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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 unprecedent 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

Forensic toxicology; synthetic cannabinoids; oral fluid; JWH122; JWH081; novel psychoactive substances

Highlights

1. Saliva samples from drug-abusers were analysed for synthetic cannabinoids
2. Six different synthetic cannabinoids at concentrations above LOD were identified
3. Positivity rate of synthetic cannabinoids was 19.7%
4. intentional synthetic cannabinoids consumption was not declared by any subject.

1. Introduction

New psychoactive substances (NPS) are defined by The United Nations Office for Drugs and Crime (UNODC) as “substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat” [1]. They represent a heterogeneous group of chemical substances, which include synthetic cannabinoids. This class of chemicals appeared for sale in European countries around 2005 before becoming available in the United States in 2008. Since then, a total of 209 new

synthetic cannabinoids have been detected in Europe and beside cathinones, they accounted for almost 60% of the number of seizures reported [2]. A 2020 survey by the National Institute on Drug Abuse (NIDA) reported the consumption of synthetic cannabinoids as high as 2.5% in adolescents [3]. They are used in a variety of ways: sprayed onto plant material and smoked, mixed into a liquid and vaped in electronic nicotine delivery devices (such as e-cigarettes) or added to herbal tea or to food and swallowed. Clinical features of synthetic cannabinoids poisoning vary and may include neurologic and psychiatric signs as well as tachypnoea, tachycardia, hypertension, severe nausea and vomiting, chest pain and heart attack, rhabdomyolysis, kidney failure [4]. While synthetic cannabinoid use is not limited to any particular demographic group, their use is similar to patterns seen for other drugs of abuse. A lot of users are people in their 20s–30s, with men more likely than women to use these substances [5]. However, large-scale information on their 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. Inevitably, this may lead to an underestimation of the problem [6].

Recently, epidemiological studies evaluating exposure to xenobiotics took the advantages of non-conventional matrices such as oral fluid [7-9] or hair [10,11], being more attractive by volunteers due to low invasiveness. On this frame,

oral fluid has become increasingly popular as alternative biological specimen for the detection of parent drugs and metabolites [12]. It proved useful to demonstrate recent exposure when it came to driving issues [13,14] and workplace drug testing [15,16]. Oral fluid was demonstrated to be a valuable matrix also for detecting specifically NPS [17,18]. In particular, synthetic cannabinoids have been detected in oral fluid samples of students [19], drivers [20] and in administration-controlled studies [21]. Unfortunately, little is known about the diffusion of this class of chemicals in high-risk subpopulations as, for example, in drugs abusers. In fact, through this subpopulation, information on the street market availability in specific areas could also be obtained.

In the present study, we considered oral fluid samples from drugs abusers in a rehabilitation centre analysed by LC–MS/MS [22]. The method was applied to provide information on drug exposure referring to 11 synthetic cannabinoids and mephedrone. Hereby, the detected drug types and relative concentrations have been reported.

2. Materials and methods

2.1. Reagents and standards

Water, acetonitrile, methanol and formic acid, all of LC-MS grade, were obtained by Sigma Aldrich (Merck KGaA, Darmstadt, Germany). NPS such as mephedrone, UR144 (1-Pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)

methanone), CP47497-C7 (2-[(1S,3R)-3-hydroxycyclohexyl]-5-(8-hydroxy-2-methyloctan-2-yl)phenol), CP47497-C8 (rel-5-(1,1-dimethyloctyl)-2-[(1R,3S)-3-hydroxycyclohexyl]-phenol), AM2201 ([1-(5-fluoropentyl)-1H-indol-3-yl]-1-naphthalenyl-methanone), JWH019 ((1-hexyl-1H-indol-3-yl)-1-naphthalenyl-methanone), JWH081 ((4-methoxy-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone), JWH122 ((4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone), JWH250 (1-(1-pentyl-1H-indol-3-yl)-2-(2-methoxyphenyl)-ethanone), JWH200 [1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]-1-naphthalenyl-methanone), HU211 (3-(1,1-dimethylheptyl)-6aS,7,10,10aS-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol) were kindly provided at 500 µg/mL in methanol by the National Health Institute and Comedical s.r.l. (Trento, Italy) within the national project News-Alert. Internal deuterated standards THC-D3 (1mg/mL) and mephedrone- D3 (1 mg/mL) were purchased by Cerilliant (Round Rock, Texas, USA) and were prepared as mixture at the final concentration of 10 µg/ml.

