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Analytically confirmed synthetic cannabinoid 5F-ADB sprayed on “herbal mixture”

I. D. Ivanov[1,2,*], S. S. Stoykova[1,2], N. T. Burdzhiev[3], I. N. Pantcheva[1], V. N. Atanasov[1,2]

[1] *Sofia University “St. Kliment Ohridski”, Faculty of Chemistry and Pharmacy, Department of Analytical Chemistry, 1, J. Bourchier Blvd., 1164 Sofia, Bulgaria*

[2] *Military Medical Academy, Department of Toxicology, Analytical Toxicology Laboratory, 3, St. G. Sofiiski Str., 1606 Sofia, Bulgaria*

[3] *Sofia University “St. Kliment Ohridski”, Faculty of Chemistry and Pharmacy, Department of Organic Chemistry and Pharmacognosy, 1, J. Bourchier Blvd., 1164 Sofia, Bulgaria*

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Synthetic cannabinoids (SCs) are new psychoactive substances, called “legal” alternative to cannabis. Their structures differ from that of Δ^9-tetrahydrocannabinol (THC), but mimic its biological effect. On the “black" market, SCs appear sprayed on "herbal mixtures" intended for smoking. It is well known that SCs are more potent cannabinoid receptors (CB_1 / CB_2) agonists in human body than THC and possess wide and severe toxicity pattern. Pharmacokinetics, pharmacodynamics and toxicity profile of most of SCs are not well studied although it is already known that they may pose a risk to human health.

In the present study we report five cases of seized “herbal mixtures” containing synthetic cannabinoid 5F-ADB (5F-MDMB-PINACA) which was identified by gas chromatography-mass spectrometry (GC-MS) and confirmed by nuclear magnetic resonance (NMR).

Keywords: *“herbal mixture”, synthetic cannabinoids, 5F-ADB, GC-MS, NMR*

INTRODUCTION

Synthetic cannabinoids (SCs) are chemical compounds which when inhaled, imitate pharmacological effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) – the main psychoactive ingredient in marijuana. Firstly, SCs appear on drug market in 2008 [1, 2], and since then hundreds different products containing various synthetic cannabinoids are annually sold. Therefore, these substances became the biggest part of the family of new psychoactive drugs [3]. Typically, the products containing SCs, are traded as powders, “herbal mixtures” or cigarettes [4, 5]. Identification of new synthetic cannabinoids is a challenge for national and international drug control due to their structural diversity (a large assortment of chemical structures which is difficult to properly identify) and unpredictable risk to human health (most of SCs are pharmacologically uncharacterized).

Generally, chemical structure of SCs consists of four major fragments – heterocyclic nucleus (core), side chain (tail), linker and lipophilic substituent (Fig. 1). In most cases heterocyclic nucleus is an indole or azaindole (indazole, benzimidazole, pyrrolopyridine), although pyrroles, napthalenes, and thiazoles have been also reported. The core is bound *via* N-atom to the side chain built by alkyl, alicyclic, heterocyclic, aromatic, or heteroaromatic moieties [6-9]. The heterocyclic nucleus and lipophilic substituent are connected through a linker, which is ketone, ester or amide group, and in some cases - alkyl ether or thiazole. A large number of lipophilic substituents exists as 8-hydroxyquinoline, naphthalene, amino acid ester, etc. [7, 10-12].

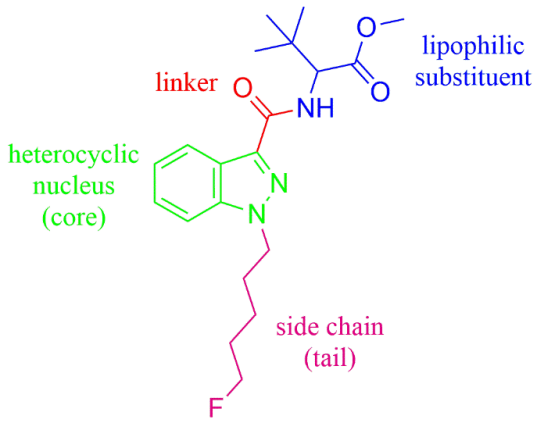

Fig. 1. Chemical structure of 5F-ADB (the four major fragments are colored differently).

