 0.5–2 h. Although there is no recommended cutoff for heroin, at a 1 μg/L cutoff, heroin was detectable 2–24 h after smoking and 5 min to 12 h after IV administration.

In 77,218 Intercept-collected OF samples primarily for workplace drug testing programs, 48 were morphine positive, 32 of which (66.7%) were positive for 6-AM at a mean (range) concentration of 416 (3–4,095) μg/L (189). The authors suggested that an OF 6-AM/morphine ratio >1 was highly suggestive of heroin use within 1 h of specimen collection.

Oxycodone

Oxycodone is a weakly basic opioid commonly prescribed for pain but also commonly abused. Following a single 20 mg controlled-released oxycodone administration (OxyContin®) to 12 individuals, OF concentrations in expectorated OF were oxycodone > noroxycodone > oxymorphone; noroxymorphone was not detected (191). Oxycodone was initially identified within 15–30 min, with a biphasic absorption pattern. Reported OF C_{max} was 133 μg/L (49.2–219) for oxycodone, 18.7 μg/L (10.3–31.8) for noroxycodone and 1.6 μg/L (1.2–2.4) for oxymorphone. Mean oxycodone detection time was 17.5 h (12–28), while oxymorphone was not detected at the 15 μg/L proposed SAMHSA cutoff. A 120–1,200 μg/L therapeutic range for oxycodone in OF was proposed to compare with a 10–1,000 μg/L blood therapeutic range (149).

Hydrocodone

In a pain management population, the median OF hydrocodone concentration was 122 μg/L (range 1.6–6,902) in samples collected with the Quantisal device (192). Prevalence of drugs and metabolites in OF was hydrocodone > norhydrocodone > hydromorphone. A clinical study documented similar prevalence following a 20 mg oral hydrocodone bitartrate dose (193). In OF collected by expectoration,

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

hydrocodone mean t_{first} was 0.27 h (0.25–0.5). OF concentrations were higher compared with blood, with a mean C_{max} of 208 μg/L (61.7–626) of hydrocodone, 12.8 μg/L (3.6–27.0) of norhydrocodone and 6.4 μg/L (2.6–18.2) of dihydrocodeine. Reported detection times at a 15 μg/L cutoff were 10.7 h (8–14) hydrocodone and 0.7 h (0–8) dihydrocodeine. In two studies evaluating OF in patients undergoing treatment for chronic pain, median OF concentrations in samples collected with the Quantisal device was 22.6 μg/L (1.4–494) and 67.8 μg/L (1.0–3,344) (158, 159).

Fentanyl

Despite frequent use of fentanyl for pain and increasing fentanyl abuse in the USA, there are few OF data to guide OF fentanyl interpretation. In patients wearing patches delivering up to 200 μg/h of fentanyl, passive drool OF concentrations were ∼50 μg/L (concentration estimated based on published figure) (19). In two chronic pain studies, median OF fentanyl concentrations (Quantisal device) were 1.2 μg/L (0.1–26.7) and 6.6 μg/L (102–5,341.3) (158, 159).

Tramadol

Although proposed SAMHSA and EWDTS guidelines do not include tramadol testing, it is being considered for inclusion by EWDTS. In 12 participants receiving 50 mg of encapsulated tramadol, median OF C_{max} (Quantisal device) was 1,181 (459–3,905) and 43 (2–158) μg/L for tramadol and *O*-desmethyltramadol, respectively (194). Lower *O*-desmethyltramadol concentrations were reported in intermediate and slow metabolizers. Tramadol OF concentrations were ∼10-fold higher than plasma concentrations, with tramadol OF t_{last} at least 48 h and *O*-desmethyltramadol for up to 32 h. Following two 100 mg tramadol doses daily over 2 days, median OF C_{max} was 7,830 μg/L in slow metabolizers (*CYP2D6*10/10** genotype) and 4,801 μg/L in the wild-type group (126).

Methadone

In 46 unstimulated expectorated OF samples collected ∼23 h after the last methadone dose from opioid-dependent patients enrolled in methadone-assisted treatment, median OF C_{max} was 105 μg/L (25–401) (133). However, participants were asked to rinse their mouth with water before providing samples, potentially diluting true OF concentrations. In another study, 16 opioid-dependent pregnant women receiving 30–110 mg/day of methadone provided unstimulated OF via the Salivette device (132). Methadone was detected in all OF samples, while the metabolites EDDP and methadol were identified in 88% and 12% of samples, respectively. No apparent dose–concentration relationship was observed. OF concentrations were 5.2–78,225, 1.0–1,791 and 5.0–281 μg/L for methadone, EDDP and methadol, respectively.

Methadone was stable in OF pooled samples collected with the Intercept and StatSure devices (168). In fortified OF, methadone was not detected in the 7-day sample but was again identified in the 14-day sample, prompting the authors to suggest that methadone was trapped in the collection pad on Day 7 and had released into the OF supernatant by Day 14 (26).

Buprenorphine

There are few studies that document the pharmacokinetics of buprenorphine in OF. In one study, OF was collected (Salivette®) from nine pregnant women receiving 2 to 24 mg/day of sublingual buprenorphine HCl (9). Buprenorphine and norbuprenorphine were detected while buprenorphine-glucuronide was infrequently identified and at concentrations of <0.6 μg/L; norbuprenorphine-glucuronide was not detected in OF. In that study, buprenorphine OF C_{max} was 672–12,300 μg/L, whereas the overall concentration range over the course of the study was 0.1–12,300 μg/L (median 15.4). High concentrations occurred due to sublingual buprenorphine contamination of the oral mucosa. A secondary peak was present after 8 h, possibly due to a buprenorphine depot in the oral mucosa, or to enterohepatic circulation. Buprenorphine fortified into OF was stable for at least 14 days with the Quantisal and Certus collection devices (26).

Benzodiazepines

Monitoring benzodiazepine exposure with OF is especially challenging due to the wide range of available benzodiazepines, variable potencies and their high protein-binding, leading to low OF concentrations. However, technological advances have improved sensitivity, with a growing body of literature documenting OF benzodiazepine disposition.

Following 10 mg of oral diazepam, OF concentrations (Intercept device) were generally <2 μg/L; diazepam metabolites were not detected (195). In six samples collected from individuals enrolled in a detoxification center, diazepam concentrations were 1–8 μg/L (196).

In eight males receiving 15 or 30 mg of oxazepam, median OF C_{max} was 11 (8–24) and 19 (15–45) μg/L, respectively (10). Oxazepam-glucuronide concentrations were lower than those of oxazepam. Oxazepam was detected for at least 8.5 h at a 0.5 μg/L cutoff, with lower concentrations in OF compared with blood. Mean OF/blood ratio was 0.05 (0.03–0.07) for oxazepam and 0.004 (0.002–0.006) for oxazepam-glucuronide.

In a study of chronic pain patients, OF alprazolam concentrations ranged from 0.7 to 46 μg/L (159). In another study with seven samples from detoxification patients, alprazolam OF concentrations were 2–25 μg/L; hydroxyalprazolam was not detected (196). In 11 volunteers administered a single oral dose of 0.5 mg of alprazolam, the median (range) C_{max} was 0.98 (0.12–23) μg/L, with t_{max} occurring at 2 (2–13) h; the median (range) detection time of alprazolam in OF was 26 (4–37) h (197).

In four volunteers administered 1 mg of flunitrazepam, expectorated OF concentrations were always below the 3 μg/L EWDTS cutoff (198). Flunitrazepam was below the 0.1 μg/L LOQ in one individual; stability may have been an issue, as no preservative was used in this participants' OF samples. Flunitrazepam C_{max} in the other three participants were 0.29, 0.57 and 0.58 μg/L. Flunitrazepam stability was improved by addition of 2% NaF; without the preservative, concentrations decreased 23% after 48 h at 4°C, although 7-aminoflunitrazepam concentrations did not increase significantly.

In OF (Intercept device) from opioid-dependent patients receiving opioid-assistance treatment, clonazepam, nitrazepam, flunitrazepam and their metabolites were detected (199). The 7-amino metabolites are more likely to be detected in OF than is the parent drug. In 1,001 clonazepam and/or 7-aminoclonazepam cases, 70.6% had both parent and metabolite in OF, 6.4% had only the parent drug and 23.0% had only the metabolite. In 211 nitrazepam cases, only the parent drug was identified in 7.6% of cases, 26.5% had only the metabolite and 65.9% had both. There were four flunitrazepam cases, one with parent and metabolite and three with only the metabolite.

