Use of synthetic cannabinoids among minors in juvenile offenders' centres

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**Abstract**

**Background:** Synthetic cannabinoids (SC) are difficult to detect in urine samples, and therefore an appropriate strategy is required to investigate its potential consumption. In this work, we have designed a study to investigate SCs consumption by minors in juvenile offenders' centres.

***Methods*:** 127 minors were selected from five juvenile offenders' centres in the Valencian Autonomous Community (Spain). 667 urine samples were collected after their therapeutic permits with stay at home. We studied the active molecules from 7 herbal blends available at the smartshop frequented by minors. Both, the herbal blends and urine samples were analysed by liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry.

***Results*:** Regarding cannabis consumption, 40.2% (N=51) of the subjects reported using organic or synthetic cannabis: 5.9% of them reported only to consume synthetic cannabis, 37.2% synthetic and organic cannabis and 56.9% only organic cannabis. The urine samples analysis revealed the absence of the parent SCs investigated, but the presence of the main metabolites from two SCs (XLR-11, UR-144): N-pentanoic acid and N-(5-hydroxypentyl). The 16 individual urine samples positives to the XLR-11 metabolites could be assigned to 6 minors, 2 of which recognize consumption whereas the remaining 4 adolescents did not recognize consumption of SCs.

***Conclusions*:** Synthetic cannabinoids, specifically, XLR-11, are consumed in juvenile offenders' centres in the Autonomous Valencian Community. Preventive and therapeutic interventions in minors housed in those centres must be implemented to reduce the

consumption of new psychoactive substances and to improve the risk-perception of these substances.

Keywords: juvenile offenders' centres; synthetic cannabinoids; drug analysis; XLR-11; UR-144.

## 1. Introduction

The consumption of different types of cannabis has gradually increased in Spain, as in most other countries, over the last decade. In Europe, cannabis is consumed by up to 23% of the population, but this figure reaches 30.4% in Spain and accounts for 29.9% of related admissions for treatment (European Monitoring Centre for Drugs and Drug Addiction, 2017). This consumption has legal, social, and health consequences for patients. The legal consequences vary depending on the judicial system: cannabis use is legalised in some areas with no repercussions, while in others its use can have civil or even criminal repercussions. In this last context, the legal status of new synthetic cannabinoids (SCs) is important. Although these substances are consumed for leisure or recreation, they are also sometimes used, among other reasons, to simulate cannabis abstinence because of administrative sanctions, in residential treatments for substance-related disorders, as a legal alternative to incarceration, or to comply with regulations in certain jobs (Bonar et al., 2014; Williams et al., 2014). In addition to the low perception of risk (Clayton et al., 2017; Haro et al., 2014, Shanks et al., 2015) and high availability and low cost of these substances, the detection of SCs and their metabolites in humans is difficult, due to the high number of existing compounds and the variability in the chemical structures. All these aspects surely contribute to the increased use of SCs.

According to the European Monitoring Centre for Drugs and Drug Addiction, more than 160 SCs have been identified in herbal blends or spices since 2008. These herb mixtures are sold as ‘legal’ cannabis substitutes and are readily available in smartshops and via the internet. According to EMCDDA, 80,000 products containing new psychoactive substances (NPS) were seized in Europe in 2015 and SCs accounted for 29% of these.

