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Oral fluid vs. Urine Analysis to Monitor Synthetic Cannabinoids and Classic Drugs Recent Exposure

Vincent Blandino, Jillian Wetzel, Jiyoung Kim, Petrit Haxhi, Richard Curtis, and Marta Concheiro

John Jay College of Criminal Justice, City University of New York, New York, USA

Abstract

Background—Urine is a common biological sample to monitor recent drug exposure, and oral fluid is an alternative matrix of increasing interest in clinical and forensic toxicology. Limited data are available about oral fluid vs. urine drug disposition, especially for synthetic cannabinoids.

Objective—To compare urine and oral fluid as biological matrices to monitor recent drug exposure among HIV-infected homeless individuals.

Methods—Seventy matched urine and oral fluid samples were collected from 13 participants. Cannabis, amphetamines, benzodiazepines, cocaine and opiates were analyzed in urine by the enzyme-multiplied-immunoassay-technique and in oral fluid by liquid chromatography tandem mass spectrometry (LC-MSMS). Eleven synthetic cannabinoids were analyzed in urine and in oral fluid by LC-MSMS.

Results—Five oral fluid samples were positive for AB-FUBINACA. In urine, 4 samples tested positive for synthetic cannabinoids PB-22, 5-Fluoro-PB-22, AB-FUBINACA, and metabolites UR-144 5-pentanoic acid and UR-144 4-hydroxypentyl. In only one case, oral fluid and urine results matched, both specimens being AB-FUBINACA positive. For cannabis, 40 samples tested positive in urine and 30 in oral fluid (85.7% match). For cocaine, 37 urine and 52 oral fluid samples were positive (75.7% match). Twenty-four urine samples were positive for opiates, and 25 in oral fluid (81.4% match). For benzodiazepines, 23 samples were positive in urine and 25 in oral fluid (85.7% match).

Conclusion/Discussion—These results offer new information about drugs disposition between urine and oral fluid. Oral fluid is a good alternative matrix to urine for monitoring cannabis, cocaine, opiates and benzodiazepines recent use; however, synthetic cannabinoids showed mixed results.

Keywords

Oral fluid; urine; synthetic cannabinoids; cannabis; cocaine; opiates; benzodiazepines

1. INTRODUCTION

Drug analysis in biological specimens provides an objective and quantitative measure of drug exposure. Because of this, drug testing is a routine praxis in forensic and clinical settings. From a clinical point of view, the analytical results help in the identification, diagnosis, treatment, and promotion of recovery for patients with drug-related health issues, such as addiction [1]. It is well known that drug use can have direct deleterious consequences on an individual's health, as well as indirect harms, for instance unsafe practices [2]. Studies have shown that drug use plays a maladaptive role in adherence to HIV treatment [3–5]. Antiretroviral therapy (ART) effectiveness relies on high and sustained levels of medication adherence. These treatments are complex with various protocols, dietary restrictions, and potential side effects. Proper treatment may be hindered by factors such as homelessness, incarceration, untreated psychiatric illnesses and drug addiction [3]. HIV positive drug users tend to have a lower uptake of treatment compared to HIV positive individuals who do not use drugs [4]. Hinkin et al [5] suggested that the acute effects of intoxication, rather than attributes that may be characteristic of the drug-using populace, leads to difficulties with medication adherence. These studies focused on classic drugs of abuse; however, currently there is no information about how the use and abuse of new psychoactive drugs, specifically the synthetic cannabinoids, affect the adherence to HIV treatment. Synthetic cannabinoids are a heterogeneous group of newly synthesized compounds that mimic the effects of cannabis. These drugs are easy to obtain (smart shops, internet) and many of them are “legal” (not scheduled substances). They show increased potency than their classic drug equivalent, and are causing serious health problems worldwide.

Urine is the most commonly used biological sample to monitor recent drug exposure. Urine offers several advantages in terms of window of detection (drug exposure can be detected for several days) and from an analytical point of view (drug concentrations are high, and there is plenty amount of sample). However, there are important disadvantages; its supervised collection intrudes on donor's privacy and/or there is risk of adulteration. Oral fluid (OF) is an alternative matrix of increasing interest in forensic and clinical toxicology. Its collection is easy, non-invasive, with low biohazard risk, and is gender-neutral during direct observation in ways that urine is not, thereby reducing the opportunity for adulteration. Disadvantages of oral fluid testing include small sample volume, reduced salivation after intake of drugs with sympathomimetic properties, and lower concentrations compared to urine [6]. Although in terms of window of detection OF shows in general a shorter window (hours) compared to urine (days) [7], the overlap between detection times in oral fluid and urine can be improved by using sensitive methods for oral fluid analysis [8]. Also, the detection window for drugs in oral fluid may be expanded after repeated ingestion of high doses of drugs of abuse [9,10].

Comparisons of paired urine and oral fluid samples, collected at the same time from the same individual, to detect recent exposure to cannabis, cocaine, opiates and amphetamines in different types of populations (pain management, long-term medication-assisted treatment, anti-doping, driving under the influence of drug cases) have been published [8,11–14]. However, scarce data are available about synthetic cannabinoids. Synthetic

cannabinoids popularity has increased in the last years. In New York City, there have been more than 6,000 synthetic cannabinoids-related emergency department visits since January 2015 [15]. Synthetic cannabinoids concentrations have been reported in urine [16,17] and in oral fluid [18,19]; however, data in paired urine and oral fluid samples are not currently available.