Benzodiazepine stability is especially important to consider when interpreting OF results. For example, there were decreases in OF diazepam collected with the Intercept device and the Salivette (25, 168). In addition, substantial benzodiazepine degradation was

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

documented for diazepam, nordiazepam, oxazepam, temazepam, bromazepam, flurazepam, lorazepam, midazolam, chlordiazepoxide, clonazepam and flunitrazepam in fortified OF with the Draeger DCD 5000 when the collection device was stored dry in the transport tube; better stability was noted when the collection device was stored with methanol in the transport tube (200).

Novel psychoactive substances

In the current era of highly available and constantly changing NPSs, OF offers some advantages over urine monitoring. As parent drugs are more common in OF, metabolic studies are not required to identify which compounds should be targeted for urine analyses. Several NPSs were identified in OF including ethylone, methylone, α-PVP, dimethylone, butylone and 4-fluoroamphetamine, with mean concentrations of 582 (41–4,105), 2,445 (40–10,027), 474 (86–1,301), 611, 497 (175–905) and 329 (281–378) μg/L, respectively (81). Not surprisingly, synthetic cathinones were unstable in OF (201). Disposition data are lacking for NPSs, and NPS potencies vary considerably and are unknown when first appearing on the market, making NPS concentrations difficult to interpret in OF and blood.

Conclusions

The technology surrounding OF collection and data describing the distribution of drugs in OF dramatically increased over the last two decades. Furthermore, validated chromatographic methods to quantify multiple analytes in OF at low concentrations are available. Controlled drug administration studies and prevalence data improved interpretation of OF test results. OF offers many advantages over other matrices, playing an important role in diverse drug testing programs. Nevertheless, it is important to consider drug-specific characteristics, variability and limitations in any interpretation of OF drug findings. A significant correlation was frequently observed between matrices (i.e., between OF and plasma or blood concentrations); however, high intra-subject and inter-subject variability precludes prediction of blood concentrations from OF concentrations.

Acknowledgments

The assistance of M. Brenton and the Centre of Forensic Sciences Library staff in retrieving numerous references is gratefully recognized. The authors also recognized Dr A. Peaire for her input.

References

1. Pedersen, A.M., Bardow, A., Jensen, S.B., Nauntofte, B. (2002) Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. *Oral Diseases*, 8, 117–129.
2. Bosker, W.M., Huestis, M.A. (2009) Oral fluid testing for drugs of abuse. *Clinical Chemistry*, 55, 1910–1931.
3. Davenport, H.W. (1977) Salivary secretion. In Davenport, H.W. (ed), *Physiology Textbook Series*. Physiology of the Digestive Tract: An Introductory Text, 4th edition. Year Book Medical Publishers, Chicago, pp. 85–94.
4. Crouch, D.J. (2005) Oral fluid collection: the neglected variable in oral fluid testing. *Forensic Science International*, 150, 165–173.
5. Lee, D., Huestis, M.A. (2014) Current knowledge on cannabinoids in oral fluid. *Drug Testing and Analysis*, 6, 88–111.
6. Aps, J.K.M., Martens, L.C. (2005) Review: the physiology of saliva and transfer of drugs into saliva. *Forensic Science International*, 150, 119–131.
7. Humphrey, S.P., Williamson, R.T. (2001) A review of saliva: normal composition, flow, and function. *The Journal of Prosthetic Dentistry*, 85, 162–169.
8. Choo, R.E., Huestis, M.A. (2004) Oral fluid as a diagnostic tool. *Clinical Chemistry and Laboratory Medicine*, 42, 1273–1287.
9. Concheiro, M., Jones, H.E., Johnson, R.E., Choo, R., Huestis, M.A. (2011) Preliminary buprenorphine sublingual tablet pharmacokinetic data in plasma, oral fluid, and sweat during treatment of opioid-dependent pregnant women. *Therapeutic Drug Monitoring*, 33, 619–626.
10. Smink, B.E., Hofman, B.J.A., Dijkhuizen, A., Lusthof, K.J., de Gier, J.J., Egberts, A.C.G. *et al.* (2008) The concentration of oxazepam and oxazepam glucuronide in oral fluid, blood and serum after controlled administration of 15 and 30 mg oxazepam. *British Journal of Clinical Pharmacology*, 66, 556–560.
11. Jenkins, A.J., Oyler, J.M., Cone, E.J. (1995) Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. *Journal of Analytical Toxicology*, 19, 359–374.
12. Newmeyer, M.N., Swortwood, M.J., Barnes, A.J., Abulseoud, O.A., Scheidweiler, K.B., Huestis, M.A. (2016) Free and glucuronide whole blood cannabinoids' pharmacokinetics after controlled smoked, vaporized, and oral cannabis administration in frequent and occasional cannabis users: identification of recent cannabis intake. *Clinical Chemistry*, 62, 1579–1592.
13. Cone, E.J., Oyler, J., Darwin, W.D. (1997) Cocaine disposition in saliva following intravenous, intranasal, and smoked administration. *Journal of Analytical Toxicology*, 21, 465–475.
14. Schepers, R.J.F., Oyler, J.M., Joseph, R.E.J., Cone, E.J., Moolchan, E.T., Huestis, M.A. (2003) Methamphetamine and amphetamine pharmacokinetics in oral fluid and plasma after controlled oral methamphetamine administration to human volunteers. *Clinical Chemistry*, 49, 121–132.
15. Desrosiers, N.A., Barnes, A.J., Hartman, R.L., Scheidweiler, K.B., Kolbrich-spargo, E.A., Gorelick, D.A. *et al.* (2013) Oral fluid and plasma 3,4-methylenedioxymethamphetamine (MDMA) and metabolite correlation after controlled oral MDMA administration. *Analytical and Bioanalytical Chemistry*, 405, 4067–4076.
16. Chiou, W.L., Pu, F.S. (1979) Creatinine VIII: saliva levels of endogenous 'true' creatinine in normal subjects. *Clinical Pharmacology and Therapeutics*, 25, 777–782.
17. Chiou, W.L., Hsu, F.H., Westenfelder, C., Kurtzman, N.A. (1977) Correlation of creatinine levels in saliva and plasma in normal subjects and renal patients. *Research Communications in Chemical Pathology and Pharmacology*, 16, 549–556.
18. Cummings, O.T., Morris, A.A., Enders, J.R., McIntire, G.L. (2016) Normalizing oral fluid hydrocodone data using calculated blood volume. *Journal of Analytical Toxicology*, 40, 486–491.
19. Heiskanen, T., Langel, K., Gunnar, T., Lillsunde, P., Kalso, E.A. (2015) Opioid concentrations in oral fluid and plasma in cancer patients with pain. *Journal of Pain and Symptom Management*, 50, 524–532.
20. Molnar, A., Lewis, J., Fu, S. (2013) Recovery of spiked Delta9-tetrahydrocannabinol in oral fluid from polypropylene containers. *Forensic Science International*, 227, 69–73.
21. Milman, G., Barnes, A.J., Schwope, D.M., Schwilke, E.W., Goodwin, R.S., Kelly, D.L. *et al.* (2011) Cannabinoids and metabolites in expectorated oral fluid after 8 days of controlled around-the-clock oral THC administration. *Analytical and Bioanalytical Chemistry*, 401, 599–607.
22. Lee, D., Milman, G., Schwope, D.M., Barnes, A.J., Gorelick, D.A., Huestis, M.A. (2012) Cannabinoid stability in authentic oral fluid after controlled cannabis smoking. *Clinical Chemistry*, 58, 1101–1109.
23. Ventura, M., Pichini, S., Ventura, R., Zuccaro, P., Pacifici, R., de la Torre, R. (2007) Stability studies of principal illicit drugs in oral fluid: preparation of reference materials for external quality assessment schemes. *Therapeutic Drug Monitoring*, 29, 662–665.
24. O'Neal, C.L., Crouch, D.J., Rollins, D.E., Fatah, A.A. (2000) The effects of collection methods on oral fluid codeine concentrations. *Journal of Analytical Toxicology*, 24, 536–542.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

