RESEARCH ARTICLE

Identification of 5F-Cumyl-PINACA, a Synthetic Cannabinoid, in the Herbal Material Used for Recreational Purposes in the Province of Trieste: Public Health Implications

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ARTICLE HISTORY

Received: April 20, 2022
Revised: August 04, 2022
Accepted: August 14, 2022

DOI:
10.2174/1389201023666220915092609

Abstract: ***Background:*** In recent years, the phenomenon of the production and trade of synthetic cannabinoids has grown, becoming a public health issue worldwide. The recent access- to the ED of the hospital of Trieste- of people who complained of episodes of hallucinations, sensation of poison- ing, tachycardia, and air hunger following the inhalation of "Che Sballo platinum", have highlighted the need to perform further analysis on the contents of the packet sold as an air freshener, produced in Koper (Slovenia).

Objective: This paper wants to be an alert about the possible consequences on health due to the spreading of "Che Sballo platinum" in the province of Trieste.

Methods: The package contents were analyzed by a multi-target screening method of MRM-IDA-EPI experiment. The result was then confirmed, and quantification was achieved *via* LC-ESI-MS/MS analysis in MRM mode using QTrap 6500 + Sinergy hydro column 100 x 2 mm 1.9 um transitions MRM1 368.3 → 250.0; MRM2 368.3 → 233.0.

Results: The initial screening tested negative for THC and showed positive results for 5F-Cumyl-PINACA. Quantitation result reported dose by the package of 8.5 mg of the compound. Formal notification was sent to the Italian Health Authorities (Notification No. 2021110205).

Conclusion: Consumption of 5F-Cumyl-PINACA results in much more potent effects than mari- juana. Lack of information about the actual concentration of the substance on the packaging does not allow drug users to have an adequate dosage, with possible toxic consequences on health. Further investigations must be done to discover the true extent of the phenomenon.

Keywords: 5F-Cumyl-PINACA, synthetic cannabinoids, new psychoactive substances, legal high, MRM-IDA-EPI, LC-ESI-MS/MS, Che Sballo platinum, intoxication.

1. INTRODUCTION

Synthetic cannabinoids (SCs) is the name given to a diverse range of substances that mimic the effect of (-)-trans-Δ9-tetrahydrocannabinol (THC), which is the main psychoactive ingredient in cannabis [1]. These compounds interact with CB_1 and CB_2 cannabinoid receptors, eliciting their cannabimimetic effects. SCs represent the "legal" substitute for cannabis and play an important role in the rapidly evolving "legal high" market. The term "legal high" refers to non-regulated new psychoactive substances (NPS), which also include SCs, sold on the open market [2]. Due to their easy availability, low cost, high potency and the perceived lack of legislative control, synthetic cannabinoids have become a major issue because of concerns regarding potential public health risks and social threats to Europe [3-5]. The desired effects of synthetic cannabinoids are similar to those produced by THC, including relaxation, euphoria and disinhibition. On the other hand, more severe adverse events are associated with SCs, not only because of their action as full agonists but also because products containing synthetic cannabinoids often contain high doses of the compounds. The combination of these two factors makes it difficult for users to control the dose they are exposed to, leading them to rap- idly administer a toxic dose unintentionally [6, 7], also with lethal outcomes [8-11]. From the beginning of 2021, three cases of transitory episodes of visual and auditory hallucina- tions (deformed room, noises), sensation of poisoning, mal-

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aise, increasing paranoia, respiratory depression, tachycardia, and air hunger were complained after smoking tea-like leaves contained in a packet of "Che Sballo platinum". It is sold as an air freshener on the open market and produced in Koper (Slovenia). The results of a complete toxicological study are reported

2. MATERIALS AND METHODS

The sample for analysis was herbal material, like small tea leaves, packed in a small bag with a clamp closure and labelled as "Che Sballo platinum", with a net weight of 2 g (Fig. **1**).

Fig. (1). Label of "Che Sballo platinum" and its contents. (*A higher resolution / colour version of this figure is available in the elec- tronic copy of the article*).