The objective of the present study was to compare urine and oral fluid as biological matrices to monitor recent exposure to synthetic cannabinoids and classic drugs (cannabis, cocaine, opiates, amphetamines, benzodiazepines) among HIV+ homeless individuals in New York City. The results from this project will be used to monitor drug exposure, including synthetic cannabinoids, among HIV+ individuals to investigate the impact of synthetic cannabinoids use on the adherence to HIV treatment.

2. MATERIALS AND METHODS

2.1 Reagents and supplies

Synthetic cannabinoids (A-796260, AB-FUBINACA, AB-PINACA, APINACA (AKB-48), JWH-018, JWH-073, MAM2201, PB-22, 5-Fluoro PB-22, UR-144, XLR-11) and metabolites standards (AB-PINACA 5-Pentanoic acid, APINACA (AKB-48) 5-Hydroxypentyl, AM2201 4-Hydroxypentyl, JWH-018 5-Pentanoic acid, JWH-019 5-Hydroxyhexyl, JWH-073 4-Butanoic acid, JWH-122 4-Hydroxypentyl, JWH-210 4-Hydroxypentyl, JWH-250 4-Hydroxypentyl, MAM2201 4-Hydroxypentyl, UR-144 4-Hydroxypentyl, UR-144 5-Pentanoic acid, XLR-11 4-Hydroxypentyl) and internal standards (IS) (JWH-250 4-Hydroxypentyl-d₅, AM2201 4-Hydroxypentyl-d₅, and JWH-210 4-Hydroxypentyl-d₅) were purchased from Cerilliant (Round Rock, TX) at 100 µg/mL in methanol or acetonitrile.

Morphine, 6-acetylmorphine (6-AM), codeine, amphetamine (A), methamphetamine (MA), 3,4-methylenedioxymphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxym-N-ethylamphetamine (MDEA), benzoylecgonine (BE), cocaine, methadone, alprazolam, clonazepam, diazepam, (–)-delta-9-tetrahydrocannabinol (THC), and the IS morphine-d₃, codeine-d₃, 6AM-d₃, amphetamine-d₅, methamphetamine-d₅, MDMA-d₅, BE-d₃, cocaine-d₃, alprazolam-d₅, clonazepam-d₄, flunitrazepam-d₇, diazepam-d₅ and THC-d₃ were purchased from Cerilliant at 100µg/mL or 1 mg/mL in methanol or acetonitrile.

Boric acid, potassium chloride, sodium hydroxide, ammonium acetate, acetic acid, acetonitrile, ethyl acetate, methanol, dichloromethane, 2-propanol, and formic acid were from Fisher Scientific (Fair Lawn, NJ). Ammonium hydroxide was from Pharmco-Aaper (Brookfield, CT). All solvents were HPLC or LC-MS grade. Water was purified by an ELGA Purelab Ultra Analytic purifier (Siemens Water Tech, Lowell, MA). Abalone beta-glucuronidase containing 100,000 units/mL was purchased from Campbell Science (Rockford, IL). 1 mL Isolute SLE+ (supported liquid extraction) cartridges were from Biotage (Charlotte, NC). Reversed phase solid phase extraction (SPE) cartridges Strata-X 60 mg 3 mL were from Phenomenex (Torrance, CA). A Kinetex C18 column (100 × 2.1 mm) combined with a guard column with the same chemistry (10 × 2.1 mm) was purchased from

Phenomenex and was used for analytical chromatography. Fisherbrand™ 4 oz. polypropylene specimen containers and 2 mL polypropylene cryotubes were purchased from Fisher. Quantisal™ buffer and Quantisal™ Oral Fluid Collection Devices were purchased from Immunalysis Corporation (Pomona, CA).

2.2 Authentic sample collection and storage

Participants were recruited for the study at the harm reduction and AIDS services non-profit organization, Boom! Health Inc., located in the Bronx, New York City). The eligibility criteria included adults who were HIV+, in antiretroviral therapy and drug users who self-identified as K2 smokers (synthetic cannabinoids use within past 7 days). The participants provided written informed consent to participate. The study was approved by The City University of New York (CUNY) Institutional Review Board. The participants visited the center weekly over a 12-week period during the Summer of 2016. At each visit, participants were interviewed about their drug use the past week, and urine and oral fluid samples were provided. Information about drug doses and specific time of intake was not available.

Urine specimens were collected by the participants in polypropylene specimen containers and shipped to the laboratory the same day. In the laboratory, the urine samples were aliquoted in three 2 mL polypropylene cryotubes, and stored in the freezer at -20°C until analysis. Oral fluid samples were collected by Quantisal oral fluid collection devices. The Quantisal collection device, with a cotton pad at the tip, was placed underneath the tongue to collect $1\text{ mL} \pm 10\%$ of oral fluid until the volume adequacy indicator turn blue. The pad was then placed in 3 mL of buffer solution to stabilize any present substances, and the samples were shipped to the laboratory the same day. In the laboratory, the oral fluid and buffer mixture were aliquoted in two 2 mL polypropylene cryotubes and stored in the freezer at -20°C until analysis.

2.3. Urine screening method

The urine samples were screened for cannabis, amphetamines, benzodiazepines, cocaine, and opiates by the enzyme-multiplied-immunoassay-technique Viva-Jr (EMIT) from Siemens Healthineers Global (Erlangen, Germany). The screening test was performed using 500 μL of urine. The cutoffs were 300 ng/mL for amphetamines, cocaine and opiates, 200 ng/mL for benzodiazepines, and 50 ng/mL for cannabis.