The current study presents five cases of seized “herbal mixtures” in Bulgaria within two-year

* To whom all correspondence should be sent.
E-mail: ahidi@chem.uni-sofia.bg

period (2017-2018). The synthetic cannabinoid 5F-ADB (5F-MDMB-PINACA; methyl-S-2-[1-(5-fluoropentyl)-1*H*-indazole-3-carboxamido]-3,3-dimethyl butanoate, Fig. 1) was identified and analytically confirmed as an active component of the dried plant materials. It belongs to the indazole-3-carboxamide family of SCs and first appeared in Europe in Hungary (January 2015) [13]. 5F-ADB shows strong agonistic activity at both human cannabinoid receptors (CB_1 and CB_2) and may significantly threaten the human health. Recently it was banned for usage and added to List I of Regulation on Classification of plants and substances as narcotics in Bulgaria.

EXPERIMENTAL

Reagents

Chromatographic grade methanol (MeOH) and ethyl acetate (EtOAc), as well as chloroform-d ($CDCl_3$) were purchased from Sigma-Aldrich (Germany).

Herbal mixtures

Herbal products seized in course of criminal investigation (five different cases) were provided for forensic study of drugs and toxic chemicals to the Analytical Toxicology Laboratory (Military Medical Academy, Sofia). "Herbal mixtures" represent unknown greenish, brownish or yellowish dried crushed plant materials with pungent smell.

GC-MS identification of active ingredients of herbal mixtures

Due to inhomogeneous distribution of possible physiologically active ingredients on "herbal mixtures", in each case a composite sample (50 mg) from different sampling points was prepared. Then MeOH (2 mL) was added, mixture was vortexed for 1 min and sonicated for 20 min. After centrifugation (3000 rpm / 5 min), the organic layer was filtered off through syringe filter (0.45 μm pore size, Millex-FH, Merck-Millipore, Germany) into a test-tube and evaporated to dryness under nitrogen. The dried extract was dissolved in EtOAc at final volume of 100 μL. 1 μL of the solution was analyzed by GC-MS (Agilent 7890B/5977A, a DB-1701 capillary column with 30 m length, 0.25 mm i.d., and 0.25 μm film thicknesses, Agilent Technologies, USA). The injector was operated in splitless mode at 270 °C. Helium gas was used as a carrier gas at a flow rate of 1.5 mL/min. The oven temperature was held at 50 °C for 5 min, ramped to 290 °C at a rate of 30 °C/min and held for 30 min. The MS detector temperature was set to 230 °C and electron energy was 70 eV. Data were collected at scan mode (50-550 m/z) and analyzed with Agilent MassHunter Workstation software (Agilent Technologies, USA). The compounds were identified by mass spectral library search (Cayman Spectral Library 2016 [14]).

NMR confirmation of sprayed ingredient on herbal mixtures

The presence and the structure of the synthetic cannabinoid identified by GC-MS analysis were additionally confirmed by NMR spectroscopy (Avance III HD 500 MHz; Bruker, Germany). Each composite sample (250 mg) was extracted in scale as described above. The resulting dried extract was reconstituted with 600 μL $CDCl_3$, filtered off through syringe filter (0.45 μm pore size) and transferred into an NMR tube. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded. Chemical shifts were adjusted to the $CDCl_3$ residual signals at δ = 7.26 ppm (^{1}H) and at δ = 77.16 ppm (^{13}C), respectively [15, 16]. Full band shape analysis of ^{1}H-NMR spectra was performed using TopSpin 3.5pl7 package (Bruker, Germany).

