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**Oral fluid analysis to monitor recent exposure to synthetic cannabinoids in a high-risk subpopulation**

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## Abstract

New psychoactive substances (NPS) represent a heterogeneous group of chemical substances. Among NPS, synthetic cannabinoids seem to have the widest diffusion in the population not limited to any particular demographic group. However, information on drug consumption relies mostly on anonymised surveys and less on clinical or analytical data. Huge efforts are constantly made to enrol subjects to gather epidemiological data on drug consumption, but it remains a big task for the perceived stigma of this anti-social behaviour. In the present study, we considered saliva samples from volunteers in a drug rehabilitation centre. Sixty-six samples have been analysed by LC-MS/MS to detect synthetic cannabinoids. While synthetic cannabinoids consumption had not been declared by any volunteer, analytical data highlighted the presence of synthetic cannabinoids at a positivity rate of almost 20%, with detection frequency HU211(5/13) > UR144/JWH122 (3/13) > JWH019/JWH081/AM2201 (1/13). Concentrations were in the range < LOQ - 0.36 ng/ml. This study enabled for the unprecedented detection of synthetic cannabinoids use in the territory of Parma (Italy) in a high-risk subpopulation. Public health consequences represented by NPS consumption is still scarce, therefore, further studies are needed to understand the real diffusion in the population.

## KEYWORDS

1  
2  
3 Forensic toxicology; synthetic cannabinoids; oral fluid; JWH122; JWH081;  
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5 novel psychoactive substances  
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## 9 **Highlights**

- 12 1. Saliva samples from drug-abusers were analysed for synthetic  
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14 cannabinoids  
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- 17 2. Six different synthetic cannabinoids at concentrations above LOD were  
18  
19 identified  
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- 22 3. Positivity rate of synthetic cannabinoids was 19.7%  
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- 25 4. intentional synthetic cannabinoids consumption was not declared by any  
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27 subject.  
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## 36 **1. Introduction**

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40 New psychoactive substances (NPS) are defined by The United Nations Office  
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42 for Drugs and Crime (UNODC) as “substances of abuse, either in a pure form  
43  
44 or a preparation, that are not controlled by the 1961 Single Convention on  
45  
46 Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which  
47  
48 may pose a public health threat” [1]. They represent a heterogeneous group of  
49  
50 chemical substances, which include synthetic cannabinoids. This class of  
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52 chemicals appeared for sale in European countries around 2005 before  
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54 becoming available in the United States in 2008. Since then, a total of 209 new  
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3 synthetic cannabinoids have been detected in Europe and beside cathinones,  
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5 they accounted for almost 60% of the number of seizures reported [2]. A 2020  
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7 survey by the National Institute on Drug Abuse (NIDA) reported the  
8  
9 consumption of synthetic cannabinoids as high as 2.5% in adolescents [3].  
10  
11 They are used in a variety of ways: sprayed onto plant material and smoked,  
12  
13 mixed into a liquid and vaped in electronic nicotine delivery devices (such as  
14  
15 e-cigarettes) or added to herbal tea or to food and swallowed. Clinical features  
16  
17 of synthetic cannabinoids poisoning vary and may include neurologic and  
18  
19 psychiatric signs as well as tachypnoea, tachycardia, hypertension, severe  
20  
21 nausea and vomiting, chest pain and heart attack, rhabdomyolysis, kidney  
22  
23 failure [4]. While synthetic cannabinoid use is not limited to any particular  
24  
25 demographic group, their use is similar to patterns seen for other drugs of  
26  
27 abuse. A lot of users are people in their 20s–30s, with men more likely than  
28  
29 women to use these substances [5]. However, large-scale information on their  
30  
31 consumption relies mostly on anonymised surveys and less on clinical or  
32  
33 analytical data. Huge efforts are constantly made to enrol subjects to gather  
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35 epidemiological data on drug consumption, but it remains a big task for the  
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37 perceived stigma of this anti-social behaviour. Inevitably, this may lead to an  
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39 underestimation of the problem [6].  
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54 Recently, epidemiological studies evaluating exposure to xenobiotics took the  
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56 advantages of non-conventional matrices such as oral fluid [7-9] or hair [10,11],  
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58 being more attractive by volunteers due to low invasiveness. On this frame,  
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3 oral fluid has become increasingly popular as alternative biological specimen  
4 for the detection of parent drugs and metabolites [12]. It proved useful to  
5  
6 demonstrate recent exposure when it came to driving issues [13,14] and  
7  
8 workplace drug testing [15,16]. Oral fluid was demonstrated to be a valuable  
9  
10 matrix also for detecting specifically NPS [17,18]. In particular, synthetic  
11  
12 cannabinoids have been detected in oral fluid samples of students [19], drivers  
13  
14 [20] and in administration-controlled studies [21]. Unfortunately, little is known  
15  
16 about the diffusion of this class of chemicals in high-risk subpopulations as, for  
17  
18 example, in drugs abusers. In fact, through this subpopulation, information on  
19  
20 the street market availability in specific areas could also be obtained.  
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23  
24 In the present study, we considered oral fluid samples from drugs abusers in a  
25  
26 rehabilitation centre analysed by LC–MS/MS [22]. The method was applied to  
27  
28 provide information on drug exposure referring to 11 synthetic cannabinoids  
29  
30 and mephedrone. Hereby, the detected drug types and relative concentrations  
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32 have been reported.  
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## 47 **2. Materials and methods**