2.4. Urine and Oral Fluid confirmatory methods

2.4.1. Instrumentation—Ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MSMS) instrument was from Shimadzu (Columbia, MD). The Nexera UHPLC system consisted of a binary LC-30AD pump, online degassing unit DGU-20A and cooled autosampler SIL-30AC. The mass spectrometer was a triple quadrupole LCMS 8030 equipped with Dual Ionization Source (DUIS) operated in positive mode. Each compound was monitored by 2 transitions in MRM mode, multiple reaction monitoring mode (Table 1). Chromatographic separations for synthetic cannabinoids and metabolites in urine, synthetic cannabinoids in oral fluid, and classic drugs in oral fluid were performed on a Kinetex C18 column (2.1 $\times$ 100 mm, 1.7 μm) with an attached guard column containing the same packing material (4.6 mm ID), using different gradients with mobile

phases A, 0.1% formic acid in water, and B, 0.1% formic acid in acetonitrile. SLE and SPE procedures were performed using a negative pressure manifold from Fisher. Evaporation under nitrogen was completed using TurboVap LV from Biotage.

2.4.2. Specimen extractions—Synthetic cannabinoids urine extraction was based on the protocol described by Scheidweiler and Huestis [20]. Briefly, 200 μ L of blank urine and 50 μ L of the IS mix was diluted with 0.3 mL of 400 mM ammonium acetate buffer pH 4.0, prior to the addition of 40 μ L of glucuronidase solution (100,000 units glucuronidase activity/mL). The tubes were incubated at 55°C for 2 h. Five hundred μ L of acetonitrile was added and the samples were centrifuged at 6,500 rpm at 4°C for 5 min. The supernatant was then transferred onto the SLE columns. After keeping equilibrium at constant pressure for 5 minutes, analytes were eluted with 2 $\times$ 3 mL of ethyl acetate. All samples were completely dried and reconstituted in 150 μ L mobile phase A:B, 85:15 (v/v), and then transferred to LC-MS autosampler vials.

In the case of synthetic cannabinoids in oral fluid, 0.8 mL of oral fluid-Quantisal samples (0.2 mL oral fluid + 0.6 mL Quantisal buffer) were spiked with 50 μ L of internal standard and 200 μ L of a 1M acetic acid solution. The samples were vortexed for approximately 10 seconds and loaded onto Isolute 1 mL SLE+ cartridges. After equilibration for 15 minutes, samples were eluted with 2 $\times$ 3 mL of ethyl acetate. Samples were evaporated and reconstituted in 150 μ L mobile phase A:B, 85:15 (v/v), and transferred to LC-MS autosampler vials.

Opiates (morphine, codeine, 6AM), amphetamines (amphetamine, methamphetamine, MDA, MDMA, MDEA), cocaine (BE, cocaine), methadone, THC and benzodiazepines (alprazolam, clonazepam, flunitrazepam and diazepam) were extracted from oral fluid-Quantisal samples following the protocol described by Concheiro et al. [21]. Briefly, one mL of borate buffer pH 9 and 25 μ L of IS mixture were added to 1 mL of oral fluid-Quantisal samples (0.25 mL oral fluid + 0.75 mL Quantisal buffer). Samples were loaded to Strata-X SPE cartridges. After washing with a mixture of water:methanol, 95:5, v/v, and water:methanol:ammonium hydroxide (60:39.5:0.5, v/v/v), the samples were eluted with dichloromethane:2-propanol, 75:25, v/v. Following evaporation, samples were reconstituted in 100 μ L mobile phase A:B, 90:10 (v/v), and transferred to LC-MS autosampler vials.

2.4.3. Method validation—Synthetic cannabinoids methods in urine and oral fluid were completely validated for the following categories: linearity, limit of detection, limit of quantification, accuracy and imprecision, extraction efficiency, matrix effect, and process efficiency by procedures described in published forensic toxicology guidelines [22–24]. Classic drugs in oral fluid validation results were previously published in Concheiro et al. [21]. For the classic drugs in oral fluid, the LOQs were 1 ng/mL, except for morphine, codeine and BE (5 ng/mL).

3. RESULTS

3.1. Synthetic cannabinoids in urine and in oral fluid confirmation methods

Eleven synthetic cannabinoids and 13 metabolites were analyzed in urine by SLE and LC-MSMS. The SLE procedure was based on a previous publication by Scheidweiler et al [20]. The chromatographic separation using a reversed-phase C18 column was achieved in less than 10 min (Figure 1). All synthetic cannabinoids had a limit of quantification of 1 ng/mL and linearity from 1 to 100 ng/mL. The imprecision was 0.7–18.5%, and the accuracy was 80.4–119%. The extraction efficiencies were from 42–107.4%, matrix effects from –11.9 to –65.3%, and process efficiencies from 23.1–104.9% (Table 2).

Eleven synthetic cannabinoids were analyzed in oral fluid collected by Quantisal devices by SLE and LC-MSMS. The chromatographic separation was achieved in less than 7 min (Figure 2). All synthetic cannabinoids showed linearity from 0.5 (LOQ) to 100 ng/mL. The accuracy for all compounds ranged between 99.6–124.6%, and imprecision values were below 13.9%. The extraction efficiencies were from 66.2–135.1%, matrix effects from –86.6 to 60.5%, and process efficiencies from 8.9–191.9% (Table 3).

3.2. Participants

Thirteen participants were recruited for the study. The participants were 28–57 years old (40.5 ± 8), predominantly male (11 male, 2 female), Hispanic (11 Hispanic, 1 black, 1 white), and homeless (10 homeless, 3 other). Regarding the health history, besides being HIV+, 6 participants had hepatitis C and 10 participants suffered from mental health problems (bipolar, depression and/or anxiety). All participants reported regular drug use, including heroin, cocaine, cannabis and synthetic cannabinoids. The goal of the study was 12 visits during 3 months (weekly visits). Six participants did 8 to 11 visits, and seven participants did 4 or less visits. A total of 70 paired urine and oral fluid samples were collected.