25. Langel, K., Engblom, C., Pehrsson, A., Gunnar, T., Ariniemi, K., Lillsunde, P. (2008) Drug testing in oral fluid-evaluation of sample collection devices. *Journal of Analytical Toxicology*, 32, 393–401.
26. Cohier, C., Megarbane, B., Roussel, O. (2017) Illicit drugs in oral fluid: evaluation of two collection devices. *Journal of Analytical Toxicology*, 41, 71–76.
27. Dickson, S., Park, A., Nolan, S., Kenworthy, S., Nicholson, C., Midgley, J. *et al.* (2007) The recovery of illicit drugs from oral fluid sampling devices. *Forensic Science International*, 165, 78–84.
28. Engblom, C., Gunnar, T., Rantanen, A., Lillsunde, P. (2007) Driving under the influence of drugs—amphetamine concentrations in oral fluid and whole blood samples. *Journal of Analytical Toxicology*, 31, 276–280.
29. Kauert, G.F., Iwersen-Bergmann, S., Toennes, S.W. (2006) Assay of Delta9-tetrahydrocannabinol (THC) in oral fluid-evaluation of the OraSure oral specimen collection device. *Journal of Analytical Toxicology*, 30, 274–277.
30. Hall, A.-J., Warner, J.V., Henman, M.G., Ferguson, W.E. (2015) Recovery of drugs of abuse from Dräger DCD5000 oral fluid collection device in Australia. *Journal of Analytical Toxicology*, 39, 140–143.
31. Immunalysis Quantisal Frequently Asked Questions—Laboratory Oral FLuid TEsting. https://ensur.invmed.com/ensur/broker/ensurbroker.aspx?code=MKT50117&cs=25664584 (7 May 2019).
32. Desrosiers, N.A., Milman, G., Mendu, D.R., Lee, D., Barnes, A.J., Gorelick, D.A. *et al.* (2014) Cannabinoids in oral fluid by on-site immunoassay and by GC-MS using two different oral fluid collection devices. *Analytical and Bioanalytical Chemistry*, 406, 4117–4128.
33. Ellefsen, K.N., Concheiro, M., Pirard, S., Gorelick, D.A., Huestis, M.A. (2016) Oral fluid cocaine and benzoylecgonine concentrations following controlled intravenous cocaine administration. *Forensic Science International*, 260, 95–101.
34. Milman, G., Barnes, A.J., Schwope, D.M., Schwilke, E.W., Darwin, W.D., Goodwin, R.S. *et al.* (2010) Disposition of cannabinoids in oral fluid after controlled around-the-clock oral THC administration. *Clinical Chemistry*, 56, 1261–1269.
35. European Workplace Drug Testing Society (2015) *European Guidelines for Workplace in Oral Fluid*. http://www.ewdts.org/data/uploads/documents/ewdts-oral-fluid-2015-11-01-v2.0.pdf. (Accessed December 2018)
36. Substance Abuse and Mental Health Services Administration, D. of H. and H.S. (2015) Mandatory Guidelines for Federal Workplace Drug Testing Programs. https://www.gpo.gov/fdsys/pkg/FR-2015-05-15/pdf/2015-11523.pdf. (Accessed December 2018)
37. NHTSA—National Highway Traffic Safety Administration and Department of Transportation (2015) *Result of the 2013–2014 National Roadside Survey of Alcohol and Drug Use by Drivers*. https://www.nhtsa.gov/sites/nhtsa.dot.gov/files/documents/13013-nrs_drug-053117-v3-tag_0.pdf (Accessed March 11, 2019).
38. Newmeyer, M.N., Swortwood, M.J., Andersson, M., Abulseoud, O.A., Scheidweiler, K.B., Huestis, M.A. (2017) Cannabis edibles: blood and oral fluid cannabinoid pharmacokinetics and evaluation of oral fluid screening devices for predicting Delta9-tetrahydrocannabinol in blood and oral fluid following cannabis brownie administration. *Clinical Chemistry*, 63, 647–662.
39. Ellefsen, K.N., Concheiro, M., Pirard, S., Gorelick, D.A., Huestis, M.A. (2016) Cocaine and benzoylecgonine oral fluid on-site screening and confirmation. *Drug Testing and Analysis*, 8, 296–303.
40. Desrosiers, N.A., Lee, D., Schwope, D.M., Milman, G., Barnes, A.J., Gorelick, D.A. *et al.* (2012) On-site test for cannabinoids in oral fluid. *Clinical Chemistry*, 58, 1418–1425.
41. Concheiro, M., Newmeyer, M.N., da Costa, J.L., Flegel, R., Gorelick, D.A., Huestis, M.A. (2015) Morphine and codeine in oral fluid after controlled poppy seed administration. *Drug Testing and Analysis*, 7, 586–591.
42. Newmeyer, M.N., Concheiro, M., da Costa, J.L., Flegel, R., Gorelick, D.A., Huestis, M.A. (2015) Oral fluid with three modes of collection and plasma methamphetamine and amphetamine enantiomer concentrations after controlled intranasal L-methamphetamine administration. *Drug Testing and Analysis*, 7, 877–883.
43. Rohrig, T.P., Moore, C.M., Stephens, K., Cooper, K., Coulter, C., Baird, T. *et al.* (2018) Roadside drug testing: an evaluation of the Alere DDS((R)) 2 mobile test system. *Drug Testing and Analysis*, 10, 663–670.
44. Logan, B.K., Mohr, A.L.A., Talpins, S.K. (2014) Detection and prevalence of drug use in arrested drivers using the Dräger Drug Test 5000 and Affiniton DrugWipe oral fluid drug screening devices. *Journal of Analytical Toxicology*, 38, 444–450.
45. Musshoff, F., Hokamp, E.G., Bott, U., Madea, B. (2014) Performance evaluation of on-site oral fluid drug screening devices in normal police procedure in Germany. *Forensic Science International*, 238, 120–124.
46. Strano-Rossi, S., Castrignano, E., Anzillotti, L., Serpelloni, G., Mollica, R., Tagliaro, F. *et al.* (2012) Evaluation of four oral fluid devices (DDS®, Drugtest 5000®, Drugwipe 5+® and RapidSTAT®) for on-site monitoring drugged driving in comparison with UHPLC-MS/MS analysis. *Forensic Science International*, 221, 70–76.
47. Beirness, D.J., Smith, D.R. (2017) An assessment of oral fluid drug screening devices. *Canadian Society of Forensic Science Journal*, 50, 55–63.
48. Niedbala, R.S., Kardos, K., Waga, J., Fritch, D., Yeager, L., Doddamane, S. *et al.* (2001) Laboratory analysis of remotely collected oral fluid specimens for opiates by immunoassay. *Journal of Analytical Toxicology*, 25, 310–315.
49. Kim, I., Barnes, A.J., Schepers, R., Moolchan, E.T., Wilson, L., Cooper, G. *et al.* (2003) Sensitivity and specificity of the Cozart microplate EIA cocaine oral fluid at proposed screening and confirmation cutoffs. *Clinical Chemistry*, 49, 1498–1503.
50. Cooper, G., Wilson, L., Reid, C., Baldwin, D., Hand, C., Spieher, V. (2004) Validation of the Cozart microplate EIA for cocaine and metabolites in oral fluid. *Journal of Analytical Toxicology*, 28, 498–503.
51. Cooper, G., Wilson, L., Reid, C., Baldwin, D., Hand, C., Spiehler, V. (2005) Comparison of GC-MS and EIA results for the analysis of methadone in oral fluid. *Journal of Forensic Sciences*, 50, 928–932.
52. Laloup, M., Tilman, G., Maes, V., De Boeck, G., Wallemacq, P., Ramaekers, J. *et al.* (2005) Validation of an ELISA-based screening assay for the detection of amphetamine, MDMA and MDA in blood and oral fluid. *Forensic Science International*, 153, 29–37.
53. Crooks, C.R., Brown, S. (2010) Roche DAT immunoassay: sensitivity and specificity testing for amphetamines, cocaine, and opiates in oral fluid. *Journal of Analytical Toxicology*, 34, 103–109.
54. Schwope, D.M., Milman, G., Huestis, M.A. (2010) Validation of an enzyme immunoassay for detection and semiquantification of cannabinoids in oral fluid. *Clinical Chemistry*, 56, 1007–1014.
55. Barnes, A.J., Kim, I., Schepers, R., Moolchan, E.T., Wilson, L., Cooper, G. *et al.* (2003) Sensitivity, specificity, and efficiency in detecting opiates in oral fluid with the Cozart Opiate Microplate EIA and GC-MS following controlled codeine administration. *Journal of Analytical Toxicology*, 27, 402–407.
56. Reichardt, E.M., Baldwin, D., Osselton, M.D. (2013) Effects of oral fluid contamination on two oral fluid testing systems. *Journal of Analytical Toxicology*, 37, 246–249.
57. Souza, D.Z., Boehl, P.O., Comiran, E., Prusch, D.S., Zancanaro, I., Fuentefria, A.M. *et al.* (2012) Which amphetamine-type stimulants can be detected by oral fluid immunoassays? *Therapeutic Drug Monitoring*, 34, 98–109.
58. Nieddu, M., Burrai, L., Baralla, E., Pasciu, V., Varoni, M.V., Briguglio, I. *et al.* (2016) ELISA detection of 30 new amphetamine designer drugs in whole blood, urine and oral fluid using Neogen(R) 'Amphetamine' and 'Methamphetamine/MDMA' Kits. *Journal of Analytical Toxicology*, 40, 492–497.
59. Mortier, K.A., Maudens, K.E., Lambert, W.E., Clauwaert, K.M., Van Bocxlaer, J.F., Deforce, D.L. *et al.* (2002) Simultaneous, quantitative determination of opiates, amphetamines, cocaine and benzoylecgonine in oral fluid by liquid chromatography quadrupole-time-of-flight mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 779, 321–330.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