### 48 **2.1. Reagents and standards**

49  
50 Water, acetonitrile, methanol and formic acid, all of LC-MS grade, were  
51  
52 obtained by Sigma Aldrich (Merck KGaA, Darmstadt, Germany). NPS such as  
53  
54 mephedrone, UR144 (1-Pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)  
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3 methanone), CP47497-C7 (2-[(1S,3R)-3-hydroxycyclohexyl]-5-(8-hydroxy-2-  
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5  
6 methyloctan-2-yl)phenol), CP47497-C8 (rel-5-(1,1-dimethyloctyl)-2-[(1R,3S)-  
7  
8  
9 3-hydroxycyclohexyl]-phenol), AM2201 ([1-(5-fluoropentyl)-1H-indol-3-yl]-1-  
10  
11 naphthalenyl-methanone), JWH019 ((1-hexyl-1H-indol-3-yl)-1-naphthalenyl-  
12  
13  
14 methanone), JWH081 ((4-methoxy-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-  
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16  
17 methanone), JWH122 ((4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-  
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19  
20 methanone), JWH250 (1-(1-pentyl-1H-indol-3-yl)-2-(2-methoxyphenyl)-  
21  
22 ethanone), JWH200 [1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]-1-naphthalenyl-  
23  
24  
25 methanone), HU211 (3-(1,1-dimethylheptyl)-6aS,7,10,10aS-tetrahydro-1-  
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27  
28 hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol) were kindly provided  
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30  
31 at 500 µg/mL in methanol by the National Health Institute and Comedical s.r.l.  
32  
33  
34 (Trento, Italy) within the national project News-Alert. Internal deuterated  
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36  
37 standards THC-D3 (1mg/mL) and mephedrone- D3 (1 mg/mL) were purchased  
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39  
40 by Cerilliant (Round Rock, Texas, USA) and were prepared as mixture at the  
41  
42  
43 final concentration of 10 µg/ml.

## 44 45 46 **2.2. Samples collection**

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49 Patients or subjects under treatment at the service for pathological  
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52 dependencies (SerDP) of Parma were enrolled as volunteers in this study.  
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55 Approval by the Ethical Committee was obtained. Volunteers were asked to  
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58 answer some short questions on their sociodemographic and anamnestic data,  
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61 as well as their abuse habits (Fig.1 in Supplemental material). Oral fluid

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3 samples were therefore collected from healthy volunteers (both male and  
4  
5 female) upon signature of the informed consent. Data were anonymised: the  
6  
7 oral fluid was collected in appropriate tubes without ID reference number to  
8  
9 avoid any possible identification or tracing back of the donor. Inclusion criteria  
10  
11 for this study were: 1) age above 18 years old; 2) alcohol and/or psychotropic  
12  
13 substances past addiction; 3) first access to treatment during 2017 or later.  
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15 Oral fluid samples were collected by spontaneous spitting of at least 2 ml. Sixty-  
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17 six oral fluid samples were collected and stored in a freezer at -20 °C until the  
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19 analysis.  
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### 31 **2.3. Samples and standards preparation**

