GC-MS Identification and Quantification of the Synthetic Cannabinoid MDMB-4en- PINACA in Cannabis-derived Material Seized in the Turin Metropolitan Area (Italy)

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Current Pharmaceutical Design, 2019, 25, Pagination 1

ARTICLE TYPE

GC-MS identification and quantification of the synthetic cannabinoid MDMB-4en-PINACA in cannabis-derived material seized in the Turin Metropolitan Area (Italy)

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ARTICLE HISTORY

Received:
Revised:
Accepted:

DOI:

Abstract

Background: the presence of the synthetic cannabinoid receptor agonist MDMB-4en-PINACA in adulterated low-THC cannabis products was recently highlighted in several reports. Moreover, numerous intoxication cases involving MDMB-4en-PINACA have been described.

Objective: In order to monitor the diffusion of cannabis products containing MDMB-4en-PINACA in our territory, a total of 358 cannabis-derived samples (213 vegetal material and 145 resins) seized in the period November 2020 – February 2021 in the western Piedmont Area (Italy) was analyzed.

Methods: General screening analyses for traditional and synthetic cannabinoids were performed by a GC-MS device operating in full scan mode (40-600 amu). The MDMB-4en-PINACA was quantified by means of a specific GC-SIM-MS protocol purposely developed and validated, while the quantification of THC, CBD, and CBN was carried out by a GC-SIM-MS method routinely employed in our laboratory.

Results: MDMB-4en-PINACA was detected in 12 out of 358 samples (3.4% of the total). Among these, the molecule was found in 11 vegetal materials and in one resin sample. Considering solely the analysis of the 213 herb products, a positive rate of 5.2% was found for the presence of MDMB-4en-PINACA in these samples. MDMB-4en-PINACA was found in the seized materials at concentration levels ranging from 0.4 up to 6.3 mg/g (mean 2.5 mg/g; median 1.7 mg/g). Concerning the traditional cannabinoids, the THC concentration was in the interval 3-43 mg/g (mean 12 mg/g; median 7 mg/g), while CBD was found at higher concentrations in all specimens, specifically in the range 47-140 mg/g (mean 87 mg/g; median 80 mg/g).

Conclusions: The adulteration of low-THC cannabis products with synthetic cannabinoid receptor agonists is a widespread phenomenon today. Since these substances are potentially more toxic than THC, their consumption poses a high risk of overdose for unaware users and a health-threatening situation. This study confirmed the sporadic presence on the market of CBD-prevalent cannabis products adulterated with MDMB-4en-PINACA.

Keywords: Synthetic cannabinoids, Cannabis, Adulterated, MDMB-4en-PINACA, NPS, GC-MS.

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1381-6128/19 $58.00+.00

1. INTRODUCTION

The number of new psychoactive substances (NPS) reported by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is growing every year. Synthetic cannabinoids receptor agonists (SCRAs) are the largest subclass of NPS reported on the European territory. Since 2008, a total of 209 new SCRAs have been detected in Europe by the EMCDDA, including 11 new substances reported for the first time in 2020 [1]. Among these, MDMB-4en-PINACA is an emerging SCRA chemically related to the 3-carboxamide indazole derived MDMB-FUBINACA (Figure 1).