3.3. Biological samples

Urine samples were screening by EMIT for classic drugs and analyzed by LC-MSMS for synthetic cannabinoids. Urine results are summarized in Table 4. The most prevalent drug group detected in urine was cannabis (57.1%), followed by cocaine (52.9%), opiates (34.3%), and benzodiazepines (32.9%). Synthetic cannabinoids were detected in 5.7% of the samples, and all samples were negative for amphetamines.

With regard to synthetic cannabinoids in urine, 4 samples (sample 2, 4, 5 and 8) out of 70 tested positive for synthetic cannabinoids. Sample 2 was positive for the parent compounds PB-22 and 5-Fluoro-PB-22 (1.4 ng/mL); sample 4 was positive for the parent compound AB-FUBINACA (3 ng/mL); sample 5 and 8 were positive for UR-144 metabolite 5-pentanoic acid (3–8.9 ng/mL), and sample 5 for UR-144 4-hydroxypentyl (1.7 ng/mL) as well.

Oral fluid samples were analyzed by 2 different confirmation methods by LC-MSMS for classic drugs and for synthetic cannabinoids. The most prevalent drug group in oral fluid was cocaine (74.3%), followed by cannabis (42.9%), opiates and benzodiazepines (35.7%

each), synthetic cannabinoids (7.1%) and amphetamines (5.7%). With regard to specific analytes, benzoylecgonine was the compound most commonly detected (67.1%), followed by cocaine (50%), THC (42.9%) and morphine (31.4%). In 5 samples, cocaine was detected (2.4–217.3 ng/mL), although BE was negative. Three samples tested positive for 6AM at low concentrations (1–5.8 ng/mL), while morphine was negative. Oral fluid results are summarized in Tables 5 and 6. For synthetic cannabinoids, 5 oral fluid samples (samples 1, 3, 4, 6 and 7) were positive for AB-FUBINACA with concentrations from 0.8 to 38.9 ng/mL.

Comparing matched urine and oral fluid samples (n=70), 40 samples tested positive for cannabis in urine and 30 were positive for THC in oral fluid, showing an overall 85.7% match. In the case of cocaine, 37 urine samples were positive and 52 oral fluid samples were positive for cocaine and/or BE (75.7% total match). Twenty-four urine samples screened positive for opiates, and 25 oral fluid samples were positive for morphine, codeine and/or 6AM (81.4% total match). For benzodiazepines, 23 samples were positive in urine and 25 oral fluid samples were positive for alprazolam, clonazepam and/or diazepam, showing an 85.7% total match. For synthetic cannabinoids, although 5 oral fluid samples and 4 urine samples were positive, in only one case, oral fluid and urine results matched for the synthetic cannabinoid AB-FUBINACA. Urine-oral fluid agreement results are shown in Table 7.

4. DISCUSSION

We collected 70 paired urine and oral fluid samples from 13 HIV+ individuals to explore the usefulness of oral fluid to monitor cannabis, opiates, cocaine, amphetamines, benzodiazepines, and synthetic cannabinoids exposure in this population. The data from this study will be utilized to explore the impact of synthetic cannabinoids use in the adherence to HIV treatment among HIV+ individuals. Urine samples were analyzed by EMIT for common drugs and LC-MSMS method for synthetic cannabinoids, and oral fluid samples by LC-MSMS methods for common drugs and for synthetic cannabinoids.

We developed a method for the determination of 11 synthetic cannabinoids in oral fluid samples collected by Quantisal devices. Several articles describing confirmation methods of different types of synthetic cannabinoids in oral fluid have been published [18,19,25–28]. The most common extraction procedure employed was SPE [19,25,26], but also a simple protein precipitation [18], sample dilution [29] and liquid-liquid extraction (LLE) [27] were applied. In the present method, we employed SLE. SLE offers advantages over SPE in being more simple and cost-effective, over LLE in being more efficient for high throughput analyses, and over protein precipitation and sample dilution in obtaining cleaner extracts that may reduce matrix effects and prolong the life of the chromatographic column. We employed 0.2 mL of oral fluid (0.8 mL oral fluid-Quantisal mix) achieving a LOQ of 0.5 ng/mL, showing a similar sensitivity compared to previous publications [18,25,26]. Some authors developed the synthetic cannabinoids methods in neat oral fluid collected by expectoration [18,26]. Although expectoration is less expensive and the oral fluid concentrations can be directly measure, its collection can be difficult, and oral fluid could be

highly viscous, yielding problems during sample preparation. We employed Quantisal devices, with a buffer that avoid specimen degradation and bacterial growth.

Comparing paired urine and oral fluid samples agreements above 75% were achieved. These results are in accordance with previous reports [8,11,12,14]. Although cannabis showed 85.7% agreement, 10 samples out of 70 were positive for cannabis in urine (50 ng/mL cutoff) and negative in oral fluid (THC LOQ 1 ng/mL). These results are a consequence of THC pharmacokinetics. THC disappears quickly from the oral fluid after smoking, while its metabolite 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THCCOOH) shows a prolonged excretion in urine, specially in chronic users [30]. Opiates, benzodiazepines and cocaine showed 81.4%, 85.7% and 75.7% agreement, respectively. Although longer windows of detection were expected in urine compared to oral fluid, some samples tested positive in oral fluid and negative in urine (opiates n=7, benzodiazepines n=6, cocaine n=16). This could be due to the lower cutoff employed for oral fluid samples (LOQ 1–5 ng/mL) than urine (200–300 ng/mL), recent use or drug accumulation in the mouth due to the repeated use and the ion trapping phenomenon in the case of basic drugs (opiates, cocaine). In the case of cocaine samples positive in oral fluid and negative in urine, BE was the only analyte detected in 13 out of 16 cases, at concentrations 8.9–54.2 ng/mL. 6AM was detected in oral fluid in samples where morphine was not detected at concentrations 1–5.8 ng/mL, as previously reported [14]. In the case of amphetamines and synthetic cannabinoids, the high number of negative samples (n=66 and 62, respectively), contributed to the high overall agreement (94 and 90%, respectively).

