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Review Article

Detection of some synthetic cannabinoids (FUB-AMB and AB-FUBINACA) in blood and urine using gas chromatography-mass spectrometry liquid–liquid extraction

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Abstract

In recent years, various types of synthetic cannabinoids have become widely distributed and are causing social and health problems in most parts of the world. Synthetic cannabinoids are currently the largest group of new psychoactive substances. Those that have been subjected to legal control are replaced by newer controlled and uncontrolled substances. Some of the most recent synthetic cannabinoids that have distributed on the market among youth are FUB-AMB and AB-FUBINACA. This study quantified blood and urine of two cases smoking tobacco mixed with AMB-FUB 0.06-0.03 ng/mL and 1.7-2.9 ng/mL AB-FUB in urine and blood respectively.

Keywords: Synthetic cannabinoids, FUB- AMB, AB-FUBINACA, Blood and Urine, GC MS

Introduction

Synthetic cannabinoids are currently the largest, most diverse and fastest growing group of new psychoactive substances.

Many of the early synthetic cannabinoids that were synthesized for use in research were named after either the scientist who first synthesized them or the institution or company where they originated. Now many synthetic cannabinoids are assigned names derived from their chemical names.

The natural cannabinoid with the strongest binding affinity to the CB1 receptor, which is linked to the psychoactive effects or "high" of marijuana [1].

These synthetic analogs often have greater binding affinity and greater potency to the CB1 receptors. There are several synthetic cannabinoid families (e.g. CP-xxx, WIN-xxx, JWH-xxx, UR-xxx, and PB-xx) classified based on the base structure [2]. Synthetic cannabinoids are a class of molecules that bind to cannabinoid receptors in the body (the same receptors to which THC and CBD attach, which are cannabinoids in cannabis plants). They are designer drugs that are commonly sprayed onto plant matter [3].

Most synthetic cannabinoids are agonists of the cannabinoid receptors. They have been designed to be similar to THC [4].

In the late 2000s, synthetic cannabinoids were identified by laboratories in "Spice" or "K2" and related herbal incense products. The compounds found in the "first generation" of synthetic cannabinoid products were primarily the C8 homologs of the nonclassical cannabinoid CP-47,497 and the aminoalkylindole, JWH-018, both of which are agonists of the CB1 and CB2receptors. The binding affinity of the synthetic cannabinoids to the CB receptors is dependent on the compound. For example, JWH-018 has a binding affinity for the CB1 receptor that is four times greater than that of THC and approximately ten times higher than THC for the CB2 receptor [5,6].

There are five major categories of synthetic cannabinoids: classical cannabinoids, non-classical cannabinoids, hybrid cannabinoids, aminoalkylindoles and eicosanoids. The indazole carboxamide including APINACA, an adamantyl indazole carboxamide, and AB PINACA, an aminocarbonyl indazole carboxamide, is an example of a new group of synthetic cannabinoids [7]. Synthetic cannabinoids are metabolized via cytochrome P450 enzymes resulting in phase I hydroxylated metabolites [8]. An alkyl side chain, when present, appears likely to undergo hydroxylation at several positions. Compounds fluorinated at the 5 position are also susceptible to oxidative defluorination and hydroxylation [9].

Metabolism of AB-PINACA by human liver microsomes suggested hydroxylation occurred primarily on the pentyl chain [10]. Synthetic cannabinoids were originally synthesized to investigate the endocannabinoid system or for their potential therapeutic benefits, but none progressed to clinical use, they are related to chemicals found in the marijuana plant. Because of this similarity, synthetic cannabinoids are sometimes misleadingly called "synthetic marijuana" (or "fake weed"), and they are often marketed as "safe," legal alternatives to that drug. These compounds are either sprayed on dried, shredded plant material so they can be smoked (herbal incense) or sold as liquids to be vaporized and inhaled in electron cigarettes and other devices (liquid incense).

The greater addictiveness and more severe adverse effects of synthetic cannabinoids in comparison to marijuana are thought to stem from the fact that many of the synthetic cannabinoids are full agonists to the cannabinoids receptors, CB1 and CB2, compared to THC, which is only a partial agonist [11].