Moreover, many novel SCs are now being identified in herbal mixtures every year, illustrating the ongoing appearance of new substances (Jia et al., 2017; Liu et al., 2017; Moore et al., 2017; Quian et al., 2017; Risseeuw et al., 2017). High-resolution mass spectrometry (HRMS) is useful for identifying SCs in herbal blends (Ibañez et al., 2013; Ibañez et al., 2014) but standardized analytical strategies are required for identification of SCs in human samples, especially in urine samples, to advance in the knowledge of this topic. This would first require metabolism studies to identify the unaltered active molecules in urine, and to elucidate their potential metabolites; in other words, to establish appropriate urinary consumption-markers for SCs. Several studies have shown that it is near impossible to detect unaltered SCs in urine samples.(Castaneto et al., 2015; Diao et a.l, 2016; Diao et al., 2017; Holm et al., 2015; Kevin et al., 2017; Scheidweiler et al., 2015; Wohlfarth et al., 2015; Thus, urine analysis of potential SC consumers should focus on searching for the major metabolites of these drugs (Diao and Huestis, 2017), using powerful techniques, such as ultra-high performance liquid chromatography (UHPLC) coupled to HRMS (Labutin, and Temerdashev, 2015; Scheidweiler et al., 2015; Zaitsu et al., 2015) or to tandem mass spectrometry (MS/MS) (Berg et al., 2016; Jang et al., 2015; Minakata, 2016).

The recent interest in cannabis consumption by healthcare professionals (Smith and Roberts, 2014) has focused, in part, on the prevention of its use by minors. Thus, in adolescents aged between 12 and 20 years, the prevalence of consumption at least once in life and its use during the previous month is 18,3% in Spain, 8% in Europe and 20% in USA; the prevalence of synthetic cannabis use is lower, with consumption percentages of 0,9% at some time in life and 0,7% during the last month in Spanish minors, compared to 5,2% in USA, 1,5% in Letonia or 0,1% in Finland (Curtis et al., 2015; European

Monitoring Centre for Drugs and Drug Addiction, 2017; Jhonson et al., 2015; Ministerio de Sanidad Servicios Sociales e Igualdad, 2018; Ralphs et al., 2017). The choice of treatment (and evaluation of treatment effectiveness) in this group of minors is complex (Hurst et al., 2011; Tait et al., 2016) and becomes even more so when considering SCs, with the previous consumption rates (Castellanos et al., 2011; Substance Abuse and Mental Health Services Administration, 2013). When minors are sent to detention centres, they become more psychopathologically, socially, and sexually vulnerable, and even risk increasing their polydrug use (Stathis et al., 2006; Young et al., 2007). This regime of deprivation of liberty is also measurable in terms of SC consumption (Johnson et al., 2011; Ralphs et al., 2017). However, the prevalence of SC use in juvenile detention centres is commonly unknown, and data about its consumption is obtained only via self-reports (Young et al., 2007), a standard procedure in the early stages of substance-use detection (Baquero et al., 2016). Given the suspected extensive use of SCs in juvenile detention centres and the difficulties associated to their detection, in this work we present our strategy to detect the presence of SC metabolites in urine samples using HRMS, including the previous analysis of the suspect herbal blends consumed to identify the SCs more frequently used. We also propose preventive interventions and treatment procedures for the possible consequences of NPS use in juvenile offenders' centres.

## 2. Methods

*Survey, clinical evaluation, and urine sample collection*

Consecutive sampling, carried out between May and October 2016, was used to select 127 subjects of either sex, aged less than 18 years and linked to one of five juvenile offenders' centres in the Valencian Autonomous Community (Spain). An inclusion criterion was that they had therapeutic permits with stay at home, which entails providing a urine sample for toxicological evaluation upon their return to the centre. Socio-demographic and health information (age, sex, the family support received, marital status, employment status, academic level, and criminal record) was collected through an ad-hoc questionnaire produced for this study. We also collected the following data about drug-use: drugs taken, route of administration, length of time suffering from substance-related disorder and its treatment. Variables associated with mental disorders and physical-health conditions such as HIV, or hepatitis B or C (HBV/HCV) infection were also recorded. The subjects participated a group interview in which they were asked about their use of NPS; if they mentioned their own consumption, we asked them about the commercial name of the NPS and how they acquired it. A total of 667 urine samples were collected from five different juvenile offenders' centres. Individual urine samples were kept at -18 ºC after collection.