Standards of NPS are freely provided by the Ministry of Health to members of the Early Warning National System (SNAP) circuit and nowadays include 78 compounds belonging to the following classes: amphetamines, arilcycloesilammines, opioids, benzimidazols, cannabinoids, cathinones, phenethilamines and benzodiazepines. ULC/MS gradient grade water, acetonitrile and methanol were all purchased from Biosolve Chimie SARL. Ammonium formate and formic acid were obtained from Sigma-Aldrich. 1 mg of herbal material was weighed in an Eppendorf vial, to which 1 mL of methanol was added and put in an ultrasonic bath for 15'. The solution was diluted 1:200 with mobile phase A for initial screening analysis.

2.1. Multi-target Screening Method (MRM-IDA-EPI)

Mass spectrometric analysis was performed on a QTRAP 6500+ hybrid triple quadrupole/linear ion trap mass spectrometer by SCIEX. Screening was performed using a hybrid in-house method using Multiple Reaction Monitoring (MRM) survey scans to achieve the most sensitive detection of a predetermined list of target compounds and, through Information Dependent Acquisition (IDA) criteria, to trigger the automated acquisition of Enhanced Product Ion (EPI) full-scan MS/MS spectra. Then, spectra were submitted for library searching to confirm the compound's identity. Our method covered more than 78 NPS compounds and cannabi- noids in scheduled mode used routinely for the drug screen- ing of seized material. Liquid chromatography was per- formed using an Exion LC-AD UHPLC system. Reversed- phase chromatographic separation was performed using a Restek Allure PFP Propyl column (5 um, 50 x 2.1 mm, 60 A) and pre-column Restek 10 x 2.1 mm, 5 um, 60 A, with mobile phase consisting of 0.2% formic acid in the water, 2 mM ammonium formate (mobile phase A), 0.2% formic acid in acetonitrile and 2mM ammonium formate (mobile phase B). The liquid gradient consisted of 10 to 90% mobile phase B in 10 minutes, followed by a hold at 90% mobile phase B for 5 minutes, and a re-equilibration at 10% phase B for 2.5 minutes. The total chromatographic run time is 18 minutes at a flow rate of 0.5 mL/min.

2.2. LC-ESI-MS/MS Target Analysis

The NPS's quantitation analysis was performed using liquid chromatography coupled to tandem mass spectrometry with electrospray ionization (LC-ESI-MS/MS). This chromatography was carried out using an XSelect CSH C18 2.5 um, 2.1 x 100 mm (Waters Corp., Milford, USA). The LC gradient initial condition was 5% of mobile phase B (1% H_2O, 99% acetonitrile, 0.1% HCOOH and 2 mM ammonium for- mate), then increased to 45% over 16 minutes, and to 100% over 4.5 minutes, held for 2 minutes and finally returned to 5%, at a flow rate of 300 uL/min.

3. RESULTS

The initial screening analysis was performed by a routine method used for drug screening of seized material, particularly by the MRM-IDA-EPI experiment (Table **1**).

Table 1. MRM transition and retention time applied for screening analysis for MRM-IDA-EPI for detection of 5F-Cumyl-PINACA.

NPS	Molecular Formula	Precursor Ion [m/z] (Nominal Mass)	Product Ions [m/z]	CE	Retention Time
5F-Cumyl-PINACA	C22H26FN3O	368.3	250.0	11	6.54

The sample was also tested for THC, with negative results. As green plant material, NPS screening, which was performed by comparing all NPS standards provided by the