Mechanism of Action

Synthetic cannabinoids are referred to as substances with structural features which allow binding to one of the known cannabinoid receptors, CB1 or CB2, present in human cells. The association of synthetic cannabinoids with the above chemical receptors leads to an increase in the concentration of another neurotransmitter known as dopamine.

When the concentration of dopamine in the brain rises, it causes a feeling of deformity after the use of these substances, and as is known as high dopamine. The expansion of the blood vessels also leads to increased body temperature and increased sweating. Dopamine is also responsible for awareness, motivation and feeling. This explains the inability of the abusers to perceive the time or to distinguish the night from the day or even the inability to remember what he was doing and what he did and this may continue to eliminate the effect of the drug. The target analyte in blood is the parent drug. Most synthetic cannabinoids are further metabolized to hydroxyl-synthetic cannabinoids. These hydroxyl metabolites are excreted in urine almost as glucuronides and should be hydrolysed in order to obtain the free metabolite.

In contrast to the pharmacological properties of synthetic cannabinoid metabolites, their toxicological properties remain less-well characterized, though some work has been done. In the present study, we developed a gas chromatography-mass spectrometry liquid–liquid extraction to determined FUB- AMB and AB-FUBINACA in the blood and urine.

AMB-FUBINACA

AMB-FUBINACA is the methyl ester analogue of AB-FUBINACA, where the terminal amide group of the 1-amino-3-methyl-1-oxobutan-2-yl is replaced with a methyl ester group.

AMB-FUBINACA (also known as FUB-AMB and FUB-MMB) N-[[1-[(4-fluorophenyl)methyl]-1H-indazol-3-yl]carbonyl]-L-valine, methyl ester.

AB-FUBINACA

AB-FUBINACA is classified as an indazole. AB-FUBINACA is based on an indazole core structure where the 1- and 3-positions of the indazole ring system are substituted.

AB-FUBINACA can also be written as AB-FUB N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide (Figures 1 and 2).

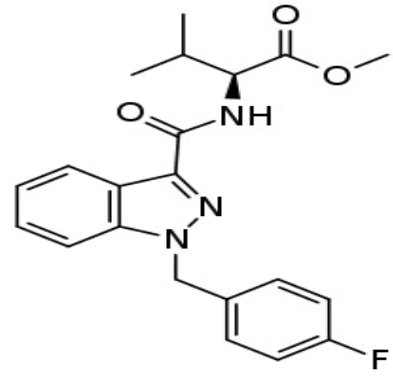

Figure 1: Chemical structure of AMB- FUBINACA.

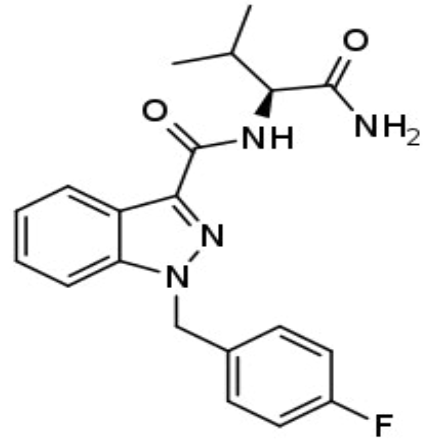

Figure 2: Chemical structure of AB-FUBINACA.

Materials and Methods

Chemicals and reagents

Standards of the synthetic cannabinoid panel were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Ammonium sulfate, hydrochloric acid, aqueous ammonia, Ethyl acetate were purchased from Algomhoria Co.

Urine preparation

Hydrolyses: 2.5mL of urine was added to 0.3mL of hydrochloric acid (20%) and heated at 60-70°C for 60 min.

Extraction: After cooling, aqueous ammonia (25%) was added to reach a pH of 8–9. The samples were extracted with 10mL of ethyl acetate and centrifuged. The organic phase was evaporated by nitrogen stream at 45°C or below.

Blood preparation

1- To 2 ml of Blood 5 ml of saturated solution of ammonium sulfate and few drops of HCl (0.1M) were added.

2- Vortexed for 3 min and then centrifuged for 5 min. After that then supernatant was collected.