*Synthetic cannabinoid detection*

The high number of urine samples received made individual analysis of each sample impracticable. Thus, to facilitate sample analysis, we prepared pooled urine samples, avoiding combining samples from different centres. Unfortunately, this process dilutes the samples (in this study, by a factor of 6–10) as a function of the number of samples the pool contains. Finally, 83 pooled urine samples were analysed. Although consumption of SCs should be monitored in urine by detecting their major metabolites (Diao et al., 2017), the

parent compound of some NPS can occasionally be detected in urine (Ibañez et al., 2016; Pozo et al., 2014); however, this was not the case in this study.

Our database included more than 200 SCs and therefore it seemed impractical to search for all their metabolic pathways. Moreover, the metabolites of the newest NPS have not yet been reported yet. Therefore, we devised an alternative strategy of searching for the specific herbal blends referred to by the minors participating in this study. During the group interviews, 59 (46.6%) of the participants mentioned consuming a SC marketed as *Hardcore* during therapy periods to avoid being caught by conventional urine analyses. They described acquiring this product in a smartshop close to the centres involved in this study; we purchased all seven herbal blends available in the smartshop (*Oro Fantastico*, *Mazazo*, *Sonrisa Absoluta, Placaje, Sonrisa*, *Hardcore,* and *Tio Tieso)* via its webpage (Fig. 1: shows the packaging of these products).

Active molecules present in these herbal blends were identified by HRMS, as described in literature (Ibañez el al., 2014). Briefly, 0.1 g of herbal blend was extracted with 2 mL of acetone in a 2 mL propylene Eppendorf tube and introduced in an ultrasonic bath for 15 min. After centrifugation at 12000 rpm for 10 min, the supernatant was diluted 1:1000 with ultrapure water. If a precipitate remained in suspension after this process, the sample was centrifuged again under the same conditions. Finally, the extract was transferred to a glass vial and 20 µL were injected into the UHPLC-HRMS system for analysis.

To detect the metabolites reported in the literature for the SCs present in the herbal blend samples, the pooled urine samples were processed using an enzymatic hydrolysis

procedure adapted from the literature (Matabosch et al., 2015; Matabosch et al., 2015), thus releasing the unconjugated compound. This process has been shown to be effective for cleaving NPS-derived glucuronides found in mice urine samples (Fabregat et al., 2017). Briefly, 1 mL of pooled urine was buffered with 0.4 mL of phosphate buffer; 16 µL of β-glucuronidase from E. coli strain K12 was added and the sample was incubated for 1 hour at 55±2°C. Samples were then frozen at -18°C for at least 3 hours, thawed, and centrifuged at 12000 rpm for 15 min to remove any lipids and proteins. Finally, the supernatant was transferred to a glass vial and 20 µL were injected into the UHPLC-HRMS system for analysis.

The herbal blends and urine samples were analysed using an ACQUITY UPLC ultra-high performance liquid chromatography (UHPLC) system (Waters, Mildford, MA, USA) coupled to a XEVO G2 QTOF hybrid quadrupole time-of-flight (QTOF) mass spectrometer (Waters Micromass, Manchester, UK) with an orthogonal Z-spray electrospray ionization (ESI) interface operating in positive ionization mode. Further details about the instrumentation can be found in literature (Fabregat et al., 2017).

*Ethical consideration*

This study was approved by the Research and Ethics Committee at the Consorcio Hospitalario Provincial (Castellon) on 26 September, 2014 (ref. 20141113), by the Conselleria de Benestar Social at the Generalitat Valenciana on 1 June, 2015 (registration 3 June, 2015, ref. 32833), and the Office of the Children's Justice Prosecutor, following the principles and requirements established in the Declaration of Helsinki and the European

Council Convention for research on humans. The confidentiality of the participants and their data was guaranteed according to Organic Law 15/1999 on the Protection of Personal Data, and the subjects and their legal guardians signed their informed consent to their participation in the study.