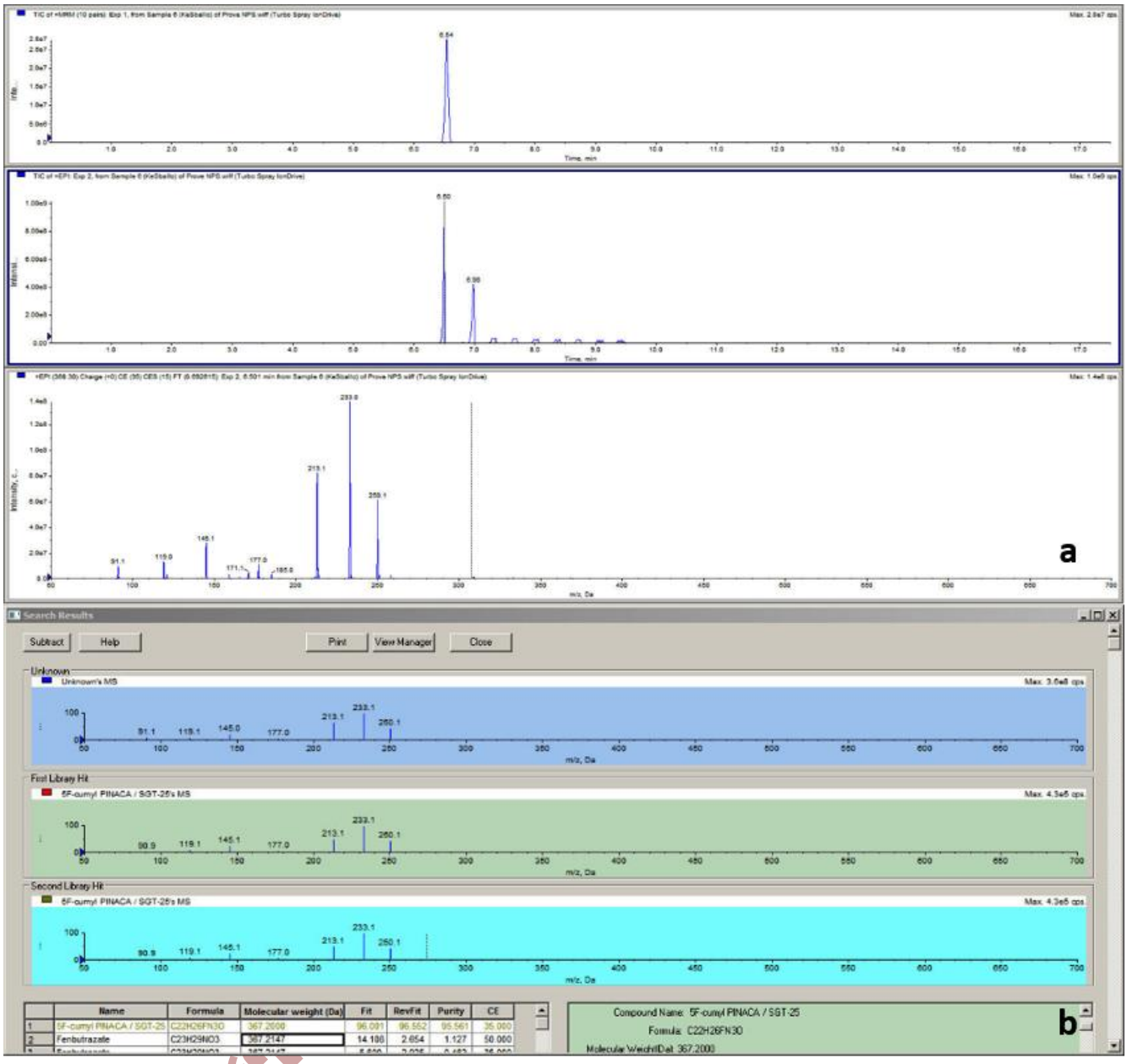

Fig. (2). (**a**). Positive identification for 5F-Cumyl-PINACA using a Targeted Screening method (MRM-IDA-EPI), which showed the chromatogram and MS/MS spectra. (**b**). Library matching for 5F-Cumyl-PINACA and reference standard spectra. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Ministry of Health, showed a positive result for 5F- CUMYL-PINACA, with an excellent match with the library, thus confirming the compound identification unambiguously (Figs. **2a**,**b**).

1 mg of sample from the package has been extracted with methanol and vortexed. As the synthetic cannabinoid is completely soluble in methanol, and not being necessary to extract it from the herbal leaves, extraction with methanol is the best matrix to work with. Then the sample was diluted 200 fold with 50% methanol and formic acid 1% and in- jected. The medium result of multiple injections is 22 ng/ml, showed in the result. Confirmation and quantitation of 5F- Cumyl-PINACA were performed *via* LC-ESI-MS/MS analysis using the following target MRM transitions and external calibration (Table **2**) (Fig. **3**).

Table 2. MRM transitions for 5F-Cumyl-PINACA applied for confirmation analysis.