60. Dams, R., Murphy, C.M., Choo, R.E., Lambert, W.E., De Leenheer, A.P., Huestis, M.A. (2003) LC-atmospheric pressure chemical ionization-MS/MS analysis of multiple illicit drugs, methadone, and their metabolites in oral fluid following protein precipitation. *Analytical Chemistry*, 75, 798–804.
61. Gunnar, T., Ariniemi, K., Lillsunde, P. (2005) Validated toxicological determination of 30 drugs of abuse as optimized derivatives in oral fluid by long column fast gas chromatography/electron impact mass spectrometry. *Journal of Mass Spectrometry: JMS*, 40, 739–753.
62. Wylie, F.M., Torrance, H., Anderson, R.A., Oliver, J.S. (2005) Drugs in oral fluid Part I. Validation of an analytical procedure for licit and illicit drugs in oral fluid. *Forensic Science International*, 150, 191–198.
63. Concheiro, M., de Castro, A., Quintela, O., Cruz, A., Lopez-Rivadulla, M. (2007) Confirmation by LC-MS of drugs in oral fluid obtained from roadside testing. *Forensic Science International*, 170, 156–162.
64. Pujadas, M., Pichini, S., Civit, E., Santamarina, E., Perez, K., de la Torre, R. (2007) A simple and reliable procedure for the determination of psychoactive drugs in oral fluid by gas chromatography–mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 44, 594–601.
65. Concheiro, M., de Castro, A., Quintela, O., Cruz, A., Lopez-Rivadulla, M. (2008) Determination of illicit and medicinal drugs and their metabolites in oral fluid and preserved oral fluid by liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 391, 2329–2338.
66. Kala, S.V., Harris, S.E., Freijo, T.D., Gerlich, S. (2008) Validation of analysis of amphetamines, opiates, phencyclidine, cocaine, and benzoylecgonine in oral fluids by liquid chromatography–tandem mass spectrometry. *Journal of Analytical Toxicology*, 32, 605–611.
67. Wood, M., Laloup, M., Ramirez Fernandez, M. del M., Jenkins, K.M., Young, M.S., Ramaekers, J.G., *et al.* (2005) Quantitative analysis of multiple illicit drugs in preserved oral fluid by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Forensic Science International*, 150, 227–238.
68. Badawi, N., Simonsen, K.W., Steentoft, A., Bernhoft, I.M., Linnet, K. (2009) Simultaneous screening and quantification of 29 drugs of abuse in oral fluid by solid-phase extraction and ultraperformance LC-MS/MS. *Clinical Chemistry*, 55, 2004–2018.
69. Fritch, D., Blum, K., Nonnemacher, S., Haggerty, B.J., Sullivan, M.P., Cone, E.J. (2009) Identification and quantitation of amphetamines, cocaine, opiates, and phencyclidine in oral fluid by liquid chromatography–tandem mass spectrometry. *Journal of Analytical Toxicology*, 33, 569–577.
70. Concheiro, M., Gray, T.R., Shakleya, D.M., Huestis, M.A. (2010) High-throughput simultaneous analysis of buprenorphine, methadone, cocaine, opiates, nicotine, and metabolites in oral fluid by liquid chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 398, 915–924.
71. Wang, I.-T., Feng, Y.-T., Chen, C.-Y. (2010) Determination of 17 illicit drugs in oral fluid using isotope dilution ultra-high performance liquid chromatography/tandem mass spectrometry with three atmospheric pressure ionizations. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 878, 3095–3105.
72. Langel, K., Gunnar, T., Ariniemi, K., Rajamaki, O., Lillsunde, P. (2011) A validated method for the detection and quantitation of 50 drugs of abuse and medicinal drugs in oral fluid by gas chromatography–mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 879, 859–870.
73. Strano-Rossi, S., Anzillotti, L., Castrignano, E., Romolo, F.S., Chiarotti, M. (2012) Ultra high performance liquid chromatography-electrospray ionization-tandem mass spectrometry screening method for direct analysis of designer drugs, 'spice' and stimulants in oral fluid. *Journal of Chromatography. A*, 1258, 37–42.
74. Zancanaro, I., Limberger, R.P., Bohel, P.O., dos Santos, M.K., De Boni, R.B., Pechansky, F. *et al.* (2012) Prescription and illicit psychoactive drugs in oral fluid–LC-MS/MS method development and analysis of samples from Brazilian drivers. *Forensic Science International*, 223, 208–216.
75. Liu, H.-C., Lee, H.-T., Hsu, Y.-C., Huang, M.-H., Liu, R.H., Chen, T.-J. *et al.* (2015) Direct injection LC-MS-MS analysis of opiates, methamphetamine, buprenorphine, methadone and their metabolites in oral fluid from substitution therapy patients. *Journal of Analytical Toxicology*, 39, 472–480.
76. Sergi, M., Montesano, C., Odoardi, S., Mainero Rocca, L., Fabrizi, G., Compagnone, D. *et al.* (2013) Micro extraction by packed sorbent coupled to liquid chromatography tandem mass spectrometry for the rapid and sensitive determination of cannabinoids in oral fluids. *Journal of Chromatography. A*, 1301, 139–146.
77. Valen, A., Leere Oiestad, A.M., Strand, D.H., Skari, R., Berg, T. (2016) Determination of 21 drugs in oral fluid using fully automated supported liquid extraction and UHPLC-MS/MS. *Drug Testing and Analysis*, 9, 808–823.
78. Di Fazio, V., Wille, S.M.R., Toennes, S.W., van Wel, J.H.P., Ramaekers, J.G. and Samyn, N. (2018) Driving under the influence of cocaine: Quantitative determination of basic drugs in oral fluid obtained during roadside controls and a controlled study with cocaine users. *Drug Testing and Analysis*, March 2018: 10.1002/dta.2379.
79. Flood, J.G., Khaliq, T., Bishop, K.A., Griggs, D.A. (2016) The new substance abuse and mental health services administration oral fluid cutoffs for cocaine and heroin-related analytes applied to an addiction medicine setting: important, unanticipated findings with LC-MS/MS. *Clinical Chemistry*, 62, 773–780.
80. Zheng, Y., Sparve, E., Bergstrom, M. (2018) A simple validated multi-analyte method for detecting drugs in oral fluid by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). *Drug Testing and Analysis*, 10, 1001–1008.
81. Mohr, A.L.A., Friscia, M. and Logan, B.K. (2016) Identification and Prevalence Determination of Novel Recreational Drugs and Discovery of their Metabolites in Blood, Urine and Oral Fluid. https://www.ncjrs.gov/pdffiles1/nij/grants/250338.pdf. (Acessed March 11, 2019).
82. Di Rago, M., Chu, M., Rodda, L.N., Jenkins, E., Kotsos, A., Gerostamoulos, D. (2016) Ultra-rapid targeted analysis of 40 drugs of abuse in oral fluid by LC-MS/MS using carbon-13 isotopes of methamphetamine and MDMA to reduce detector saturation. *Analytical and Bioanalytical Chemistry*, 408, 3737–3749.
83. Huestis, M.A. (2009) A new ultraperformance-tandem mass spectrometry oral fluid assay for 29 illicit drugs and medications. *Clinical Chemistry*, 55, 2079–2081.
84. Maurer, H.H. (2005) Advances in analytical toxicology: the current role of liquid chromatography–mass spectrometry in drug quantification in blood and oral fluid. *Analytical and Bioanalytical Chemistry*, 381, 110–118.
85. Desrosiers, N.A., Scheidweiler, K.B., Huestis, M.A. (2015) Quantification of six cannabinoids and metabolites in oral fluid by liquid chromatography–tandem mass spectrometry. *Drug Testing and Analysis*, 7, 684–694.
86. Wood, M., De Boeck, G., Samyn, N., Morris, M., Cooper, D.P., Maes, R.A.A. *et al.* (2003) Development of a rapid and sensitive method for the quantitation of amphetamines in human plasma and oral fluid by LC-MS-MS. *Journal of Analytical Toxicology*, 27, 78–87.
87. Kankaanpaa, A., Gunnar, T., Ariniemi, K., Lillsunde, P., Mykkanen, S., Seppala, T. (2004) Single-step procedure for gas chromatography–mass spectrometry screening and quantitative determination of amphetamine-type stimulants and related drugs in blood, serum, oral fluid and urine samples. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 810, 57–68.
88. Scheidweiler, K.B., Huestis, M.A. (2006) A validated gas chromatographic-electron impact ionization mass spectrometric method for methylenedioxymethamphetamine (MDMA), methamphetamine and metabolites in oral fluid. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 835, 90–99.
89. Moore, C., Coulter, C., Crompton, K. (2007) Achieving proposed federal concentrations using reduced specimen volume for the extraction of amphetamines from oral fluid. *Journal of Analytical Toxicology*, 31, 442–446.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