Although uncommon for synthetic cannabinoids, parent compounds AB-FUBINACA, PB-22 and 5-Fluoro-PB-22 were detected in urine at low concentrations (1.4–3 ng/mL). Parent compounds are rarely present in urine, being their corresponding metabolites the most common target compounds [16,17]. However, Vikingsson et al. [31] investigated AB-FUBINACA biomarkers in urine from 28 authentic cases, and AB-FUBINACA parent compound was detected in 54% of these cases. In the present study, metabolites for AB-FUBINACA, MAM2201, PB-22 and 5-Fluoro-PB-22 could not be included in the urine method due to the lack of reference material.

In oral fluid samples, the only synthetic cannabinoid detected was AB-FUBINACA (n=5). Although PB-22, 5-Fluoro-PB-22 or UR-144 were detected in urine samples, these compounds were negative in paired oral fluid samples. PB-22 and 5-Fluoro-PB-22 showed significant ion suppression in oral fluid (–70.7% and –86.6% respectively), but both compounds showed similar suppression effects in oral fluid samples from 10 different sources (PB-22 CV = 20.7%, 5-Fluoro-PB-22 CV = 11.1%). Also, critical validation parameters that could be affected by this ion suppression effect, such as limit of detection and quantification, accuracy and precision, were not compromised in oral fluid samples from different sources. Because of this, the analytical performance may not be the reason of discrepancy between results in urine and oral fluid. In our method, only the parent compounds were investigated, as previously recommended in the literature [18,32]. However, Amaratunga et al. [19] showed that the 4-hydroxy UR-144 metabolite was detected in oral fluid at higher concentration than the parent compound, and some samples were positive for this metabolite and negative for the parent compound.

In the present study, only one sample showed a positive agreement between urine and oral fluid for the synthetic cannabinoid AB-FUBINACA. That sample showed a higher AB-FUBINACA concentration in oral fluid (38.9 ng/mL) than in urine (3 ng/mL), suggesting recent drug exposure. Unfortunately, data about time of exposure was not available.

These data provide further evidence to employ OF as a reliable alternative to urine to monitor drug exposure. OF is a good alternative matrix to urine for monitoring cannabis, cocaine, opiates and benzodiazepines recent use; however, synthetic cannabinoids showed mixed results. Monitoring additional metabolites in both matrices, urine and OF, may improve the agreement between them for the detection of synthetic cannabinoids exposure.

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References

1. Jarvis M, Williams J, Hurford M, Lindsay D, Lincoln P, Giles L, et al. Appropriate Use of Drug Testing in Clinical Addiction Medicine. *Jouranal Addict Med*. 2017; 11(3):163–73.
2. Degenhardt L, Hall W. Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *Lancet*. 2012; 379(9810):55–70. [PubMed: 22225671]
3. Reddon H, Milloy MJ, Simo A, Montaner J, Wood E, Kerr T. Methadone maintenance therapy decreases the rate of antiretroviral therapy discontinuation among HIV-positive illicit drug users. *AIDS Behav*. 2014; 18(4):740–6. [PubMed: 23918244]
4. Calcagno C, Lobatto ME, Robson PM, Millon A. Treatment of medical, psychiatric, and substance-use comorbidities in people infected with HIV who use drugs. 2016; 28(10):1304–14.
5. Hinkin CH, Barclay TR, Castellon SA, Levine AJ, Durvasula RS, Marion SD, et al. Drug use and medication adherence among HIV-1 infected individuals. *AIDS Behav*. 2007; 11(2):185–94. [PubMed: 16897351]
6. Bosker WM, Huestis MA. Oral fluid testing for drugs of abuse. *Clin Chem*. 2009; 55(11):1910–31. [PubMed: 19745062]
7. Drummer OH. Introduction and review of collection techniques and applications of drug testing of oral fluid. *Ther Drug Monit*. 2008; 30(2):203–6. [PubMed: 18367981]
8. Vindenes V, Yttredal B, Oiestad EL, Waal H, Bernard JP, Mørland JG, et al. 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. *J Anal Toxicol*. 2011; 35:32–9. [PubMed: 21219701]
9. Lee D, Milman G, Barnes AJ, Goodwin RS, Hirvonen J, Huestis MA. Oral fluid cannabinoids in chronic, daily cannabis smokers during sustained, monitored abstinence. *Clin Chem*. 2011; 57(8): 1127–36. [PubMed: 21677094]
10. Jufer R, Walsh SL, Cone EJ, Sampson-Cone A. Effect of repeated cocaine administration on detection times in oral fluid and urine. *J Anal Toxicol*. 2006; 30(7):458–62. [PubMed: 16959139]
11. Miller KL, Puet BL, Roberts A, Hild C, Carter J, Black DL. Urine drug testing results and paired oral fluid comparison from patients enrolled in long-term medication-assisted treatment in Tennessee. *J Subst Abuse Treat*. 2017; 76:36–42. [PubMed: 28131519]
12. Heltsley R, DePriest A, Black D, Crouch D, Robert T, Marshall L, et al. Oral fluid drug testing of chronic pain patients. II. Comparison of paired oral fluid and urine specimens. 2012; 36:75–80.