NPS	Molecular Formula	Precursor Ion [*m/z*]	Product Ions [*m/z*]	CE	DP
5F-Cumyl-PINACA	C22H26FN3O	368.3	250.0	11	90
			233.0	11	90

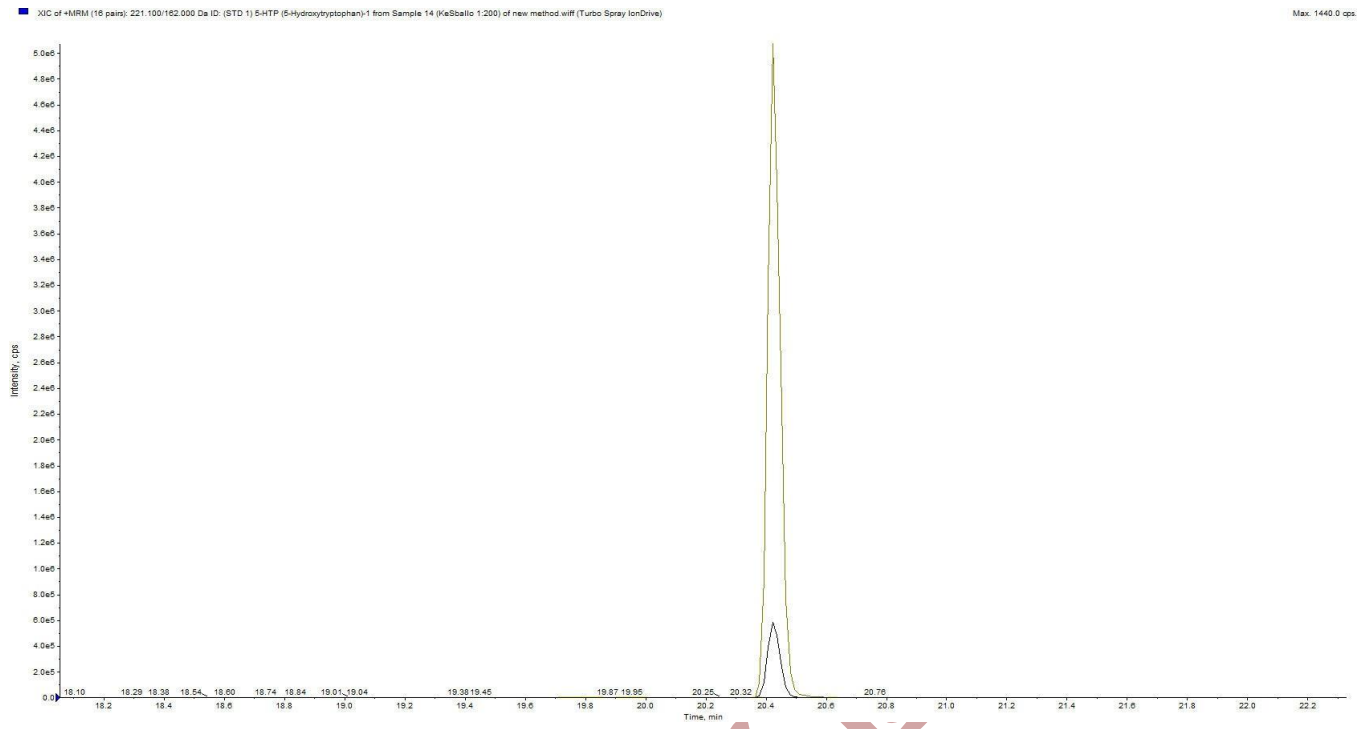

Fig. (3). MRM transitions for quantitation of "Che Sballo platinum" sample diluted 1:200. Quantifier MRM 368.3 → 250.0; qualifier MRM 368.3 → 233.0. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Five calibration points was used for the calibration, by proper dilution of stock solution (0.005 mg/ml): working calibrators of 100 ng/ml; 50 ng/ml, 25 ng/ml, 5 ng/ml, 0 ng/ml were prepared. Linear regression was excellent (r^2 = 0.9992), adopting a weighting factor of 1/x, and accuracy and repeatability were respectively 96.9% and 98%. Four calibration points were used for quantitation by dilution of standard (50 ng/mL, 25 ng/mL, 5 ng/mL, 0 ng/mL) with mobile phase A and injection of 2 uL (Fig. **4**).