90. Peters, F.T., Samyn, N., Kraemer, T., Riedel, W.J., Maurer, H.H. (2007) Negative-ion chemical ionization gas chromatography–mass spectrometry assay for enantioselective measurement of amphetamines in oral fluid: application to a controlled study with MDMA and driving under the influence cases. *Clinical Chemistry*, 53, 702–710.
91. Souza, D.Z., Boehl, P.O., Comiran, E., Mariotti, K.C., Pechansky, F., Duarte, P.C.A.V. *et al.* (2011) Determination of amphetamine-type stimulants in oral fluid by solid-phase microextraction and gas chromatography–mass spectrometry. *Analytica Chimica Acta*, 696, 67–76.
92. Newmeyer, M.N., Concheiro, M., Huestis, M.A. (2014) Rapid quantitative chiral amphetamines liquid chromatography–tandem mass spectrometry: method in plasma and oral fluid with a cost-effective chiral derivatizing reagent. *Journal of Chromatography. A*, 1358, 68–74.
93. Borg, D., Kolb, E., Lantigua, C., Stripp, R. (2018) Chiral analysis of methamphetamine in oral fluid samples: a method to distinguish licit from illicit drug use. *Journal of Analytical Toxicology*, 42, 25–32.
94. Concheiro, M., de Castro, A., Quintela, O., Cruz, A., Lopez-Rivadulla, M. (2004) Development and validation of a method for the quantitation of Delta9tetrahydrocannabinol in oral fluid by liquid chromatography electrospray-mass-spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 810, 319–324.
95. Teixeira, H., Proenca, P., Verstraete, A., Corte-Real, F., Vieira, D.N. (2005) Analysis of Delta9-tetrahydrocannabinol in oral fluid samples using solid-phase extraction and high-performance liquid chromatography-electrospray ionization mass spectrometry. *Forensic Science International*, 150, 205–211.
96. Laloup, M., Ramirez Fernandez, M. del M., Wood, M., De Boeck, G., Henquet, C., Maes, V., *et al.* (2005) Quantitative analysis of Delta9-tetrahydrocannabinol in preserved oral fluid by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography. A*, 1082, 15–24.
97. Day, D., Kuntz, D.J., Feldman, M., Presley, L. (2006) Detection of THCA in oral fluid by GC-MS-MS. *Journal of Analytical Toxicology*, 30, 645–650.
98. Moore, C., Coulter, C., Rana, S., Vincent, M., Soares, J. (2006) Analytical procedure for the determination of the marijuana metabolite 11-nor-Delta9-tetrahydrocannabinol-9-carboxylic acid in oral fluid specimens. *Journal of Analytical Toxicology*, 30, 409–412.
99. Moore, C., Rana, S., Coulter, C. (2007) Simultaneous identification of 2-carboxy-tetrahydrocannabinol, tetrahydrocannabinol, cannabinol and cannabidiol in oral fluid. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 852, 459–464.
100. Quintela, O., Andrenyak, D.M., Hoggan, A.M., Crouch, D.J. (2007) A validated method for the detection of Delta 9-tetrahydrocannabinol and 11-nor-9-carboxy-Delta 9-tetrahydrocannabinol in oral fluid samples by liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry. *Journal of Analytical Toxicology*, 31, 157–164.
101. Teixeira, H., Verstraete, A., Proenca, P., Corte-Real, F., Monsanto, P., Vieira, D.N. (2007) Validated method for the simultaneous determination of Delta9-THC and Delta9-THC-COOH in oral fluid, urine and whole blood using solid-phase extraction and liquid chromatography–mass spectrometry with electrospray ionization. *Forensic Science International*, 170, 148–155.
102. Milman, G., Barnes, A.J., Lowe, R.H., Huestis, M.A. (2010) Simultaneous quantification of cannabinoids and metabolites in oral fluid by two-dimensional gas chromatography mass spectrometry. *Journal of Chromatography. A*, 1217, 1513–1521.
103. Bylda, C., Leinenbach, A., Thiele, R., Kobold, U., Volmer, D.A. (2012) Development of an electrospray LC-MS/MS method for quantification of Delta(9)-tetrahydrocannabinol and its main metabolite in oral fluid. *Drug Testing and Analysis*, 4, 668–674.
104. Coulter, C., Garnier, M., Moore, C. (2012) Analysis of tetrahydrocannabinol and its metabolite, 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid, in oral fluid using liquid chromatography with tandem mass spectrometry. *Journal of Analytical Toxicology*, 36, 413–417.
105. He, X., Kozak, M., Nimkar, S. (2012) Ultra-sensitive measurements of 11-nor-delta(9)-tetrahydrocannabinol-9-carboxylic acid in oral fluid by microflow liquid chromatography–tandem mass spectrometry using a benchtop quadrupole/Orbitrap mass spectrometer. *Analytical Chemistry*, 84, 7643–7647.
106. Molnar, A., Lewis, J., Doble, P., Hansen, G., Prolov, T., Fu, S. (2012) A rapid and sensitive method for the identification of delta-9-tetrahydrocannabinol in oral fluid by liquid chromatography–tandem mass spectrometry. *Forensic Science International*, 215, 92–96.
107. Lee, P.D., Chang, Y.-J., Lin, K.-L., Chang, Y.-Z. (2012) Simultaneous determination of Delta9-tetrahydrocannabinol and 11-nor-9-carboxy-Delta9-tetrahydrocannabinol in oral fluid using isotope dilution liquid chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 402, 851–859.
108. Fabritius, M., Giroud, C. (2013) Letter to the editor regarding 'Simultaneous determination of 9-tetrahydrocannabinol and 11-nor-9-carboxy-9-tetrahydrocannabinol in oral fluid using isotope dilution liquid chromatography tandem mass spectrometry'. *Analytical and Bioanalytical Chemistry*, 405, 435–437.
109. Concheiro, M., Lee, D., Lendoiro, E., Huestis, M.A. (2013) Simultaneous quantification of Delta(9)-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography–high resolution mass spectrometry. *Journal of Chromatography. A*, 1297, 123–130.
110. Scheidweiler, K.B., Himes, S.K., Chen, X., Liu, H.-F., Huestis, M.A. (2013) 11-Nor-9-carboxy-9-tetrahydrocannabinol quantification in human oral fluid by liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 405, 6019–6027.
111. Wille, S.M.R., Di Fazio, V., Ramirez-Fernandez, M.d.M., Kummer, N., Samyn, N. (2013) Driving under the influence of cannabis: pitfalls, validation, and quality control of a UPLC-MS/MS method for the quantification of tetrahydrocannabinol in oral fluid collected with StatSure, Quantisal, or Certus collector. *Therapeutic Drug Monitoring*, 35, 101–111.
112. Fabritius, M., Staub, C., Mangin, P., Giroud, C. (2013) Analysis of cannabinoids in oral fluid by liquid chromatography–tandem mass spectrometry. *Forensic Toxicology*, 31, 151–163.
113. Barnes, A.J., Scheidweiler, K.B., Huestis, M.A. (2014) Quantification of 11-nor-9-carboxy-delta9-tetrahydrocannabinol in human oral fluid by gas chromatography–tandem mass spectrometry. *Therapeutic Drug Monitoring*, 36, 225–233.
114. Niedbala, R.S., Kardos, K.W., Fritch, D.F., Kunsman, K.P., Blum, K.A., Newland, G.A. *et al.* (2005) Passive cannabis smoke exposure and oral fluid testing. II. Two studies of extreme cannabis smoke exposure in a motor vehicle. *Journal of Analytical Toxicology*, 29, 607–615.
115. Niedbala, S., Kardos, K., Salamone, S., Fritch, D., Bronsgeest, M., Cone, E.J. (2004) Passive cannabis smoke exposure and oral fluid testing. *Journal of Analytical Toxicology*, 28, 546–552.
116. Gross, S.J., Worthy, T.E., Nerder, L., Zimmermann, E.G., Soares, J.R., Lomax, P. (1985) Detection of recent cannabis use by saliva delta 9-THC radioimmunoassay. *Journal of Analytical Toxicology*, 9, 1–5.
117. Cone, E.J., Bigelow, G.E., Herrmann, E.S., Mitchell, J.M., LoDico, C., Flegel, R. *et al.* (2015) Nonsmoker exposure to secondhand cannabis smoke. III. Oral fluid and blood drug concentrations and corresponding subjective effects. *Journal of Analytical Toxicology*, 39, 497–509.
118. Moore, C., Coulter, C., Uges, D., Tuyay, J., van der Linde, S., van Leeuwen, A. *et al.* (2011) Cannabinoids in oral fluid following passive exposure to marijuana smoke. *Forensic Science International*, 212, 227–230.
119. Lee, D., Vandrey, R., Mendu, D.R., Anizan, S., Milman, G., Murray, J.A. *et al.* (2013) Oral fluid cannabinoids in chronic cannabis smokers during oral delta9-tetrahydrocannabinol therapy and smoked cannabis challenge. *Clinical Chemistry*, 59, 1770–1779.
120. Clauwaert, K., Decaestecker, T., Mortier, K., Lambert, W., Deforce, D., Van Peteghem, C. *et al.* (2004) The determination of cocaine, benzoylecgonine, and cocaethylene in small-volume oral fluid samples by liquid chromatography–quadrupole-time-of-flight mass spectrometry. *Journal of Analytical Toxicology*, 28, 655–659.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