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33 The standard mix solution was prepared by individual methanolic stock  
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35 solutions at a final concentration of 500 ng/ml and then appropriate  
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37 intermediate solutions were prepared in methanol by dilution.  
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40  
41 Pooled drug-free oral fluid samples from anonymised volunteers were used to  
42  
43 prepare calibration curve in the range LOQ-100 ng/ml. Blank samples were run  
44  
45 in between to check for any carry-over. Samples and calibrators were prepared  
46  
47 in the abovementioned blank matrix preventively checked free from any drug  
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49 or alcohol, collected from healthy volunteers by spiking 50 ng of Internal  
50  
51 Standard mix to 100 µl of oral fluid and then added of 200 µl of methanol.  
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57 Samples were centrifuged at 10000 rpm for 5 minutes for protein removal and  
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59 100 µl of supernatant was transferred to the LC vial.  
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## 2.4. Analytical method

Analyses were carried out by LC-MS/MS composed of an Agilent 1100 (Agilent Technologies, Santa Clara, CA, USA) coupled to API4000 MS/MS (SCIEX, Framingham, MA USA) equipped with a Turbo Ion Spray interface for pneumatically assisted electrospray. Separations were performed by using a Pursuit XRs Ultra 100 × 2.0 mm, 3 μm column with mobile phase A consisting of water and mobile phase B consisting of methanol/acetonitrile, 95/5 (v/v), both added of 10 Mm formic acid. The optimized gradient was as follow: 15% B, hold for 2 min; from 15 to 80% B in 1.5 min, hold for 1 min; from 80 to 100% B in 1 min; 100% B, hold for 5.5 min; then back to the starting condition in 0.5 min. The flow rate and injection volume were set at 0.2 ml/min and 2 μl, respectively. Mass spectrometric acquisition was in positive ion mode with acquisition parameters (declustering potential and collision energies) optimized for each compound by individual direct infusion to the MS. Acquisition was in Selected Reaction Monitoring (SRM) by acquiring two transitions for each compound. Limit of detection (LOD) and limit of quantification (LOQ) for NPS were in the range 0.001- 0.83 ng/ml and 0.003- 2.7 ng/ml, respectively [22].

## 3. Results

### 3.1. Questionnaire results

Data obtained by the survey are summarized as follow. Male represented the majority (n=53, 80.3%) of the subjects, and their average age was 41 years old with first access to therapies at 27 years old. Italians accounted for 87.9% (n=58). Unemployment rate was 62.1% of the subjects. Education level was generally poor with almost half subjects (47.0%) limited at the compulsory school, while 42.0% had diploma.

Opioids abuse accounted for 69.7%, followed by cannabinoids (46.0%) and to a lesser extent to stimulants and alcohol users. Methadone and benzodiazepines were the most frequent associations for opioid abuse treatment. Synthetic cannabinoids use was not declared by any subject.

Statistical details of the questionnaire results are not of interest to this study and therefore they will not be analysed and discussed.

### 3.2. Toxicological results

Sixty-six saliva samples from volunteers were collected and analysed for new psychoactive substances of the synthetic cannabinoid type and mephedrone.

Results are presented in table 1. Thirteen out of 66 samples (19.7%) were found positive to six different synthetic cannabinoids namely JWH019, JWH081, JWH122, AM2201, HU211 and UR144 at concentrations above LOD.

One sample showed the simultaneous presence of JWH081 and JWH122 at concentrations of 0.29 ng/ml and 0.36 ng/ml, respectively. Detection frequency

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3 was HU211(5/13) > UR144/JWH122 (3/13) > JWH019/JWH081/AM2201  
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6 (1/13).  
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#### 10 11 **4. Discussion**