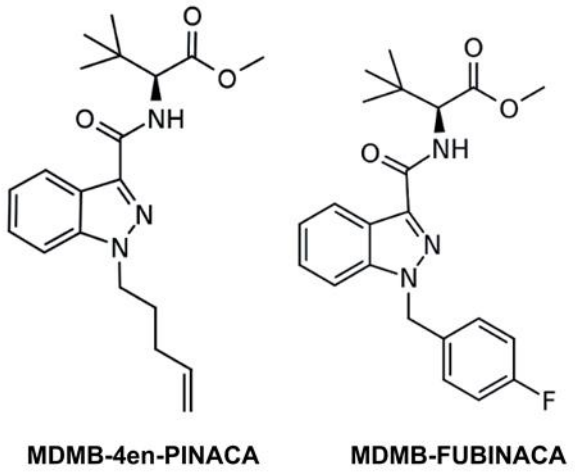

Fig. 1 Chemical structures of MDMB-4en-PINACA and MDMB-FUBINACA

SCRAs were initially marketed for sale as herbal incenses or smoking mixtures, and occasionally as bulk forms of powders [2]. These substances were sold mainly in the forms of "legal highs" to avoid provisions of existing drug laws [3]. In recent years, alongside smoking mixtures, new dosage forms, including e-liquids for vaping using electronic cigarettes and paper or blotters impregnated with SCRAs, have appeared on the drug market [4,5]. Moreover, the presence of SCRAs, including MDMB-4en-PINACA, was recently reported in cannabis-derived products, mainly those containing very low THC levels [6]. Cannabis-derived products with low content of THC while often rich in CBD, can be legally sold and purchased in several countries [7,8]. As a consequence, the marketing of adulterated low THC-cannabis products to unaware users highlights the new and potentially risk of the inadvertent consumption of potent SCRAs, including MDMB-4en-PINACA. As a matter of fact, several fatal and non-fatal intoxications involving MDMB-4en-PINACA were described in the recent literature, highlighting the potency and the danger of this synthetic substance [9].

In this study, the identification and quantification of MDMB-4en-PINACA in cannabis-derived samples is reported. General screening analyses for traditional and synthetic cannabinoids were performed by a full-scan GC-MS method, while the quantitative analysis was carried out by means of a GC-SIM-MS protocol purposely developed and validated. The procedure was applied to 358 cannabis-derived samples seized by the Police in the period November 2020 – February 2021 in the western Piedmont Area (Italy), in order to monitor the diffusion of adulterated cannabis products containing MDMB-4en-PINACA in the territory.

2. EXPERIMENTAL

2.1 Seized Samples

A total of 358 cannabis-derived samples was analyzed for the presence of natural cannabinoids and other psychoactive substances. The samples were constituted of vegetal material (dried flowers and leaves; n=213) and resins (n=145) seized by the Italian Police in the western Piedmont Area (Northern Italy) in the period November 2020 and February 2021. The seized materials were sent to our laboratory for forensic identification at the request of Prosecutor's Offices.

2.2 Chemicals and Reagents

Ethanol, methanol and theophylline (analytical grade) were purchased from Merck KGaA (Milan, Italy). MDMB-4en-PINACA was obtained from LGC Standards (Milan, Italy). Standard stock solution of the MDMB-4en-PINACA reference material (1000 mg/L in methanol) was stored at –20°C until used.

2.3 Sample Preparation

About 100 mg of pulverized vegetal material or resin were weighted in a test tube and added with 10 mL of ethanol containing 250 mg/L of theophylline as internal standard. After sonication in an ultrasound bath for 30 min at 55°C, the samples were vortex mixed for 10 min. After extraction, the tubes were centrifuged at 4,000 rpm for 5 min, and a 1 µL aliquot of the solution was injected into the GC–MS system.

2.4 Apparatus and Methods

Preliminary drug screening analysis on the seized material was performed using a 6890N GC apparatus (Agilent Technologies, Milan, Italy) equipped with a 30 m fused-silica capillary column (J&W Scientific HP-5MS) with a 0.25 mm inner diameter and a 0.250 mm film thickness. Helium was employed as the carrier gas at a constant pressure of 15.50 psi. The GC oven temperature was set at 150°C for 1 min and then raised to 315°C with a 30°C/min heating rate. The oven temperature was then maintained at 315°C for 4 min, for a total run time of 11.60 min. The GC injector and transfer line were maintained at 230°C. Full scan spectra in the interval 40–600 amu were acquired using a 5975 inert mass-selective detector (Agilent Technologies, Milan, Italy) operating in the EI mode at 70 eV. The qualitative identification of unknown compounds was performed by comparing the full scan spectra obtained with those recorded in the available spectra libraries (SWGDRUG version 3.9 and CaymanSpectraLib ver. 09222020). For the MDMB-4en-PINACA confirmation analysis, a dedicated GC–SIM–MS procedure was developed and validated. Chromatographic conditions were the same described above, while the quantitative determination was performed by monitoring the diagnostic ions at m/z 213 (target ion), 357, 301 and 298 (qualifiers) for MDMB-4en-PINACA, whereas for the internal standard (theophylline), the diagnostic ions at