## 3. Results

*Socio-demographic, health, and new psychoactive substance consumption data*

The average participant age was 16.2 years (*SD* = 1.27), and 84.2% were male; 82.2% were Spanish, 11.8% South American, 4% African, and 2% from other European Union countries. Concerning marital status, 82.4% were single, 13.7% married or equivalent, and 3.9% divorced or separated; 1.77% were parents. Regarding their academic level, 33.3% had a primary school certificate, 12.8% a secondary school certificate or basic vocational studies, 41% had not completed primary school, and 12.8% were uneducated; 3.9% had worked in the past. The average of times sent to juvenile offenders' centres was 1.61 (*SD* = 1.35) with an average length of stay of 14.78 months (*SD* = 9.22); during the time they had spent in juvenile offenders' centres, 3.9% of them were not in contact with their family.

None of the participants reported any physical health problem but 2% of them had had a diagnosis of attention deficit and hyperactivity disorder. Regarding cannabis consumption, 40.2% (N = 51) of the minors reported using organic or synthetic cannabis; 5.9% of them reported only to consume synthetic cannabis, 37.2% synthetic and organic cannabis and 56.9% organic cannabis, including marihuana or hash. All the subjects using

cannabis smoked it, with a mean of 4.14 years' consumption (*SD* = 2.08) and 0.34 years' (*SD* = 0.62) treatment for the cannabis-related disorder. In relation to the consumption of other NPS, 2% reported consumption of mephedrone and 5.9% of 3,4-methylenedioxy-methamphetamine (MDMA).

*Identification of synthetic cannabinoids in urine samples*

Of the seven herbal blends acquired for this study, we had already analysed *Oro fantastico*, *Mazazo*, *Placaje,* and *Sonrisa absoluta* (**Fig. 1A**), and had identified four SCs in them: JWH-081, JWH-250, JWH-203, and JWH-019 [12]; our repeated analysis in this study found no differences in their composition. We also analysed the new herbal products, *Hardcore, Sonrisa,* and *Tio tieso* (Fig. 1B) by UHPLC-HRMS and cross-referenced the suspect peaks against our SC library (Ibañez et al., 2014). Based on the observed accurate-mass data on fragmentation and information in the literature, we tentatively identified four new SCs: XLR-11, UR-144, an UR-144 N-(5-chloropentyl) analogue, and 5F-AKB48 (5F-APINACA), as shown in Table 1.

As an illustrative example, analysis of the herb mixture *Hardcore* was as follows: three chromatographic peaks were present in the base peak intensity chromatogram (BPI) of the low-energy function (Fig. 2B), at 13.40 min ($[M+H]^+$ *m/z* 330.2222), 13.99 min ($[M+H]^+$ *m/z* 346.1931), and 14.54 min ($[M+H]^+$ *m/z* 312.2327). These peaks were tentatively identified as XLR-11, an UR-144 N-(5-chloropentyl) analogue, and UR-144, based on the accurate-mass collision-induced dissociation (CID) fragments observed in the high-energy function (**Fig. 2A**). The fragments observed for XLR-11 and UR-144 also coincided with fragmentation profiles reported in the literature (Sobolevsky et al., 2012;

Wohlfarth et al., 2013), while fragmentation of the UR-144 N-(5-chloropentyl) analogue was justified based on the XLR-11 and UR-144 fragments. After CID ion evaluation, a plausible fragmentation pathway was proposed for the three SCs found in the *Hardcore* herbal blend (Fig. S1, supplementary information).

Eight SC were identified in total in the herbal blends analysed (Table 1). Then, a searching in the literature was made to establish a target list of two to four major metabolites for each SC (Jang et al., 2015; Scheidweiler, 2015). No metabolites were found for the UR-144 N-(5-chloropentyl) analogue, a fact that was considered not much relevant because this compound was only found in *Hardcore* and at a very low abundance in comparison to the other two SCs (XLR-11 and UR-144) present in this product. Table 2 shows the 19 metabolites selected as target compounds to be investigated in urine samples based on the literature search. It can be seen that UR-144 and XLR-11, the two main components of *Hardcore,* share two metabolites, and only XLR-11 presented an additional specific one.