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15 Hundreds of NPS have emerged in the drug market over the last decade, but  
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17 prevalence and use are still mostly unknown. The aim of this study was to  
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19 determine synthetic cannabinoids in oral fluid samples to monitor drug diffusion  
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21 in high-risk subjects. Oral fluid was selected over urine as non-invasive matrix  
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23 since parent drug represents the analytical target, which helps in method  
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25 development. Moreover, oral fluid collection is perceived by the volunteers as  
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27 faster and less affected by privacy issues.  
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34 A total of 66 subjects participated to the study, which represents the first  
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36 preliminary attempt to include a high-risk subpopulation in the monitoring of  
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38 NPS in the Italian scenario. An anonymised survey was initially provided to ask  
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40 people for history and abused substances. The survey results revealed a  
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42 relatively young population with first access to addiction centre in their late 20s,  
43  
44 mostly males with low income and education. Opioids and cannabinoids were  
45  
46 declared as abused substances, but no information on synthetic cannabinoids  
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48 use was obtained. Differently from the survey, analytical data highlighted the  
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50 presence of synthetic cannabinoids in the analysed oral fluids at a positivity  
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52 rate of 19.7%. In previously published studies the detected positivity rate for  
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3 NPS in oral fluid was around 7-8% in non-high-risk populations [14,20] (i.e.,  
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6 drivers) reaching up to 39% in case of high-risk classes [23].  
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9 In our study the detected concentrations were mostly below LOQ (qualitative  
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11 data) or at very low concentrations for JWH081 and JWH122, confirming that  
12  
13 analytical methods must be very sensitive to detect the use of synthetic  
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15 cannabinoids in this specimen. In literature, pharmacokinetic studies on saliva  
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17 detected parent compounds maximum concentrations in the order of 2.2-2036  
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19 ng/ml reached within few minutes (5- 20 minutes) after smoking [21,24] and  
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21 remaining detectable up to some hours (6-12 hours) at concentration below 0.2  
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23 ng/ml. It is also known that contamination of the oral cavity may lead to  
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25 extremely high concentrations of the drug at the end of inhalation followed by  
26  
27 a steep decline during the next hours. Therefore, concentrations measured in  
28  
29 our study are probably representative of a detection time from few hours up to  
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31 12 hours since inhalation. Finally, the fact that synthetic cannabinoids  
32  
33 consumption was not declared by any volunteer may also suggest that  
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35 exposure might have been unintentional or unknown. In fact, synthetic  
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37 cannabinoids could also be used as adulterant in common cannabis, whose  
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39 consumption was instead declared by 46.0% of the subjects.  
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51 A major limitation of our study remains the impossibility to comment on the  
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53 metabolic profiles of the detected compounds due to the unavailability of  
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55 reference material for detecting metabolites and information on the time of  
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57 consumption of the drugs.  
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## 5. Conclusions

In the present study, 66 saliva samples from patients under treatment by the addiction center SerDP of Parma have been analysed to detect synthetic cannabinoids exposure. Analytical data highlighted the presence of this class of substances in the analysed oral fluids at a positivity rate of almost 20%, with detection frequency HU211(5/13) > UR144/JWH122 (3/13) > JWH019/JWH081/AM2201 (1/13). Concentrations were in the range below LOQ - 0.36 ng/ml. One sample showed the simultaneous presence of JWH081 and JWH122. Synthetic cannabinoids consumption was not declared by any volunteer, this could be due either to a persistent resistance in referring this kind of abuse, or to an unawareness of adulterants in common drugs such as NPS. Synthetic cannabinoids use has been detected for the first time in the territory of Parma (Italy) in a high-risk subpopulation. It is the intention of the authors to continue the study expanding it to a larger number of subjects.

## References

1. United Nations Office on Drugs and Crime (UNODC). Global smart update-2016. <https://www.unodc.org/documents/scientific/Global-SMART-Update-2016-vol-16.pdf>. Accessed February 27, 2022.
2. European Monitoring Centre for Drugs and Drug Addiction (EMCCDA). European Drug Report 2021: Trends and Developments-2021. [https://www.emcdda.europa.eu/publications/edr/trends-developments/2021\\_en](https://www.emcdda.europa.eu/publications/edr/trends-developments/2021_en). Accessed February 27, 2022.
3. National Institute on Drug Abuse (NIDA). Synthetic Cannabinoids (K2/Spice)-2020. <https://www.drugabuse.gov/drug-topics/synthetic-cannabinoids-k2spice/synthetic-cannabinoids-k2spice-trends-statistics>. Accessed February 27, 2022.
4. Presley BC, Gurney SM, Scott KS, Kacinko SL, Logan BK. Metabolism and toxicological analysis of synthetic cannabinoids in biological fluids and tissues. *Forensic Sci Rev* 2016;28(2):103-169.
5. Centers for Disease Control and Prevention. Synthetic Cannabinoids: an overview for healthcare providers-2018. <https://www.cdc.gov/nceh/hsb/chemicals/sc/healthcare.html>. Accessed February 27, 2022.
6. Miller P, Curtis A, Jenkinson R, Droste N, Bowe SJ, Pennay A. Drug use in Australian nightlife settings: estimation of prevalence and validity