121. Fiorentin, T.R., D'Avila, F.B., Comiran, E., Zamboni, A., Scherer, J.N., Pechansky, F. *et al.* (2017) Simultaneous determination of cocaine/crack and its metabolites in oral fluid, urine and plasma by liquid chromatography–mass spectrometry and its application in drug users. *Journal of Pharmacological and Toxicological Methods*, 86, 60–66.
122. Jones, J., Tomlinson, K., Moore, C. (2002) The simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, 6-acetylmorphine, and oxycodone in hair and oral fluid. *Journal of Analytical Toxicology*, 26, 171–175.
123. Campora, P., Bermejo, A.M., Tabernero, M.J., Fernandez, P. (2006) Use of gas chromatography/mass spectrometry with positive chemical ionization for the determination of opiates in human oral fluid. *Rapid Communications in Mass Spectrometry: RCM*, 20, 1288–1292.
124. Moore, C., Rana, S., Coulter, C. (2007) Determination of meperidine, tramadol and oxycodone in human oral fluid using solid phase extraction and gas chromatography–mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 850, 370–375.
125. Tuyay, J., Coulter, C., Rodrigues, W., Moore, C. (2012) Disposition of opioids in oral fluid: importance of chromatography and mass spectral transitions in LC-MS/MS. *Drug Testing and Analysis*, 4, 395–401.
126. Yu, H., Hong, S., Jeong, C.-H., Bae, J.-W., Lee, S. (2018) Development of a linear dual column HPLC-MS/MS method and clinical genetic evaluation for tramadol and its phase I and II metabolites in oral fluid. *Archives of Pharmacal Research*, 41, 288–298.
127. Shaparin, N., Mehta, N., Kunkel, F., Stripp, R., Borg, D., Kolb, E. (2017) A novel chronic opioid monitoring tool to assess prescription drug steady state levels in oral fluid. *Pain Medicine (Malden, Mass.)*, 18, 2162–2169.
128. George, R., Lobb, M., Haywood, A., Khan, S., Hardy, J., Good, P. *et al.* (2016) Quantitative determination of the enantiomers of methadone in human plasma and saliva by chiral column chromatography coupled with mass spectrometric detection. *Talanta*, 149, 142–148.
129. Miguez-Diez, E., Modamio, P., Lastra, C.F., Arrufat, F.X., Serra, M., Reig, S. *et al.* (2013) Development and validation of a rapid and reliable high-performance liquid chromatography method for methadone quantification in human plasma and saliva. *Clinical Chemistry and Laboratory Medicine*, 51, e309–e312.
130. Martins, L.F., Yegles, M., Wennig, R. (2008) Simultaneous enantioselective quantification of methadone and of 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine in oral fluid using capillary electrophoresis. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 862, 79–85.
131. Rosas, M.E.R., Preston, K.L., Epstein, D.H., Moolchan, E.T., Wainer, I.W. (2003) Quantitative determination of the enantiomers of methadone and its metabolite (EDDP) in human saliva by enantioselective liquid chromatography with mass spectrometric detection. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 796, 355–370.
132. Gray, T.R., Dams, R., Choo, R.E., Jones, H.E., Huestis, M.A. (2011) Methadone disposition in oral fluid during pharmacotherapy for opioid-dependence. *Forensic Science International*, 206, 98–102.
133. Hsu, Y.-C., Chen, B.-G., Yang, S.-C., Wang, Y.-S., Huang, S.-P., Huang, M.-H. *et al.* (2013) Methadone concentrations in blood, plasma, and oral fluid determined by isotope-dilution gas chromatography–mass spectrometry. *Analytical and Bioanalytical Chemistry*, 405, 3921–3928.
134. Fucci, N. and De Giovanni, N. (2008) Stability of methadone and its main metabolite in oral fluid. *Drug Metabolism Letters*, 2, 125–129.
135. Kintz, P., Villain, M., Concheiro, M., Cirimele, V. (2005) Screening and confirmatory method for benzodiazepines and hypnotics in oral fluid by LC-MS/MS. *Forensic Science International*, 150, 213–220.
136. Quintela, O., Cruz, A., de Castro, A., Concheiro, M., Lopez-Rivadulla, M. (2005) Liquid chromatography-electrospray ionisation mass spectrometry for the determination of nine selected benzodiazepines in human plasma and oral fluid. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 825, 63–71.
137. Link, B., Haschke, M., Wenk, M., Krahenbuhl, S. (2007) Determination of midazolam and its hydroxy metabolites in human plasma and oral fluid by liquid chromatography/electrospray ionization ion trap tandem mass spectrometry. *Rapid Communications in Mass Spectrometry: RCM*, 21, 1531–1540.
138. Moore, C., Coulter, C., Crompton, K., Zumwalt, M. (2007) Determination of benzodiazepines in oral fluid using LC-MS-MS. *Journal of Analytical Toxicology*, 31, 596–600.
139. Ngwa, G., Fritch, D., Blum, K., Newland, G. (2007) Simultaneous analysis of 14 benzodiazepines in oral fluid by solid-phase extraction and LC-MS-MS. *Journal of Analytical Toxicology*, 31, 369–376.
140. Jang, M., Chang, H., Yang, W., Choi, H., Kim, E., Yu, B.-H. *et al.* (2013) Development of an LC-MS/MS method for the simultaneous determination of 25 benzodiazepines and zolpidem in oral fluid and its application to authentic samples from regular drug users. *Journal of Pharmaceutical and Biomedical Analysis*, 74, 213–222.
141. Rodrigues, W.C., Catbagan, P., Rana, S., Wang, G., Moore, C. (2013) Detection of synthetic cannabinoids in oral fluid using ELISA and LC-MS-MS. *Journal of Analytical Toxicology*, 37, 526–533.
142. Oiestad, E.L., Johansen, U., Christophersen, A.S., Karinen, R. (2013) Screening of synthetic cannabinoids in preserved oral fluid by UPLC-MS/MS. *Bioanalysis*, 5, 2257–2268.
143. Rocchi, R., Simeoni, M.C., Montesano, C., Vannutelli, G., Curini, R., Sergi, M. *et al.* (2018) Analysis of new psychoactive substances in oral fluids by means of microextraction by packed sorbent followed by ultra-high-performance liquid chromatography–tandem mass spectrometry. *Drug Testing and Analysis*, 10, 865–873.
144. de Castro, A., Lendoiro, E., Fernandez-Vega, H., Steinmeyer, S., Lopez-Rivadulla, M., Cruz, A. (2014) Liquid chromatography tandem mass spectrometry determination of selected synthetic cathinones and two piperazines in oral fluid. Cross reactivity study with an on-site immunoassay device. *Journal of Chromatography. A*, 1374, 93–101.
145. Mohamed, K.M., Al-Hazmi, A.H., Alasiri, A.M., Ali, M.E.-S. (2016) A GC-MS method for detection and quantification of cathine, cathinone, methcathinone and ephedrine in oral fluid. *Journal of Chromatographic Science*, 54, 1271–1276.
146. Williams, M., Martin, J., Galettis, P. (2017) A validated method for the detection of 32 bath salts in oral fluid. *Journal of Analytical Toxicology*, 41, 659–669.
147. Quintela, O., Crouch, D.J., Andrenyak, D.M. (2006) Recovery of drugs of abuse from the Immunalysis Quantisal oral fluid collection device. *Journal of Analytical Toxicology*, 30, 614–616.
148. Dams, R., Choo, R.E., Lambert, W.E., Jones, H., Huestis, M.A. (2007) Oral fluid as an alternative matrix to monitor opiate and cocaine use in substance-abuse treatment patients. *Drug and Alcohol Dependence*, 87, 258–267.
149. Moore, C., Kelley-Baker, T., Lacey, J. (2012) Interpretation of oxycodone concentrations in oral fluid. *Journal of Opioid Management*, 8, 161–166.
150. Drummer, O.H., Gerostamoulos, D., Chu, M., Swann, P., Boorman, M., Cairns, I. (2007) Drugs in oral fluid in randomly selected drivers. *Forensic Science International*, 170, 105–110.
151. Newmeyer, M.N., Desrosiers, N.A., Lee, D., Mendu, D.R., Barnes, A.J., Gorelick, D.A. *et al.* (2014) Cannabinoid disposition in oral fluid after controlled cannabis smoking in frequent and occasional smokers. *Drug Testing and Analysis*, 6, 1002–1010.
152. Substance Abuse and Mental Health Services Administration, D. of H. and H.S. (2004) Proposed Revisions to Mandatory Guidelines for Federal Workplace Drug Testing Programs. https://www.govinfo.gov/content/pkg/FR-2004-04-13/pdf/04-7984.pdf (Accessed March 11, 2019).
153. DRUID—Driving under the Influence of Drugs Alcohol and Medicines (2011) Per Se Limits—Methods of Defining Cut-Off Values for Zero Tolerance. https://biblio.ugent.be/publication/1988464. (Accessed March 11, 2019).
154. Standards Australia (2006) *Procedures for Specimen Collection and Detection and Quantitation of Drugs in Oral Fluid. Australian Stan-*