RESULTS AND DISCUSSION

Identification of SCs in "herbal mixtures" represents a major challenge for forensic chemists and toxicologists due to rapid change of their structures and dynamic appearance of new synthesized compounds. In the present study, five different herbal products were submitted for forensic expertise.

At first, a GC-MS screening of MeOH extracts from each of the provided "herbal mixtures" was performed. In each of the resulting total ion chromatograms, a peak with retention time at 16.1 min was detected. The compound was identified by comparison of the obtained mass spectral data with reference data from Cayman Spectral Library. Thus, the active substance in the five herbal products seized was identified as 5F-ADB. It should be mentioned that the identified SC possesses temperature-stable core skeleton and does not undergo thermal degradation during GC-MS analysis to produce analytical artifacts like 1*H*-indole-3-carboxylate compounds [17, 18]. Representative total ion chromatogram of "herbal mixture" and corresponding mass spectrum are shown on Fig. 2 and Fig. 3, respectively.

The presence of 5F-ADB in "herbal mixtures" and its chemical structure were confirmed by ^{1}H- and ^{13}C-NMR spectroscopy. Both spectra contain also additional signals due to water-insoluble compounds derived from the plant materials. However, all chemical shifts and multiplicities assigned to SC correspond to those previously reported in the literature [8, 9]. Representative ^{1}H-NMR spectrum (Fig. 4) clearly confirms the presence of the synthetic cannabinoid 5F-ADB

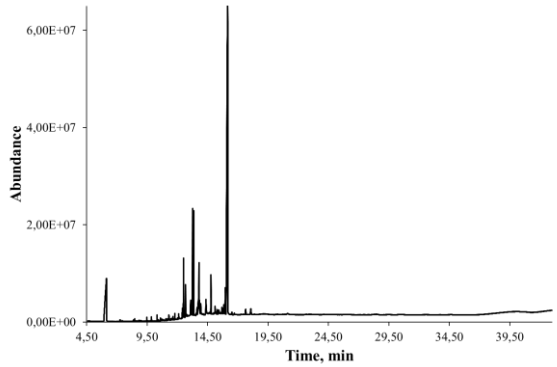

Fig. 2. Representative total ion chromatogram of methanol extract of "herbal mixture".

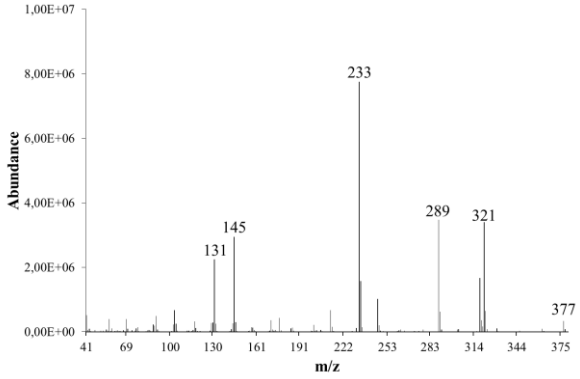

Fig. 3. The mass spectrum of the peak eluted at Rt = 16.1 min.