m/z 180 (target ion), 95 and 68 were chosen. The determination of THC, CBD and CBN were carried out by a GC-MS method routinely employed in our laboratory.

2.5 Method Validation

Dried leaves of industrial *Cannabis Sativa* (THC and CBD < 0.2% w/w) were used as a blank matrix. A standard calibration curve was prepared by analyzing six calibrators at 0.5, 1.0, 2.5, 5.0, 7.5 and 10 mg/g final concentration. The occurrence of possible interferences from endogenous substances present in the herbal material was checked by monitoring the signal to noise ratio (S/N) for the MDMB-4en-PINACA diagnostic ions at the expected retention time. LOD values were estimated as the analyte concentration whose response provided a S/N value equal to 3, as determined from the least abundant among qualifier ions. Estimated LOD numerical value was extrapolated from S/N values of the lowest concentration level (LCL) using the corresponding calibration curve. This calculated LOD was then experimentally confirmed by analyzing spiked samples with MDMB-4en-PINACA. The LOQ value was initially estimated as the analyte concentration whose response provided an S/N value equal to ten. The operational LOQ was set at the lowest concentration of the calibration curves (0.5 mg/g), which was positively tested for precision and accuracy requirements, as reported below. Within-batch precision (expressed as percent variation coefficient, CV%) and accuracy (expressed as bias %), were assessed by extracting and analyzing a series of five blank matrix samples fortified with MDMB-4en-PINACA at the LCL. Acceptance criteria acceptance criteria were set as < 20% coefficient of variation for the precision and < ±20% for bias.

3. RESULTS AND DISCUSSION

The calibration plot showed good linearity in the range 0–100 mg/L, with a determination coefficient of 0.999. The SIM chromatograms from five blank samples showed no interfering signals (i.e., S/N ratio lower than 3) at the retention time of MDMB-4en-PINACA, indicating that the method is selective and free from matrix interferences. The calculated LOD was 0.1 mg/L and the LOQ was fixed at 0.5 mg/g. The results show a satisfactory within-batch precision (CV%: 4.6) and accuracy (bias%: -12.4) at 0.5 mg/g.

The MDMB-4en-PINACA was detected in 12 out of 358 samples (3.4% of the total). Among these, the molecule was found in 11 vegetal materials and in one resin sample. Considering solely the analysis of the 213 herbal products, a positive rate of 5.2% was found for the presence of MDMB-4en-PINACA in these samples. MDMB-4en-PINACA was found in the seized material at a concentration ranging between 0.04 and 0.63% w/w (0.4-6.3 mg/g; mean 2.5 mg/g; median 1.7 mg/g). Moreover, all the tested samples showed the presence of the phytocannabinoids THC and CBD. The THC concentration was in the interval 0.3-4.3 % w/w (3-43 mg/g; mean 12 mg/g; median 7 mg/g), while CBD was found at higher concentrations in all specimens, specifically in the range 4.7-14.0% w/w (47-140 mg/g; mean 87 mg/g; median 80 mg/g). These findings suggested that cannabis-derived products are coming mainly from CBD-prevalent Cannabis recently spread as industrial hemp in several countries. The positive cases were summarized in Table 1. Considering the vegetal material, in 10 cases the amount of the seized sample was in the range of 0.2-28 g (mean 5.1 g; median 2.7 g), suggesting the possession of the material for personal use. In the remained case, around 5 kilos of Cannabis flowers were seized by law enforcement.

Table **1**. Summary of the cannabis-derived material positive to MDMB-4en-PINACA.