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

dard 4760–2006. https://www.saiglobal.com/PDFTemp/Previews/OSH/AS/AS4000/4700/4760-2006.pdf (Accessed March 11, 2019).
155. Walsh, J.M., Verstraete, A.G., Huestis, M.A. and Morland, J. (2008) Guidelines for research on drugged driving. Addiction (Abingdon, England), 103, 1258–1268.
156. Committee, C.S. of F.S.D. and D. (2017) Canadian Society of Forensic Science Drugs and Driving Committee (DDC) Drug Screening Equipment—Oral Fluid Standards and Evaluation Procedures. https://www.csfs.ca/wp-content/uploads/2017/11/Approval-Standards-for-Drug-Screening-Equipment.pdf. https://www.csfs.ca/wp-content/uploads/2017/11/Approval-Standards-for-Drug-Screening-Equipment.pdf. (Accessed December 2018)
157. Cone, E.J., Presley, L., Lehrer, M., Seiter, W., Smith, M., Kardos, K.W. *et al.* (2002) Oral fluid testing for drugs of abuse: positive prevalence rates by Intercept immunoassay screening and GC-MS-MS confirmation and suggested cutoff concentrations. *Journal of Analytical Toxicology*, 26, 541–546.
158. Heltsley, R., DePriest, A., Black, D.L., Robert, T., Marshall, L., Meadors, V.M. *et al.* (2011) Oral fluid drug testing of chronic pain patients. I. Positive prevalence rates of licit and illicit drugs. *Journal of Analytical Toxicology*, 35, 529–540.
159. Heltsley, R., Depriest, A., Black, D.L., Crouch, D.J., Robert, T., Marshall, L. *et al.* (2012) Oral fluid drug testing of chronic pain patients. II. Comparison of paired oral fluid and urine specimens. *Journal of Analytical Toxicology*, 36, 75–80.
160. Conermann, T., Gosalia, A.R., Kabazie, A.J., Moore, C., Miller, K., Fetsch, M. *et al.* (2014) Utility of oral fluid in compliance monitoring of opioid medications. *Pain Physician*, 17, 63–70.
161. Kunkel, F., Fey, E., Borg, D., Stripp, R., Getto, C. (2015) Assessment of the use of oral fluid as a matrix for drug monitoring in patients undergoing treatment for opioid addiction. *Journal of Opioid Management*, 11, 435–442.
162. Vindenes, V., Yttredal, B., Oiestad, E.L., Waal, H., Bernard, J.P., Morland, J.G. *et al.* (2011) Oral fluid is a viable alternative for monitoring drug abuse: detection of drugs in oral fluid by liquid chromatography–tandem mass spectrometry and comparison to the results from urine samples from patients treated with methadone or buprenorphine. *Journal of Analytical Toxicology*, 35, 32–39.
163. Chu, M., Gerostamoulos, D., Beyer, J., Rodda, L., Boorman, M., Drummer, O.H. (2012) The incidence of drugs of impairment in oral fluid from random roadside testing. *Forensic Science International*, 215, 28–31.
164. Simonsen, K.W., Steentoft, A., Hels, T., Bernhoft, I.M., Rasmussen, B.S., Linnet, K. (2012) Presence of psychoactive substances in oral fluid from randomly selected drivers in Denmark. *Forensic Science International*, 221, 33–38.
165. Vindenes, V., Lund, H.M.E., Andresen, W., Gjerde, H., Ikdahl, S.E., Christophersen, A.S. *et al.* (2012) Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers. *Forensic Science International*, 219, 165–171.
166. Gjerde, H., Langel, K., Favretto, D., Verstraete, A.G. (2015) Detection of illicit drugs in oral fluid from drivers as biomarker for drugs in blood. *Forensic Science International*, 256, 42–45.
167. Barnes, A.J., Scheidweiler, K.B., Kolbrich-Spargo, E.A., Gorelick, D.A., Goodwin, R.S., Huestis, M.A. (2011) MDMA and metabolite disposition in expectorated oral fluid after controlled oral MDMA administration. *Therapeutic Drug Monitoring*, 33, 602–608.
168. Lund, H.M.E., Oiestad, E.L., Gjerde, H., Christophersen, A.S. (2011) Drugs of abuse in oral fluid collected by two different sample kits—stability testing and validation using ultra performance tandem mass spectrometry analysis. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 879, 3367–3377.
169. Andas, H.T., Enger, A., Oiestad, A.M.L., Vindenes, V., Christophersen, A.S., Huestis, M.A. *et al.* (2016) Extended detection of amphetamine and methamphetamine in oral fluid. *Therapeutic Drug Monitoring*, 38, 114–119.
170. Huestis, M.A., Cone, E.J. (2004) Relationship of Delta 9-tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis. *Journal of Analytical Toxicology*, 28, 394–399.
171. Swortwood, M.J., Newmeyer, M.N., Andersson, M., Abulseoud, O.A., Scheidweiler, K.B., Huestis, M.A. (2017) Cannabinoid disposition in oral fluid after controlled smoked, vaporized, and oral cannabis administration. *Drug Testing and Analysis*, 9, 905–915.
172. Anizan, S., Milman, G., Desrosiers, N.A., Barnes, A.J., Gorelick, D.A., Huestis, M.A. (2013) Oral fluid cannabinoid concentrations following controlled smoked cannabis in chronic frequent and occasional smokers driving under influence of drugs. *Analytical and Bioanalytical Chemistry*, 405, 8451–8461.
173. Toennes, S.W., Ramaekers, J.G., Theunissen, E.L., Moeller, M.R., Kauert, G.F. (2010) Pharmacokinetic properties of delta9-tetrahydrocannabinol in oral fluid of occasional and chronic users. *Journal of Analytical Toxicology*, 34, 216–221.
174. Toennes, S.W., Schneider, K., Wunder, C., Kauert, G.F., Moeller, M.R., Theunissen, E.L. *et al.* (2013) Influence of ethanol on the pharmacokinetic properties of Delta9-tetrahydrocannabinol in oral fluid. *Journal of Analytical Toxicology*, 37, 152–158.
175. Wille, S.M.R., Di Fazio, V., Toennes, S.W., van Wel, J.H.P., Ramaekers, J.G., Samyn, N. (2015) Evaluation of Delta(9)-tetrahydrocannabinol detection using DrugWipe5S((R)) screening and oral fluid quantification after Quantisal collection for roadside drug detection via a controlled study with chronic cannabis users. *Drug Testing and Analysis*, 7, 178–186.
176. Scheidweiler, K.B., Andersson, M., Swortwood, M.J., Sempio, C., Huestis, M.A. (2017) Long-term stability of cannabinoids in oral fluid after controlled cannabis administration. *Drug Testing and Analysis*, 9, 143–147.
177. Odell, M.S., Frei, M.Y., Gerostamoulos, D., Chu, M., Lubman, D.I. (2015) Residual cannabis levels in blood, urine and oral fluid following heavy cannabis use. *Forensic Science International*, 249, 173–180.
178. Lee, D., Schwope, D.M., Milman, G., Barnes, A.J., Gorelick, D.A., Huestis, M.A. (2012) Cannabinoid disposition in oral fluid after controlled smoked cannabis. *Clinical Chemistry*, 58, 748–756.
179. Milman, G., Schwope, D.M., Gorelick, D.A., Huestis, M.A. (2012) Cannabinoids and metabolites in expectorated oral fluid following controlled smoked cannabis. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 413, 765–770.
180. de Castro, A., Lendoiro, E., Fernandez-Vega, H., Lopez-Rivadulla, M., Steinmeyer, S., Cruz, A. (2014) Assessment of different mouthwashes on cannabis oral fluid concentrations. *Drug Testing and Analysis*, 6, 1011–1019.
181. Lee, D., Milman, G., Barnes, A.J., Goodwin, R.S., Hirvonen, J., Huestis, M.A. (2011) Oral fluid cannabinoids in chronic, daily cannabis smokers during sustained, monitored abstinence. *Clinical Chemistry*, 57, 1127–1136.
182. Houwing, S., Smink, B.E., Legrand, S.-A., Mathijssen, R.P.M., Verstraete, A.G., Brookhuis, K.A. (2012) Repeatability of oral fluid collection methods for THC measurement. *Forensic Science International*, 223, 266–272.
183. Niedbala, R.S., Kardos, K.W., Fritch, D.F., Kardos, S., Fries, T., Waga, J. *et al.* (2001) Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. *Journal of Analytical Toxicology*, 25, 289–303.
184. Anizan, S., Bergamaschi, M.M., Barnes, A.J., Milman, G., Desrosiers, N.A., Lee, D. *et al.* (2015) Impact of oral fluid collection device on cannabinoid stability following smoked cannabis. *Drug Testing and Analysis*, 7, 114–120.
185. Scheidweiler, K.B., Spargo, E.A.K., Kelly, T.L., Cone, E.J., Barnes, A.J., Huestis, M.A. (2010) Pharmacokinetics of cocaine and metabolites in human oral fluid and correlation with plasma concentrations after controlled administration. *Therapeutic Drug Monitoring*, 32, 628–637.
186. Jufer, R., Walsh, S.L., Cone, E.J., Sampson-Cone, A. (2006) Effect of repeated cocaine administration on detection times in oral fluid and urine. *Journal of Analytical Toxicology*, 30, 458–462.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020