13. Strano-Rossi S, Colamonici C, Botrè F. Parallel analysis of stimulants in saliva and urine by gas chromatography/mass spectrometry: Perspectives for “in competition” anti-doping analysis. *Anal Chim Acta*. 2008; 606(2):217–22. [PubMed: 18082653]
14. Vindenes V, Lund HME, Andresen W, Gjerde H, Ikdahl SE, Christophersen AS, et al. Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers. *Forensic Sci Int*. 2012; 219(1–3):165–71. [PubMed: 22284072]
15. New York City Health and Mental Hygiene Department (DOHMH). Jun 16. 2017 <http://www1.nyc.gov/site/doh/health/health-topics/k2.page>
16. Castaneto MS, Scheidweiler KB, Gandhi A, Wohlfarth A, Klette L, Martin TM, et al. Quantitative urine confirmatory testing for synthetic cannabinoids in randomly collected urine specimens. *Drug Test Anal*. 2016; 7(6):483–93.
17. Borg D, Tverdovsky A, Stripp R. A fast and comprehensive analysis of 32 synthetic cannabinoids using agilent triple quadrupole LC-MS-MS. *J Anal Toxicol*. 2017; 41(1):6–16. [PubMed: 27681343]
18. Kneisel S, Speck M, Moosmann B, Corneillie TM, Butlin NG, Auwärter V. LC/ESI-MS/MS method for quantification of 28 synthetic cannabinoids in neat oral fluid and its application to preliminary studies on their detection windows. *Anal Bioanal Chem*. 2013; 405(14):4691–706. [PubMed: 23535743]
19. Amaratunga P, Thomas C, Lemberg BL, Lemberg D. Quantitative measurement of XLR11 and UR-144 in oral fluid by LC-MS-MS. *J Anal Toxicol*. 2014; 38(6):315–21. [PubMed: 24812645]
20. Scheidweiler KB, Huestis MA. Simultaneous quantification of 20 synthetic cannabinoids and 21 metabolites, and semi-quantification of 12 alkyl hydroxy metabolites in human urine by liquid chromatography-tandem mass spectrometry. *J Chromatogr A*. 2014; 1327:105–17. [PubMed: 24418231]
21. Concheiro M, De Castro A, Quintela Ó, Cruz A, López-Rivadulla M. Determination of illicit and medicinal drugs and their metabolites in oral fluid and preserved oral fluid by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2008; 391(6):2329–38. [PubMed: 18483722]
22. Scientific working group for forensic toxicology (SWGTOX) standard practices for method validation in forensic toxicology. *J Anal Toxicol*. 2013; 37:452–74. [PubMed: 23934984]
23. Wille SMR, Coucke W, Baere T, Peters FT. Update of Standard Practices for New Method Validation in Forensic Toxicology. *Curr Pharm Des*. 2017 Jul 14. Epub ahead of print. doi: 10.2174/1381612823666170714154444
24. Peters FT, Wissenbach DK, Busardò FP, Marchei E, Pichini S. Method development in Forensic Toxicology. *Curr Pharm Des*. 2017 Jun 22. Epub ahead of print. doi: 10.2174/1381612823666170622113331
25. Coulter C, Garnier M, Moore C. Synthetic cannabinoids in oral fluid. *J Anal Toxicol*. 2011; 35(7): 424–30. [PubMed: 21871150]
26. De Castro A, Piñeiro B, Lendoiro E, Cruz A, López-Rivadulla M. Quantification of selected synthetic cannabinoids and 89-tetrahydrocannabinol in oral fluid by liquid chromatography-tandem mass spectrometry. *J Chromatogr A*. 2013; 1295:99–106. [PubMed: 23680386]
27. Kneisel S, Auwärter V, Kempf J. Analysis of 30 synthetic cannabinoids in oral fluid using liquid chromatography-electrospray ionization tandem mass spectrometry. *Drug Test Anal*. 2013; 5(8): 657–69. [PubMed: 23081933]
28. Øiestad EL, Johansen U, Christophersen AS, Karinen R. Screening of synthetic cannabinoids in preserved oral fluid by UPLC–MS/MS. *Bioanalysis*. 2013; 5(18):2257–68. [PubMed: 24053241]
29. Strano-Rossi S, Anzillotti L, Castrignano E, Romolo FS, Chiarotti M. Ultra high performance liquid chromatography-electrospray ionization-tandem mass spectrometry screening method for direct analysis of designer drugs, “spice” and stimulants in oral fluid. *J Chromatogr A*. 2012; 1258:37–42. [PubMed: 22939380]
30. Desrosiers NA, Lee D, Concheiro-Guisan M, Scheidweiler KB, Gorelick DA, Huestis MA. Urinary cannabinoid disposition in occasional and frequent smokers: Is THC-glucuronide in sequential urine samples a marker of recent use in frequent smokers? *Clin Chem*. 2014; 60(2):361–72. [PubMed: 24185550]

31. Vikingsson S, Green H, Brinkhagen L, Mukhtar S, Josefsson M. Identification of AB-FUBINACA metabolites in authentic urine samples suitable as urinary markers of drug intake using liquid chromatography quadrupole tandem time of flight mass spectrometry. *Drug Test Anal.* 2016; 8(9): 950–6. [PubMed: 26560240]
32. Znalezioná J, Ginterová P, Petr J, Ondra P, Válka I, Ševík J, et al. Determination and identification of synthetic cannabinoids and their metabolites in different matrices by modern analytical techniques - a review. *Anal Chim Acta.* 2015; 874:11–25. [PubMed: 25910441]

Figure 1.