Case ID	Type	THC (% w/w)	CBD (% w/w)	MDMB-4en-PINACA	
				(% w/w)	(mg/g)
1	Dry herb	0.7	8.0	0.14	**1.4**
2	Dry herb	0.7	8.1	0.25	**2.5**
3	Dry herb	0.5	7.1	0.06	**0.6**
4	Dry herb	0.3	5.7	0.70	**0.7**
5	Dry herb	0.7	9.2	0.63	**6.3**
6	Dry herb	0.6	6.0	0.05	**0.5**
7	Dry herb	0.7	11.3	0.55	**5.5**
8	Dry herb	4.3	4.7	0.12	**1.2**
9	Dry herb	0.8	9.8	0.20	**2.0**
10	Dry herb	3.2	5.9	0.17	**1.7**
11	Dry herb	1.7	14.4	0.40	**0.4**
12	Resin	0.5	14.1	0.43	**4.3**

A recent study published by Oomen et al. highlighted the occurrence of Cannabis-derived material adulterated with MDMB-4en-PINACA in eight different European countries (Austria, France, Germany, Italy, Luxembourg, Nederland, Switzerland and Wales) [6]. In particular, drug-checking services operating in Europe under the TEDI network (Trans European Drug Information network) reported that from the analysis of 1142 samples sold as cannabis in herbal, resin and e-liquid form in the period April 2020-April 2021, 270 turned out positive for the presence of MDMB-4en-PINACA. Moreover, all cannabis samples contained THC levels below 1% w/w, confirming that the main source of the adulterated plant material is industrial hemp which contains low levels of THC.

On the other hand, few data are currently available in the literature about the MDMB-4en-PINACA concentration in adulterated samples. Ozturk et al. reported the detection of MDMB-4en-PINACA in a herbal product from Turkey in April 2019 [10]. In a seizure of three 'Spice' samples that occurred in Manchester (UK) in July 2020, MDMB-4en-PINACA was identified at a concentration between 1.56% and 2.09% w/w (15.6-20.9 mg/g) [9]. Liu et al. reported the presence of MDMB-4en-PINACA in an herbal blend seized by the local police of Beijing (China) at a concentration of 3.3 mg/g (analysis by GC-MS) [11]. Recently, considering the increased diffusion of vaping, e-cigarette liquids adulterated with SCRAs appeared on the market sold as "e-Liquids". Moreover, impregnated papers are becoming an alternative way of drug consumption, including SCRAs [4]. During the analysis of 360 seized paper samples from 3 Scottish prisons in the period June 2018-September 2019, 22 samples (6.1%) were found to contain MDMB-4en-PINACA. The reported concentration was in the interval <0.07–0.58 mg/cm^2 [12]. In September 2020, MDMB-4en-PINACA was detected in 97 separate seizures of

impregnated papers. MDMB-4en-PINACA was detected alone, or in combination with other SCRAs, namely 4F-MDMB-BINACA, 5F-MDMB-PICA, 4F-MDMB-BINACA, 5FMDMB-PICA, and 5F-EMB-PICA [9]. Although MDMB-4en-PINACA has not been formally studied in humans, recent in vitro studies revealed the high affinity of this molecule for the CB1 receptor agonist [13,14]. Because of its high potency and the high doses that unaware users may be exposed to, a high risk of severe poisonings due to MDMB-4en-PINACA intake exists, as documented by multiple fatal/non-fatal intoxication cases involving MDMB-4en-PINACA recently described in the literature [9,14,15].

CONCLUSIONS

In this study we reported the presence on the market of cannabis products adulterated with the synthetic cannabinoid MDMB-4en-PINACA. GC-MS analysis demonstrated to be a valid tool for screening the presence of MDMB-4en-PINACA in seized material and for its quantitation. Considering the high potency of this molecule, a risk for an unintentional or unaware intake of MDMB-4en-PINACA among cannabis users exists, with high risk of intoxication for this population.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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