2.2. Samples collection

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

samples were therefore collected from healthy volunteers (both male and female) upon signature of the informed consent. Data were anonymised: the oral fluid was collected in appropriate tubes without ID reference number to avoid any possible identification or tracing back of the donor. Inclusion criteria for this study were: 1) age above 18 years old; 2) alcohol and/or psychotropic substances past addiction; 3) first access to treatment during 2017 or later.

Oral fluid samples were collected by spontaneous spitting of at least 2 ml. Sixty-six oral fluid samples were collected and stored in a freezer at -20 °C until the analysis.

2.3. Samples and standards preparation

The standard mix solution was prepared by individual methanolic stock solutions at a final concentration of 500 ng/ml and then appropriate intermediate solutions were prepared in methanol by dilution.

Pooled drug-free oral fluid samples from anonymised volunteers were used to prepare calibration curve in the range LOQ-100 ng/ml. Blank samples were run in between to check for any carry-over. Samples and calibrators were prepared in the abovementioned blank matrix preventively checked free from any drug or alcohol, collected from healthy volunteers by spiking 50 ng of Internal Standard mix to 100 µl of oral fluid and then added of 200 µl of methanol.

Samples were centrifuged at 10000 rpm for 5 minutes for protein removal and 100 µl of supernatant was transferred to the LC vial.

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

was HU211(5/13) > UR144/JWH122 (3/13) > JWH019/JWH081/AM2201 (1/13).

4. Discussion

Hundreds of NPS have emerged in the drug market over the last decade, but prevalence and use are still mostly unknown. The aim of this study was to determine synthetic cannabinoids in oral fluid samples to monitor drug diffusion in high-risk subjects. Oral fluid was selected over urine as non-invasive matrix since parent drug represents the analytical target, which helps in method development. Moreover, oral fluid collection is perceived by the volunteers as faster and less affected by privacy issues.

A total of 66 subjects participated to the study, which represents the first preliminary attempt to include a high-risk subpopulation in the monitoring of NPS in the Italian scenario. An anonymised survey was initially provided to ask people for history and abused substances. The survey results revealed a relatively young population with first access to addiction centre in their late 20s, mostly males with low income and education. Opioids and cannabinoids were declared as abused substances, but no information on synthetic cannabinoids use was obtained. Differently from the survey, analytical data highlighted the presence of synthetic cannabinoids in the analysed oral fluids at a positivity rate of 19.7%. In previously published studies the detected positivity rate for

NPS in oral fluid was around 7-8% in non-high-risk populations [14,20] (i.e., drivers) reaching up to 39% in case of high-risk classes [23].

In our study the detected concentrations were mostly below LOQ (qualitative data) or at very low concentrations for JWH081 and JWH122, confirming that analytical methods must be very sensitive to detect the use of synthetic cannabinoids in this specimen. In literature, pharmacokinetic studies on saliva detected parent compounds maximum concentrations in the order of 2.2-2036 ng/ml reached within few minutes (5- 20 minutes) after smoking [21,24] and remaining detectable up to some hours (6-12 hours) at concentration below 0.2 ng/ml. It is also known that contamination of the oral cavity may lead to extremely high concentrations of the drug at the end of inhalation followed by a steep decline during the next hours. Therefore, concentrations measured in our study are probably representative of a detection time from few hours up to 12 hours since inhalation. Finally, the fact that synthetic cannabinoids consumption was not declared by any volunteer may also suggest that exposure might have been unintentional or unknown. In fact, synthetic cannabinoids could also be used as adulterant in common cannabis, whose consumption was instead declared by 46.0% of the subjects.

A major limitation of our study remains the impossibility to comment on the metabolic profiles of the detected compounds due to the unavailability of reference material for detecting metabolites and information on the time of consumption of the drugs.

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.

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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