Linear regression was 0.9992. Quantitation result reported dose by the package of 8.5 mg of 5F-cumyl-PINACA mixed with the herbal material contained in the package (2 g net weight). Finally, the results of the toxicological analysis were formally notified to the Italian Health Authorities (Notification No. 2021110205)

4. DISCUSSION

Since their first identification in 2008 - through the detection of the synthetic cannabinoid JWH-018 [12] -, synthetic cannabinoids have become the largest group of substances monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), with 209 reported between 1 January 2008 and 31 December 2020 [6]. The increasing number, their chemical diversity and the speed of their emergence make this group of substances particularly challenging in terms of detection, especially in view of the fact that most synthetic cannabinoid metabolites are not commercially available [13, 14]. It is common for SCs to be mar- keted by head shops or the more popular online stores, whichsell them under the names of "herbal smoking mixtures," "herbal incense", air freshener, bath salts, powders, tablets ormore recently, as vaping solutions (in the form of e-liquids) [12, 15]. The most common method of administration is inhalation. When smoked, these substances are immediately absorbed by the lungs and, due to their high lipophilic na- ture, redistributed to other organs, such as the brain, in a short time. On the other hand, oral intake reduces absorption and bioavailability due to the first-pass effect [16]. In terms of look, smell and flavor, SCs are very similar to "genuine" illicit cannabis; however, many of them are more potent than THC, and this could explain the higher risk of poisoning: while THC acts as a partial agonist, most synthetic cannabi- noids act as full agonists, causing significantly stronger (side) effects [17]. There are several factors associated with SCs' health effects, such as (1) the intrinsic properties of the substances, (2) the metabolic pathway, and (3) the way these compounds are produced. Four constituents comprise the fundamental structure of synthetic cannabinoids: tail, core, linker and linked group. A key aspect is that the n-pentyl side chain, if present, is crucial to determine affinity, selec- tivity, and potency at cannabinoid receptors. Concerning the affinity, which mainly depends on functional group substitu- tion, Gamage *et al.* [18] stated that all compounds tested in their study exhibited high affinity at CB_1 and CB_2 receptors, with 5F-Cumyl-PINACA as the most affinitive compound for CB_1, and 5F-APINACA for CB_2. Moreover, all parent compounds showed modest selectivity for the receptors, with a 5-fold greater selectivity for CB_1 and CB_2, respectively, by 5F-Cumyl-PINACA and 5F-APINACA. Additionally, 5F-Cumyl-PINACA was very potent at stimulating CB_2 receptors. Although parent compounds are usually hardly detectable in biological matrices - due to the extensive metabolism -in the literature, only a few studies analyze the pharmacology of SCs metabolites' [13]. Generally, synthetic cannabinoids undergo hepatic metabolism *via* cytochrome P450