187. Kim, I., Barnes, A.J., Oyler, J.M., Schepers, R., Joseph, R.E.J., Cone, E.J. *et al.* (2002) Plasma and oral fluid pharmacokinetics and pharmacodynamics after oral codeine administration. *Clinical Chemistry*, 48, 1486–1496.
188. Samano, K.L., Clouette, R.E., Rowland, B.J., Sample, R.H.B. (2015) Concentrations of morphine and codeine in paired oral fluid and urine specimens following ingestion of a poppy seed roll and raw poppy seeds. *Journal of Analytical Toxicology*, 39, 655–661.
189. Presley, L., Lehrer, M., Seiter, W., Hahn, D., Rowland, B., Smith, M. *et al.* (2003) High prevalence of 6-acetylmorphine in morphine-positive oral fluid specimens. *Forensic Science International*, 133, 22–25.
190. Phillips, S.G., Allen, K.R. (2006) Acetylcodeine as a marker of illicit heroin abuse in oral fluid samples. *Journal of Analytical Toxicology*, 30, 370–374.
191. Cone, E.J., DePriest, A.Z., Heltsley, R., Black, D.L., Mitchell, J.M., LoDico, C. *et al.* (2015) Prescription opioids. III. Disposition of oxycodone in oral fluid and blood following controlled single-dose administration. *Journal of Analytical Toxicology*, 39, 192–202.
192. Cao, J.M., Ma, J.D., Morello, C.M., Atayee, R.S., Best, B.M. (2014) Observations on hydrocodone and its metabolites in oral fluid specimens of the pain population: comparison with urine. *Journal of Opioid Management*, 10, 177–186.
193. Cone, E.J., DePriest, A.Z., Heltsley, R., Black, D.L., Mitchell, J.M., LoDico, C. *et al.* (2015) Prescription opioids. IV: Disposition of hydrocodone in oral fluid and blood following single-dose administration. *Journal of Analytical Toxicology*, 39, 510–518.
194. Meyer, M.R., Rosenborg, S., Stenberg, M., Beck, O. (2015) First report on the pharmacokinetics of tramadol and *O*-desmethyltramadol in exhaled breath compared to plasma and oral fluid after a single oral dose. *Biochemical Pharmacology*, 98, 502–510.
195. Laloup, M., Fernandez, M.D.M.R., Wood, M., Maes, V., De Boeck, G., Vanbeckevoort, Y. *et al.* (2007) Detection of diazepam in urine, hair and preserved oral fluid samples with LC-MS-MS after single and repeated administration of Myolastan and Valium. *Analytical and Bioanalytical Chemistry*, 388, 1545–1556.
196. Arvidsson, M., Ullah, S., Franck, J., Dahl, M.-L. and Beck, O. (2018) Drug abuse screening with exhaled breath and oral fluid in adults with substance use disorder. *Drug Testing and Analysis*, March 2018: 10.1002/dta.2384.
197. Temte, V., Kjeldstadli, K., Bruun, L.D., Birdal, M., Bachs, L., Karinen, R. *et al.* An experimental study of diazepam and alprazolam kinetics in urine and oral fluid following single oral doses. *Journal of Analytical Toxicology*. In: *December 2018: 10.1093/jat/bky062*, 2018.
198. Samyn, N., De Boeck, G., Cirimele, V., Verstraete, A., Kintz, P. (2002) Detection of flunitrazepam and 7-aminoflunitrazepam in oral fluid after controlled administration of rohypnol. *Journal of Analytical Toxicology*, 26, 211–215.
199. Vindenes, V., Strand, D.H., Koksaeter, P., Gjerde, H. (2016) Detection of nitrobenzodiazepines and their 7-amino metabolites in oral fluid. *Journal of Analytical Toxicology*, 40, 310–312.
200. Kempf, J., Wuske, T., Schubert, R., Weinmann, W. (2009) Pre-analytical stability of selected benzodiazepines on a polymeric oral fluid sampling device. *Forensic Science International*, 186, 81–85.
201. Miller, B., Kim, J., Concheiro, M. (2017) Stability of synthetic cathinones in oral fluid samples. *Forensic Science International*, 274, 13–21.

Downloaded from https://academic.oup.com/jat/article-abstract/43/6/415/5524345 by guest on 13 March 2020