Extraction

3- Drops of aqueous ammonia (25%) was added till pH becomes 8-9.

4- 20 ml of ethyl acetate was added and the mixture was shake n for 4 min using falcon tube.

5- The organic layer was transfered into glass tubes and evaporates it by nitrogen stream at 45°C.

Instrumental

Gas chromatography (Agilent 6890) coupled to a mass spectrometry detector (Agilent 5973) with column HP 5-MS, (0.25mm × 60m × 0.25μm) non-polar chromatographic capillary column (Agilent Technologies).

Column

Agilent HP-5-MS column; 0.25mm × 60m × 0.25μm capillary, 60m × 250μm × 0.25 μm nominal.

Mode: Constant flow

Gas type: Helium

Helium pressure: 27.5 Psi

Helium flow: 1.4 ml/min

The separation was performed by applying the following thermal program

Oven temperature

Initial temperature: 100°C, hold time: 2 min, ramp 50°C/min to 280°C, hold time 14.45: Run time 22.6min

Inlet

Mode: Splitless

Temperature: 250°C

Pressure: 27.5Psi

Total flow: 1.4 ml/min

Gas saver: Off

MS spect

Aux temperature: 280°C

Resolution mode: SCAN

MS source temp: 230°C

MS quad temp: 150°C

Solvent delay: 5min

Retention time: 14.43min. ± 2%

Resolution mode Ions

The characteristic ions are 109,253,324 and 145

The Selected ion group used for qualification analysis for analyte to match with GC-MS Cayman Spectral library.

Injector

Injection volume 2 μL

Syringe size :10 μL (Auto or Manual injection)

Result and Discussions

FUB- AMB and AB-FUBINACA are synthetic cannabinoids of the class of substituted indazole-3-carboxamides.

First, the blood and urine were examined for the absence of narcotic drugs and psychotropic substances from the groups of amphetamines and its derivatives, benzodiazepine derivatives, cannabinoids, opioids and cocaine.

Urine is the preferred specimen because the collection of urine samples is easy and non-invasive, and the concentrations of analytes are often higher when compared with sample of blood or oral fluid. Moreover, the detection time in urine is longer than blood (days or weeks).

The parent compounds may not be detectable in urine. Hydrolysis with acid is required in the procedure of urine sample preparation for FUB- AMB and AB-FUBINACA and other synthetic cannabis compounds because these were found conjugated form excreted.

Blood used for the determination of the concentrations and ratios of parent and its metabolite that could yield useful information relating to acute or chronic use. But protein blood precipitation has been found to be more useful without precipitation. Blank control of the sample solutions without the target FUB- AMB and AB-FUBINACA of urine and blood were not detected in the solutions used as control blank.

The characteristic fragment ions and the fragmentation pathways of two synthetic cannabinoids FUB- AMB and AB-FUBINACA were detected in urine and blood, parent structure were analyzed carefully as shown in Figures 3-6.

Urine and blood samples from two men aged 35 and 44 years, smoking synthetic cannabis after putting in tobacco were collected. Results shown concentration of AMB-FUB in urine - 0.06 and