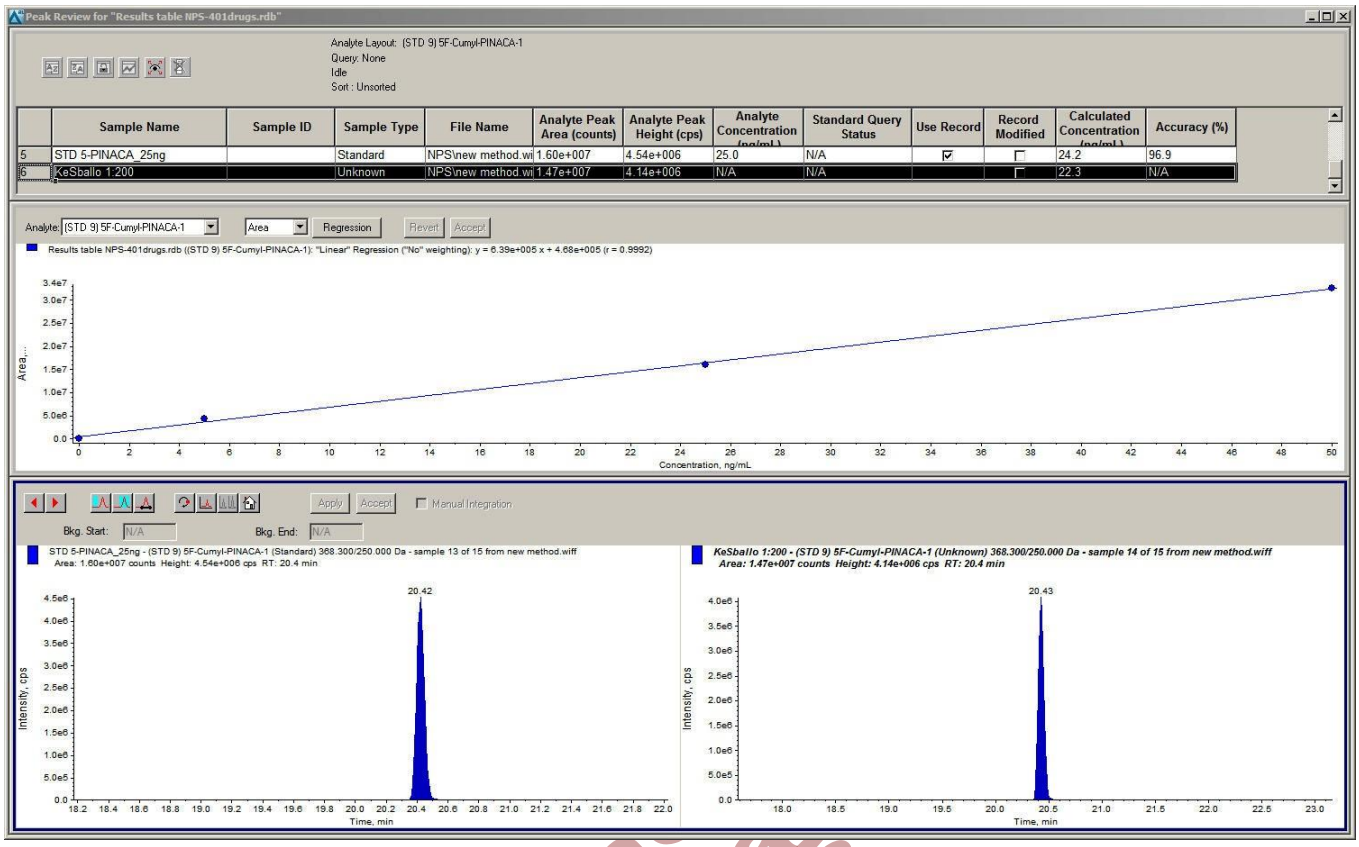

Fig. (4). Calibration curve from 0 ng/mL to 50 ng/mL used for quantitation 5F-Cumyl-PINACA and side-to-side chromatograms of 25 ng/mL of standard and sample. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

enzymes, resulting in phase I hydroxylated metabolites (oxidative metabolism). Especially for compounds fluorinated at the 5-position, they are susceptible to oxidative defluorination and hydroxylation, while alkyl side chain, when present, would appear to undergo hydroxylation at several points. Although hydroxylation reduces the affinity at both CB_1 and CB_2, it does not seem to affect the efficacy of most synthetic cannabinoids, but, on the contrary, increases its potency. Metabolites are subsequently conjugated by UDP-glucuronosyltransferases (conjugative metabolism) and excreted as glucuronic acid conjugates in human urine [19, 20]. The metabolic products of the process described above, combined with any defects in the pathway, determine the toxicity of SCs [21]. For example, polymorphisms of cytochrome P450 determine differences in phase I or, because of glucuronidation, metabolites may even lose their agonist activity and become antagonists (for example, JWH-018) [18, 22, 23]. Adverse events more commonly associated withthe use of synthetic cannabinoids are related to central nerv- ous system effects (anxiety, agitation, panic attacks, irritabil- ity, aggression, changes in mood and perception, confusion, lethargy, paranoia, hallucination and coma), cardiovascular effects (tachycardia, arrhythmia, hypertension, dyspnea and chest pain), and gastrointestinal effects (nausea and vomiting), also combined with dry mouth and bloodshot eyes [24, 25]. Severe poisoning - such as stroke, seizure, heart attack, breakdown of muscle tissue, kidney damage, psychosis and severe or prolonged vomiting - are also common [26-28], and fatalities linked to the consumption of these substances have been recorded [7-11, 16]. Little is known about their chronic use, but symptoms suggestive of dependence and withdrawal have also been reported [29, 30]. Although many European countries have taken legal action against thismarket, availability continues to be high [31, 32]. The sei- zure of synthetic cannabinoids in powder form and the dis- covery of processing facilities in Europe indicate that the products are packaged on the continent. Regarding this phe- nomenon, between 2008 and 2015, there has been a consis- tent rise of newly synthesised cannabinoids in European States placed on the market, with an average of 27 cannabi- noids appearing each year between 2011 and 2015. The trend had changed since 2016, when the average number of SCs settled around 10 [6]. More specifically, since that year, a total of 7 synthetic cannabinoids have been formally risk- assessed by the EMCDDA: MDMB-CHMICA, AB- CHMINACA, ADB-CHMINACA, 5F-MDMB-PINACA, CUMYL-4CN-BINACA, MDMB-4en-PINACA, and 4F-MDMB-BICA. In Italy, 128 NPSs were reported in 2020, and 20 of these were synthetic cannabinoids. To better understand the phenomenon in terms of toxicological effects and health/social risks, the consumption data analysis cannot be ignored. Intoxication cases have been increasing and, at the same time, in recent years, the consumption of NPS has been spreading, also among the very young, with particular regard to synthetic cannabinoids, second only to cannabis as the most frequently consumed substances [33]. The Euro-