- 1  
2  
3 of self-report. *Addiction* 2015;110(11):1803-1810. doi:  
4  
5  
6 10.1111/add.13060.  
7  
8  
9 7. da Cunha KF, Oliveira KD, Huestis MA, Costa JL. Screening of 104  
10  
11 new psychoactive substances (NPS) and other drugs of abuse in oral  
12  
13 fluid by LC-MS-MS. *J Anal Toxicol* 2020;44(7):697-707. doi:  
14  
15  
16 10.1093/jat/bkaa089.  
17  
18  
19 8. Martinez L, La Maida N, Papaseit E, Perez-Manà C, Poyatos L,  
20  
21 Pellegrini M, Pichini S, Ventura M, Galindo L, Busardò FP, Farrè M.  
22  
23 Acute pharmacological effects and oral fluid concentrations of the  
24  
25 synthetic cannabinoids JWH-122 and JWH-210 in humans after self-  
26  
27 administration: an observational study. *Front Pharmacol* 2021;12:  
28  
29 705643. doi: 10.3389/fphar.2021.705643. eCollection 2021.  
30  
31  
32  
33  
34  
35 9. Mulet CT, Tarifa A, De Caprio AP. Comprehensive analysis of synthetic  
36  
37 cannabinoids and metabolites in oral fluid by online solid-phase  
38  
39 extraction coupled to liquid chromatography-triple quadrupole-mass  
40  
41 spectrometry. *Anal Bioanal Chem* 2020;412(28):7937-7953. doi:  
42  
43  
44 10.1007/s00216-020-02926-9.  
45  
46  
47  
48  
49 10. Salomone A, Palamar JJ, Gerace E, Di Corcia D, Vincenti M. Hair  
50  
51 Testing for Drugs of Abuse and New Psychoactive Substances in a  
52  
53 High-Risk Population. *J Anal Toxicol* 2017;41(5):376-381. doi:  
54  
55  
56 10.1093/jat/bkx020.  
57  
58  
59  
60

- 1  
2  
3 11. Leung KW, Wong ZCF, Ho JYM, Yip AWS, Cheung JKH, Ho KKL,  
4  
5 Duan R, Tsim KWK. Surveillance of drug abuse in Hong Kong by hair  
6  
7 analysis using LC-MS/MS. *Drug Test Anal* 2018;10(6):977-983. doi:  
8  
9 10.1002/dta.2345.  
10  
11
- 12 12. Wille SMR, Eliaerts J, Di Fazio V, Samyn N. Challenges  
13  
14 concerning new psychoactive substance detection in oral fluid.  
15  
16 *Toxicologie Analytique et Clinique* 2017; 29(1): 11-17. doi:  
17  
18 10.1016/j.toxac.2016.12.004.  
19  
20
- 21 13. Busardò FP, Pichini S, Pellegrini M, Montana A, Lo Faro AF,  
22  
23 Zaami S, Graziano S. Correlation between blood and oral fluid  
24  
25 psychoactive drug concentrations and cognitive impairment in driving  
26  
27 under the influence of drugs. *Current Neuropharmacology* 2018;  
28  
29 16(1):84-96. doi: 10.2174/1570159X15666170828162057.  
30  
31
- 32 14. Richeval C, Dumestre-Toulet V, Wiart JF, Vanhoye X, Humbert L,  
33  
34 Nachon-Phanithavong M, Allorge D, Gaulier JM. New psychoactive  
35  
36 substances in oral fluid of drivers around a music festival in south-west  
37  
38 France in 2017. *Forensic Sci Int* 2019; 297:265-269. doi:  
39  
40 10.1016/j.forsciint.2019.02.029.  
41  
42
- 43 15. Department of Health and Human Services. Substance Abuse and  
44  
45 Mental Health Services Administration. Mandatory Guidelines for  
46  
47 Federal Workplace Drug Testing Programs-2008.  
48  
49  
50  
51  
52  
53  
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1  
2  
3 <http://www.gpo.gov/fdsys/pkg/FR-2008-11-25/pdf/E8-26726.pdf>

4  
5  
6 (accessed February 24,2022).