After data re-processing of urine samples, which all were negative for parent SCs, positive detections were returned only for two SC metabolites: N-pentanoic acid and N-(5-hydroxypentyl). These compounds were found in 9 pooled urine samples. Fig. 3 shows the tentative identification of both metabolites (A, N-pentanoic acid; B, N-(5-hydroxypentyl)) based on the accurate-mass fragmentation observed for one of the pooled urine samples. The presence of these metabolites indicated that the subjects had consumed either XLR-11 or UR-144. We could not differentiate between these two SCs because these metabolites were common to both of them. The extracted-ion chromatogram at the exact mass of N-(6-hydroxyindole), another metabolite of XLR-11, showed a chromatographic peak, but the

fragmentation pattern did not fit with that reported in previous works, with the hydroxylation point appearing to be on the tetramethylcyclopropane ring (Fig. S2). However, our data supported previous findings, where the main metabolites reported in urine samples from XLR-11 consumers were N-pentanoic acid and N-(5-hydroxypentyl) (Jang et al., 2016).

*Identification of specific participants with urine samples positive for XLR-11 or UR-144*

The 9 pooled urine samples positive for XLR-11/UR-144 metabolites corresponded to 72 individual urine samples from three different juvenile offenders' centres. The individual samples were then treated with enzymatic hydrolysis and analysed by UHPLC-HRMS searching for additional minor metabolites described in urine from XLR-11 consumers (Jang et al., 2016). The 12 phase-I metabolites described in the literature corresponded to six elemental compositions because in some cases the same biotransformations occurred on different moieties of the molecule. For example, four metabolites corresponded to hydroxylation on different carbon atoms of the tetramethylcyclopropane ring. **Table 3** shows the XLR-11 metabolites used as target compounds for the screening of individual urine samples.

There were positive results for 16 out of the 72 individual urine samples for five of these minor metabolites (**Table 3**); only M2 was not detected in any of these positive samples. To obtain cleaner spectra and enhance reliability in the metabolites identification, additional MS/MS experiments were performed to obtain the accurate-mass product ion spectra, comparing the fragmentation observed with that described in the literature (Jang et al., 2016). This allowed confirming the identity of the metabolites. In the particular case of

M5, two chromatographic peaks were observed corresponding to two different hydroxylation points in the degraded tetramethylcyclopropane ring. With the information available, it was not possible to determine the exact position of the hydroxyl group. The MS/MS spectra of the detected metabolites at 10, 20, 30, and 40 eV collision energies, and the fragment-structure justifications are detailed in the Supplementary Information (**Fig. S3-S8**).

Finally, with all information obtained, the 16 individual urine samples positives to the XLR-11 metabolites could be assigned to 6 minors based on the anonymous urine sample codification. Only two adolescents recognized consumption in the administered questionnaires, while the remaining 4 did not recognize any SCs consumption.

## 4. Discussion

The consumption of SCs, which escape conventional detection systems, seems not very common in the juvenile offenders' centres from the Valencian region, but a few cases have been found in this work. The anonymous survey results from this study indicate that some minors consume SCs because they elude routine drug analysis for cannabis. In the present study, 29 out of 127 participants admitted they had used SCs at some time, although our survey did not record when the consumption had occurred. Therefore, indication of SC consumption in the survey did not necessarily imply that the urine sample collected would produce a positive result. Although 29 of the participants were self-reported SC users at some time, this questionnaire-based interview information should be cross-referenced with toxicological analyses. Analysis of the herbal blends reported to have been consumed by the participants allowed the identification of several SCs in those samples. Subsequent