Total ion chromatogram of 11 synthetic cannabinoids and 13 metabolites in urine sample at 15 ng/mL. 1, AB-PINACA 5-Pentanoic acid; 2, A-796260; 3, AB-FUBINACA; 4, JWH-250 4-Hydroxypentyl; 5, JWH-073 4-Butanoic acid; 6, AB-PINACA; 7, JWH-018 5-Pentanoic acid; 8, AM2201 4-Hydroxypentyl; 9, MAM2201 4-Hydroxypentyl; 10, JWH-019 5-Hydroxyhexyl; 11, UR-144 5-Pentanoic acid; 12, JWH-122 4-Hydroxypentyl; 13, XLR-11 4-Hydroxypentyl; 14, UR-144 4-Hydroxypentyl; 15, 5-Fluoro PB-22; 16, JWH-210 4-Hydroxypentyl; 17, APINACA (AKB-48) 5-Hydroxypentyl; 18, PB-22; 19, MAM2201; 20, JWH-073; 21, XLR-11; 22, JWH-018; 23, UR-144; 24, APINACA (AKB-48).

Figure 2.

Total ion chromatogram of 11 synthetic cannabinoids in oral fluid sample at 2.5 ng/mL. 1, A-796260; 2, AB-FUBINACA; 3, AB-PINACA; 4, 5-Fluoro PB-22; 5, PB-22; 6, MAM2201; 7, JWH-073; 8, XLR-11; 9, JWH-018; 10, UR-144; 11, APINACA (AKB-48).

Liquid chromatography-tandem mass spectrometry parameters for synthetic cannabinoids and metabolites. The underlined product (m/z) is the quantifier transition.

Table 1

Analyte	Precursor (m/z)	Product (m/z)	Q1 (V)	CE	Q3 (V)
5-Fluoro PB-22	377	143.85	-25	-42	-15
		<u>232.10</u>	-25	-16	-17
A-796260	355.1	114.1	-17	-32	-23
		<u>125</u>	-17	-24	-26
AB-FUBINACA	369	109.05	-10	-45	-22
		<u>253.05</u>	-10	-27	-18
AB-PINACA	331	144.95	-15	-42	-15
		<u>215.10</u>	-15	-26	-25
AB-PINACA 5-Pentanoic acid	261.1	217.1	-16	-31	-10
		<u>227.1</u>	-16	-32	-24
AM2201 4-Hydroxypentyl	376	127.05	-16	-49	-27
		<u>155</u>	-16	-27	-30
AM2201 4-Hydroxypentyl-d ₅	381.1	126.95	-10	-53	-28
		<u>154.95</u>	-10	-29	-30
APINACA (AKB-48)	366.1	<u>135.1</u>	-10	-24	-14
		93.15	-10	-54	-18
APINACA (AKB-48) 5-Hydroxypentyl	382.1	79	-18	-55	-16
		<u>135.15</u>	-18	-27	-29
JWH 210 4-Hydroxypentyl	386.1	154.9	-10	-40	-29
		<u>183</u>	-10	-26	-20
JWH-018	341.8	126.95	-22	-48	-26
		<u>154.95</u>	-22	-27	-30

Analyte	Precursor (m/z)	Product (m/z)	Q1 (V)	CE	Q3 (V)
JWH-018 5-Pentanoic acid	372	126.9	-10	-49	-13
		<u>154.85</u>	-10	-24	-17
JWH-019 5-Hydroxyhexyl	372	127	-10	-49	-26
		<u>154.95</u>	-10	-25	-30
JWH-073	327.8	127	-21	-50	-27
		<u>154.9</u>	-21	-24	-11
JWH-073 4-Butanoic acid	358	126.95	-10	-49	-27
		<u>154.95</u>	-10	-23	-16
JWH-122 4-Hydroxypentyl	372	140.95	-10	-47	-29
		<u>169.05</u>	-10	-24	-18
JWH-210 4-Hydroxypentyl-d ₅	390.9	154.9	-13	-44	-11
		<u>183</u>	-13	-29	-19
JWH-250 4-Hydroxypentyl	351.8	91.15	-22	-55	-19
		<u>121</u>	-22	-25	-26
JWH-250 4-Hydroxypentyl-d ₅	357	91.1	-10	-55	-10
		<u>121.1</u>	-10	-25	-26
MAM2201	373.8	141.05	-16	-45	-29
		<u>168.95</u>	-16	-29	-18
MAM2201 4-Hydroxypentyl	390	140.9	-17	-50	-30
		<u>168.95</u>	-17	-27	-12
PB-22	358.8	143.95	-16	-37	-15
		<u>214</u>	-16	-17	-10
UR-144	311.9	54.95	-13	-43	-22
		<u>124.90</u>	-13	-24	-27
UR-144 4-Hydroxypentyl	328.1	55	-30	-44	-22

Analyte	Precursor (m/z)	Product (m/z)	Q1 (V)	CE	Q3 (V)
UR-144 5-Pentanoic acid	342	125.05	-30	-22	-26
		55.1	-15	-45	-23
		125	-15	-24	-28
XLR-11	330	55.1	-15	-46	-22
		125	-15	-26	-27
XLR-11 4-Hydroxypentyl	346.1	55.1	-15	-42	-22
		125	-15	-25	-26

Table 2

Mean extraction efficiencies (n=5), matrix effects (n=10) and process efficiencies (n=5) for 11 synthetic cannabinoids and 13 metabolites at quality control 15 ng/mL in urine samples.