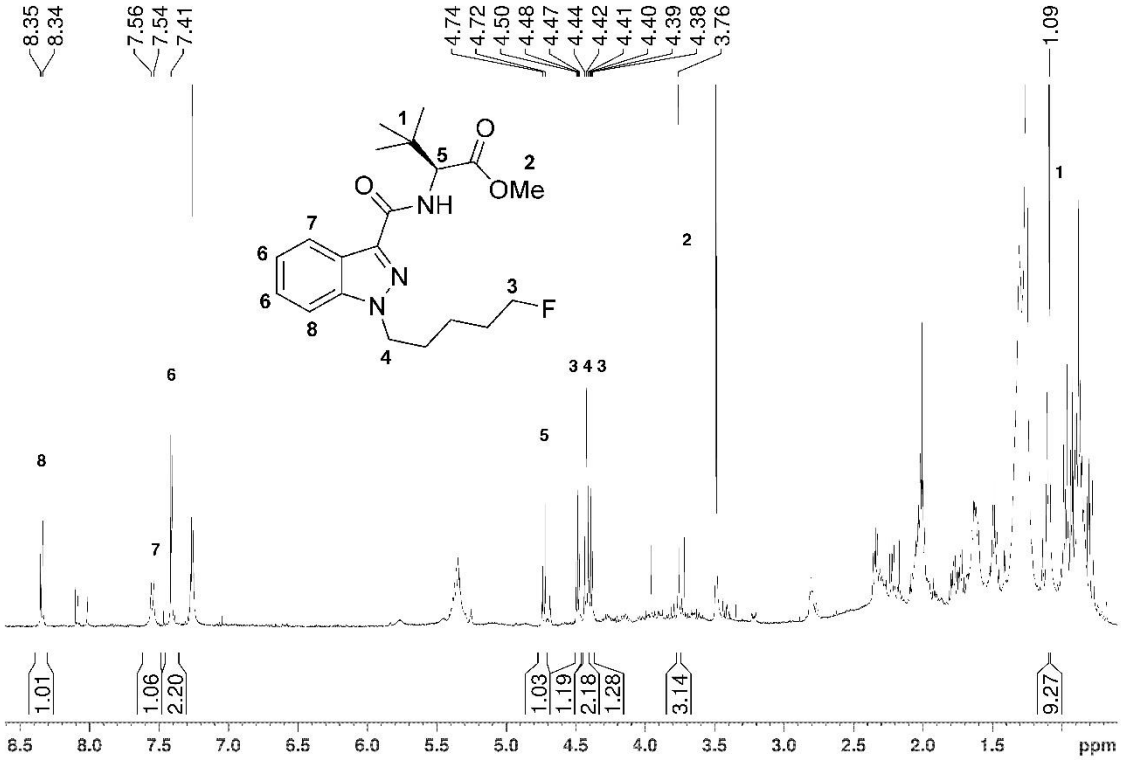

Fig. 4. ^{1}H-NMR spectrum of "herbal mixture" extract.

The singlets for protons belonging to methyl and ester methyl groups in lipophilic substituent were observed at 1.09 ppm and 3.76 ppm, respectively. The signals for protons of CH_2F group were recorded as doublet of triplets (4.43 ppm) due to the spin coupling of fluorine. The doublet at 4.73 ppm was assigned to the α-hydrogen of the amino acid moiety. Protons of indole heterocycle were observed at 7.42 ppm (singlet), 7.54 ppm and 8.35 ppm (doublets), respectively.

On the other hand, the ^{13}C-NMR spectra of herbal extracts are not informative as corresponding ^{1}H-NMR data due to the presence of a number of signals attributed to the carbon atoms of unknown ingredients of the plant materials, small amount of provided "herbal mixtures" and low concentration of sprayed synthetic cannabinoid.

In the all five cases studied, identification of 5F-ADB using GC-MS and confirmation of its structure by ^{1}H-NMR spectroscopy were efficient for the purposes of toxicological expertise. GC-MS is an analytical method, which provides chromatographic and mass spectral data for identification and characterization of unknown compounds. In addition, the data obtained by second independent analytical method (NMR spectroscopy) corroborate well with the findings achieved by GC-MS technique. Combination of these two methods is a powerful tool for identification and characterization of unidentified substances, as well as for

confirmation of presence of already known chemical compounds.

CONCLUSIONS

Five different "herbal mixtures" seized as criminal evidence in the country were analyzed for the presence of drugs, presumably sprayed on plant materials. In all cases plant materials contained the synthetic cannabinoid 5F-ADB. Identification and structural characterization of SCs are important stages in the case of analysis of new synthesized physiologically active substances in herbal blends intended for smoking.

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