pean data, extracted from the recent European School Survey Project on Alcohol and Other Drugs Report (ESPAD), show that, until 2019, 3.1% of the ESPAD students who had been reported as synthetic cannabinoid users at least once in their lifetime range from 1.1% in Slovakia to 5.2% in France [34]. In 2019, Italy was in line with the European average, with a percentage of 2.8%. However, the Italian ESPAD study, carried out annually, shows worrying data, with a prevalence of synthetic cannabinoids' use among participating students reaching 5% in 2021, with an increase of 2.2% compared to 2019 [35]. In the north-east of Italy, the 2020 Report "*Consumo, dipendenza da sostanze e comportamenti di addiction in Friuli Venezia Giulia*" highlights the fact that, until that year, there were no official alerts concerning the consumption of SCs [36]. 5F-Cumyl-PINACA (chemical name: 1-(5-fluoropentyl)-N-(1-methyl-1-phenylethyl)-1H-indazole-3-carboxamide) was first identified in September 2014 and then reported to the European information system and database of new drugs (EDND) by the Swedish National Laboratory of Forensic Science [12, 37]. In the literature, several studies have been conducted since 2015 to evaluate the relative potency of cumyl derivatives as compared to other synthetic cannabinoids: in particular, the 5F-Cumyl-PINACA turn out to be the most potent synthetic cannabinoid when compared to the other cumyl derivatives [38]. Only one case of mild toxicity from 5F-Cumyl-PINACA with hospital presentation is reported, though the recent episodes of misuse of "Che Sballo platinum" recorded in the province of Trieste constitute an alert with regard to the increasing phenomenon of 5F-Cumyl-PINACA misuse, due to its more powerful effects and easy accessibility on the web [39]. The findings of our study should be interpreted in the light of some limitations. Certainly, one aspect that must be stressed is the small sample size. Unfortunately, obtaining accurate epidemiological data is not possible because the information has been extrapolated from spontaneous reports made by patients. Furthermore, at the same time, the analysis was carried out on the content of a single bag because only in one case the patient proceeded to deliver what he/she claimed to have consumed.

CONCLUSION

Since their first appearance on the market, synthetic cannabinoids have become a major health issue [40]. Their ready availability on the web, their higher psychoactivity compared to THC and the possibility to apply minor structure modifications to evade scheduling laws have led to exponential growth in production and trade over time. The SCs currently available have shown a broad range of effects on humans, both centrally and peripherally. However, products containing synthetic cannabinoids rarely state the correct ingredients and/or their concentrations. Consequently, people who use such products will be unaware that they are using this substance and will be unable to obtain accurate dosage information. Moreover, to better understand health consequences, another key point is their metabolism, as changes in the metabolic pathway may affect toxicity. As regards 5F-Cumyl-PINACA, *in vitro* studies of cumyl derivatives metabolism showed extensive biotransformation, with hydroxylation, dihydroxylation and oxidative defluorination - for the fluorinated compounds - as the main steps [13]. Our study has confirmed that this substance has a much more powerful effect than marijuana and that it is easily available on the market since it is sold as an air freshener with a captivating name ("Che Sballo platinum", literally translated "What a Trip platinum") and packaging, though devoid of any information regarding ingredients or concentration. Our analysis has confirmed the presence of 5F-Cumyl-PINACA within the content of "Che Sballo platinum", at a dose by the package of 8.5 mg. The authors want to alert the scientific community about the possible dangerous consequences of the misuse of 5F-Cumyl-PINACA on health, even though greater efforts are required to obtain confirmation of the real extent of this growing phenomenon worldwide.

LIST OF ABBREVIATIONS

SCs	=	Synthetic cannabinoids
THC	=	Tetra Hydro Cannabinol
NPS	=	New psychoactive substances
IDA	=	Information Dependent Acquisition
EPI	=	Enhanced Product Ion

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datsupporting this study's findings are available from the corresponding author, S.D., on special request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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