urine analysis demonstrated the presence of major metabolites from XLR-11 and UR-144, supporting the consumption of the suspect products by some participants. Some SCs metabolites remain present for only 14 hours in urine after consumption (Crews, 2013), and in the case of XLR-11/UR-144, for only 3 hours (Lemos, 2014), this being a limiting factor in monitoring the consumption of these products In our study, SC consumption was only detected in 6 of the 29 self-referred cases. Therefore, to increase the detection rate we argue that is important to focus the analysis in the appropriate target compounds. Considering the high number of SCs reported until now and the even higher number of potential metabolites, some of them being still unknown, we emphasize the importance to focus the investigation on major metabolites of the active compounds identified in the products within the "distribution area" of the minors. Both, toxicological and consumption information, can then be obtained from users in a synchronised way (Lemos, 2014). The use of advanced analytical techniques, such as LC-HRMS, allow to detect more SC consumers via their urine samples from among those who do not recognise their consumption via surveys or interviews. In our study, we detected 4 additional cases of the remaining minors who, in the group interviews and questionnaires, denied the use of synthetic cannabinoids.

The time factor not only determines the detection and analysis procedure, but is also the key in preventive and guidance interventions. The effects of consuming SCs are widely documented: indeed, many of these compounds are more toxic than cannabis itself, with reports of several poisonings and deaths and emergency hospital admissions for intoxications (European Monitoring Centre for Drugs and Drug Addiction, 2017), as well as repercussions at the respiratory, reproductive, cardiovascular, neurological, and

psychiatric levels (Forrester et al., 2012; Nordstrom and Levin, 2017; Werse and Morgensten, 2012), described among military personnel (Danovitch and Gorelick, 2012; Davis et al., 2015), mining workers (Blevions et al., 2016), and prison inmate (Keyes et al., 2016). In addition, the consumption of NPS usually increases polydrug consumption (Pinto et al., 2015). Regarding the specific effects reported for the consumption of XLR-11, its use is associated with an increased risk of death, hypertension, lack of horizontal and vertical gaze nystagmus, non-convergence of the eyes, dilated pupil size, tachycardia, and even genetic alterations, as well as with psychiatric symptoms such as anxiety, hallucinations, irritability, seizures, and agitation (Adamowicz et al., 2017; Joseph et al., 2017; Lemos, 2014 Ferk et al., 2016; Shanks et al., 2015; Sun and Dey, 2014).

These secondary effects justify interventions, like those used for any substance consumption treatment, to increase the perception of risk. Changing this perception has proven important in treating disorders related both to cannabis (Kilmer et al., 2007), and SC use because consumers tend to have a low perception of risk and have normalised their consumption (Clayton et al., 2017; Shanks et al., 2015). We propose using the specific information available on the SCs detected at juvenile offenders' centres at any time to guide the information given about the risks of consumption. This would allow users to evolve from a pre-contemplative to a fully contemplative psychological state, which would require varying degrees of subsequent therapeutic intervention. Some other factors also determine the urgency of therapeutic interventions, such as the youth of the consumers and their deprivation of liberty status, which determines their level of control and supervision (Castellanos et al., 2015; Stathis et al., 2006; Young et al., 2007). In addition, we propose that any interventions implemented should cover the consumption of all NPS drugs,

including SCs and other synthetic drugs. Moreover, the therapeutic team should include professionals from the juvenile offenders' centre, as well as external NPS specialists (Farrow, 1984; Sthatis et al., 2006) and experienced analytical chemists in using HRMS. Minors deprived of liberty, and who do not report NPS consumption or present detectable NPS metabolites in their urine, should also participate in these interventions as a preventive measure (Warren et al., 2017). The main objective of these interventions should never be punitive, but rather preventing NPS use, rehabilitation, and increasing the welfare of minors.

## 5. Conclusions

The results from our study have demonstrated that SCs (specifically, XLR-11) are occasionally consumed in juvenile offenders' centres in the Autonomous Valencian Community. The procedure for detecting SCs consumption must be efficiently synchronised, so that information obtained from interviews and questionnaires should be matched to the smartshops where the consumers obtained these substances from, as well as their toxicological urine analysis results within few hours of their last consumption. Preventive and therapeutic interventions in minors housed in juvenile offenders' centres must be implemented to reduce the consumption of NPS and to adjust the risk-perception of these substances, both in consumers and non-consumers.

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