- 7  
8  
9 16. Cooper G, Moore C, George C, Pichini S. Guidelines for  
10  
11 European workplace drug testing in oral fluid. *Drug Test Anal*  
12  
13 2011;3(5):269-276. doi: 10.1002/dta.284.
- 14  
15  
16 17. Andresen Bergström M, Lövgren H, Abrahamsson A, Eriksson  
17  
18 EK, Lindbjer Andersson M, Komorowska M, Axelsson MAB. Rethinking  
19  
20 Drug Analysis in Healthcare: High-Throughput Analysis of 71 Drugs of  
21  
22 Abuse in Oral Fluid using Ion Mobility - High Resolution Mass  
23  
24 Spectrometry. *J Anal Toxicol* 2021. doi: 10.1093/jat/bkab114.
- 25  
26  
27 18. Leere Øiestad E, Leere Øiestad AM, Gjelstad A, Karinen R. Oral  
28  
29 fluid drug analysis in the age of new psychoactive substances.  
30  
31 *Bioanalysis* 2016;8(7):691-710. doi: 10.4155/bio-2015-0027.
- 32  
33  
34 19. Anzillotti L, Marezza F, Calò L, Andreoli R, Agazzi S, Bianchi F,  
35  
36 Careri M, Cecchi R. Determination of synthetic and natural  
37  
38 cannabinoids in oral fluid by solid-phase microextraction coupled to gas  
39  
40 chromatography/mass spectrometry: A pilot study. *Talanta*  
41  
42 2019;201:335-341. doi: 10.1016/j.talanta.2019.04.029.
- 43  
44  
45 20. Richeval C, Wille SMR, Nachon-Phanithavong M, Samyn N,  
46  
47 Allorge D, Gaulier JM. New psychoactive substances in oral fluid of  
48  
49 French and Belgian drivers in 2016. *Int J Drug Policy* 2018; 57:1-3. doi:  
50  
51 10.1016/j.drugpo.2018.03.013.
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21. Toennes SW, Geraths A, Pogoda W, Paulke A, Wunder C, Theunissen EL, Ramaekers JG. Pharmacokinetic properties of the synthetic cannabinoid JWH-018 in oral fluid after inhalation. *Drug Test Anal* 2018;10(4):644-650. doi: 10.1002/dta.2310.
22. Calò L, Anzillotti L, Maccari C, Cecchi R, Andreoli R. Validation of a Bioanalytical Method for the Determination of Synthetic and Natural Cannabinoids (New Psychoactive Substances) in Oral Fluid Samples by Means of HPLC-MS/MS. *Front Chem* 2020;8:439. doi: 10.3389/fchem.2020.00439.
23. da Cunha KF, Oliveira KD, Cardoso MS, Arantes ACF, Coser PHP, Lima LN, Maluf ACS, Comis MAC, Huestis MA, Costa JL. Prevalence of new psychoactive substances (NPS) in Brazil based on oral fluid analysis of samples collected at electronic music festivals and parties. *Drug Alcohol Depend* 2021; 227:108962. doi: 10.1016/j.drugalcdep.2021.108962.
24. La Maida N, Pellegrini M, Papaseit E, Pérez-Mañá C, Poyatos L, Ventura M, Galindo L, Busardò FP, Pichini S, Farré M, Marchei E. Determination of the Synthetic Cannabinoids JWH-122, JWH-210, UR-144 in Oral Fluid of Consumers by GC-MS and Quantification of Parent Compounds and Metabolites by UHPLC-MS/MS. *Int J Mol Sci* 2020;21(24):9414. doi: 10.3390/ijms21249414.

TABLE 1.

Substance	LOD (ng/ml)	LOQ (ng/ml)	Results > LOD (n.)	Results > LOQ (n.)	Concentration (ng/ml)
Mephedrone	0.025	0.085	-	-	-
JWH019	0.002	0.005	1	-	-
JWH081	0.001	0.003	1	1	0.29*
JWH122	0.001	0.004	3	1	0.36*
JWH200	0.056	0.185	-	-	-
JWH250	0.378	1.260	-	-	-
AM2201	0.002	0.006	1	-	-
HU211	0.076	0.255	5	-	-
UR144	0.002	0.005	3	-	-
CP47497-C7	0.830	2.700	-	-	-
CP47497-C8	0.600	1.970	-	-	-

**Table 1.** Results of 66 saliva samples analysed for synthetic cannabinoids. Limit of detection (LOD) and limit of quantification (LOQ) are also indicated for each compound.

\*from the same subject

FIGURE 1

Date					
ID number					
Gender	M	F	ND		
Age					
Nationality					
Education	SS	D	UD	HE	N
Job	Unemployed	Fixed-term job	Permanent job		
Penalties	yes		no		
Current pharmaceutical therapy					
Age of first access to the addiction centre					
Abused substances at the entrance					
Current abused substances/new psychoactive substances					
Frequency of abuse					
Alcohol abuse	yes		no		
Note					

Fig. 1: Questionnaire overview sheet. SS: secondary school; D: Diploma; UD: University Degree; HE: higher education; N: none