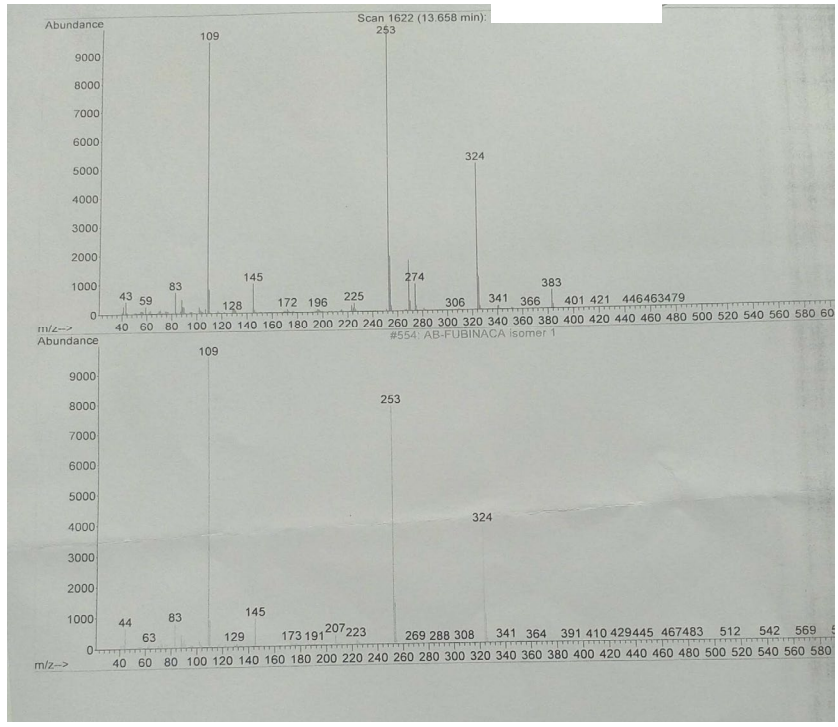

Figure 3: main fragmentation of AB- Fubinaca in Urine (LLE).

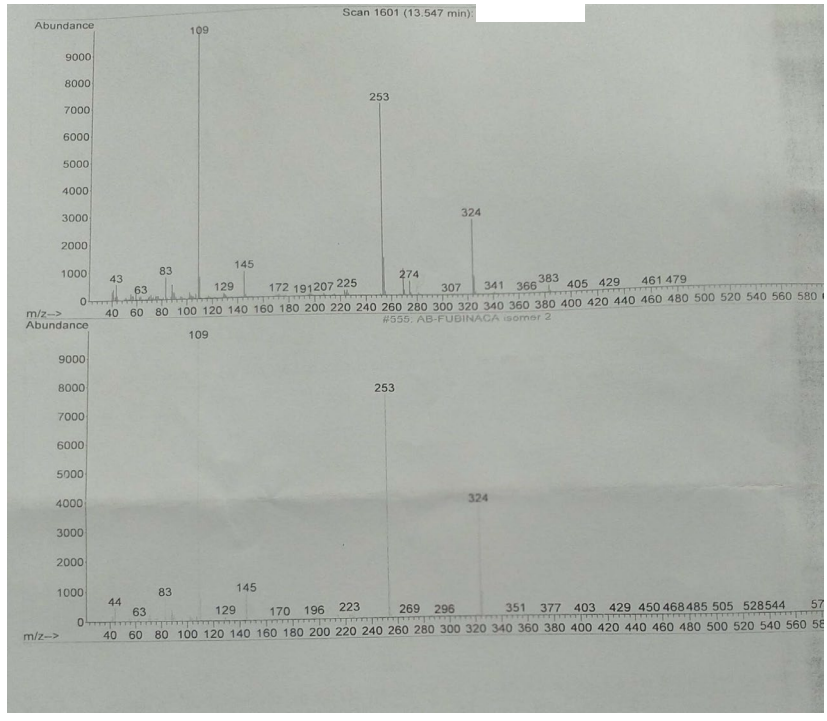

Figure 4: main fragmentation of AB- Fubinaca in Blood (LLE).