Analyte	Extraction Efficiency	Matrix Effect	Process Efficiency
5-Fluoro PB-22	77.6	-27.4	56.4
A-796260	90.7	-46.6	48.5
AB-FUBINACA	93.9	-42.3	54.2
AB-PINACA	98.4	-11.9	86.7
AB-PINACA 5-Pentanoic acid	83.2	26.1	104.9
AM2201 4-Hydroxypentyl	97	-22.2	75.5
APINACA (AKB-48)	89.7	-13	78.1
APINACA (AKB-48) 5-Hydroxypentyl	105.7	-28.9	75.1
JWH 210 4-Hydroxypentyl	107.4	-40.9	63.5
JWH-018	59.9	-57.1	25.7
JWH-018 5-Pentanoic acid	95.6	-22.7	73.9
JWH-019 5-Hydroxyhexyl	100.7	-25.7	74.9
JWH-073	53.5	-56.8	23.1
JWH-073 4-Butanoic acid	94.8	-8.9	86.4
JWH-122 4-Hydroxypentyl	100	-36.2	63.8
JWH-250 4-Hydroxypentyl	95	-36.1	60.8
MAM2201	83.9	-57.5	35.7
MAM2201 4-Hydroxypentyl	90.7	-23.6	69.3
PB-22	91.2	-65.3	31.6
UR-144	42	-26.7	30.8
UR-144 4-Hydroxypentyl	104	-41.7	60.7
UR-144 5-Pentanoic acid	102.5	-37.1	64.5
XLR-11	47.3	-51.1	23.2
MAM2201 4-Hydroxypentyl	90.7	-23.6	69.3

Table 3

Mean extraction efficiencies (n=5), matrix effects (n=10) and process efficiencies (n=5) for 11 synthetic cannabinoids at quality control 2.5 ng/mL in oral fluid-Quantisal buffer samples.

Compound	Extraction Efficiency	Matrix Effects	Process Efficiency
5-Fluoro_PB-22	66.2	−86.6	8.9
A-796260	93.1	−55.9	41.1
AB-FUBINACA	98.6	−40.9	58.3
AB-PINACA	97.5	−43.2	55.4
APINACA (AKB-48)	135.1	23.6	167
JWH-018	108.8	56.7	170.5
JWH-073	102.3	−42.5	58.8
MAM2201	106	−26.2	78.2
PB-22	103.3	−70.7	30.3
UR-144	119.6	60.5	191.9
XLR-11	113.7	−57.1	48.8

Table 4

Urine samples (n=70) positive results for cannabis, cocaine, opiates, benzodiazepines, amphetamines and synthetic cannabinoids (AB-FUBINACA, PB-22, 5-Fluoro-PB-22, UR-144 metabolites). Cutoffs in urine were 1 ng/mL for synthetic cannabinoids, 50 ng/mL for cannabis, 200 ng/mL for benzodiazepines and 300 ng/mL for cocaine, opiates and amphetamines.

Group of Drugs	# of positive	%
Cannabis	40	57.1
Cocaine	37	52.9
Opiates	24	34.3
Benzodiazepines	23	32.9
Amphetamines	0	0
Synthetic cannabinoids	4	5.7

Table 5

Oral fluid samples (n=70) positive results for cannabis (THC), cocaine (cocaine and/or BE), opiates (morphine, codeine and/or 6AM), benzodiazepines (alprazolam, clonazepam, and/or diazepam), amphetamines (amphetamine, methamphetamine and/or MDA) and synthetic cannabinoids (AB-FUBINACA). The limit of quantification (LOQ) for synthetic cannabinoids was 0.5 ng/mL, and for the classic drugs was 1 ng/mL, except for morphine, codeine and BE (5 ng/mL).

Group of Drugs	# of positive	% of positive
Cannabis	30	42.9
Cocaine	52	74.3
Opiates	25	35.7
Benzodiazepines	25	35.7
Amphetamines	4	5.7
Synthetic cannabinoids	5	7.1

Table 6

Oral fluid samples (n=70) positive results (concentrations and number of samples) for THC, cocaine, BE, morphine, codeine, 6AM, alprazolam, clonazepam, diazepam, amphetamine, methamphetamine, MDA and synthetic cannabinoids (AB-FUBINACA). The limit of quantification (LOQ) for synthetic cannabinoids was 0.5 ng/mL and upper limit of quantification (ULOQ) was 100 ng/mL, and for the classic drugs the LOQ was 1 ng/mL, except for morphine, codeine and BE (5 ng/mL), and ULOQ was 200 ng/mL.

Analyte	Concentration range (ng/mL)	# of positive	% of positive
Morphine	4.8 – >200	22	31.4
Codeine	5.1 – 75.4	5	7.1
6AM	1 – >200	15	21.4
Amphetamine	2	1	1.4
Methamphetamine	1.3 – 10.6	2	2.9
MDA	1.4 – 3.4	2	2.9
BE	1.6 – 199.3	47	67.1
Cocaine	1 – >200	35	50
Alprazolam	1.4 – >200	20	28.6
Clonazepam	1 – 80.2	15	21.4
Diazepam	5– 14.7	3	4.3
THC	1.1 – >200	30	42.9
Synthetic cannabinoids (AB-FUBINACA)	0.8 – 38.9	5	7.1

Table 7

Matched urine-oral fluid samples results for cannabis, cocaine, opiates, benzodiazepines, amphetamines and synthetic cannabinoids. Total number of paired samples was 70. OF: oral fluid, UR: urine.

Group of Drugs	#OF+ & UR+	#OF+ & UR-	#OF- & UR+	#OF- & UR-	Agreement (%)
Cannabis	30	0	10	30	85.7
Cocaine	36	16	1	17	75.7
Opiates	18	7	6	39	81.4
Benzodiazepines	19	6	4	41	85.7
Amphetamines	0	4	0	66	94
Synthetic cannabinoids	1	4	3	62	90