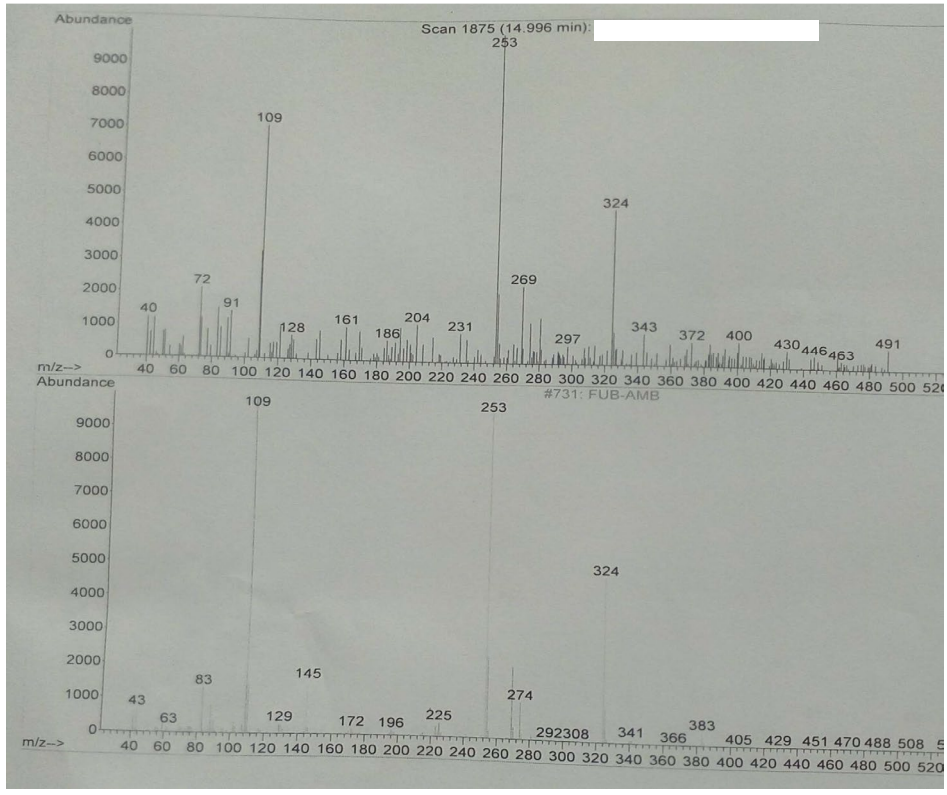

Figure 5: main fragmentation of FUB- AMB in Urine (LLE).

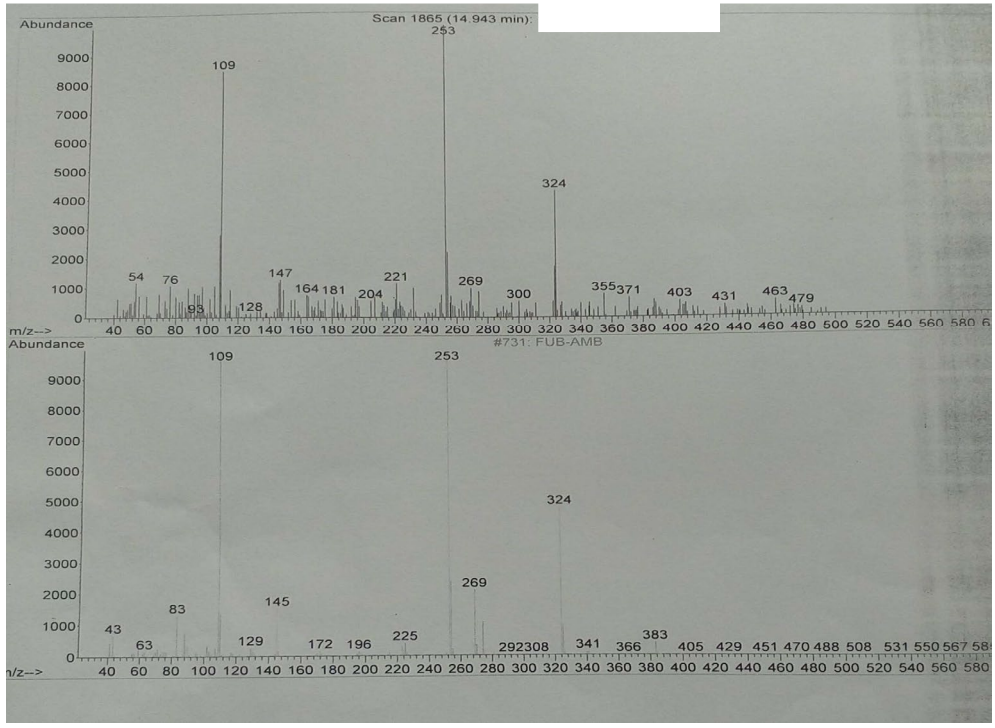

Figure 6: main fragmentation of FUB- AMB in Blood (LLE).

blood of AMB-FUB 0.03 ng/mL while urine concentration of AB-FUB is 1.7 and blood is 2.9 ng/mL.

Conclusion

The presented study of FUB- AMB and AB-FUBINACA are dangerous to health and may lead to fatal intoxication.

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