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Identification of Synthetic Cannabinoid 5F-ADB (5F-MDMB-PINACA) and Its Metabolite in Urine Sample Using Liquid Chromatography–High Resolution Mass Spectrometer (LC-HRMS)

Rieska Dwi Widayati*, Tanti, Erlana Nindya Maulida, Martin Luther Silubun

Quality and Research Division, The Center of Narcotics Laboratory of National Narcotics Board Republic of Indonesia. Jl. HR Edi Sukma Km 21, Ds. Wates Jaya, Cigombong, Bogor, West Java

Corresponding email:* rieska.dwi@bnn.go.id

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ABSTRACT

Synthetic cannabinoids are commonly known as Gorillas Tobacco, Hanomans Tobacco or Ganeshas Tobacco in Indonesia. Those products are camouflaged as a tobacco related to the number of smokers in Indonesia. The 5F-ADB (5F-MDMB-PINACA) has become an issue since 2016. It was undetectable by conventional drug testing methodology such as immunoassay method. GC-MS as a routine method analysis is not recommended also for detecting the metabolites from biological specimen with low concentration. The paper report LC-HRMS based method for identification of 5F-ADB and its metabolites in urine sample. Various of volume injections (1, 3, and 6 µL) was studied. Sample was acidified with concentrate of HCl, then undergo extraction with EXtrelut® Column NT3 prior to LC-HRMS analysis. The full method was operated for MS/dd-MS2 identification. The 5F-ADB and its ester hydrolysis metabolite, 5F-ADB metabolite 7 ($C_{19}H_{27}FN_3O_3^+$) was detected in urine sample.

Keywords**:** synthetic cannabinoids**,** EXtrelut® column NT3, LC-HRMS**,** 5F-ADB**,** 5F-ADB metabolite 7

INTRODUCTION

Rapid increasing in the number, type and availability of new psychoactive substances (NPS) across the globe had become global issues. NPS are a new threat for Indonesia and the rest of the world. United Nations Office on Drugs and Crime through World Drug Report 2019 confirmed 892 NPS identified from 2009 to the end of 2018 [1]. A continues increasing of NPS on the illicit market in Indonesia is also observed. Up to February 2020, 76 NPS were identified and 72 of them have been scheduled in Indonesia due to high potential of abuse and no medical benefits. From the total 76 NPS identified, 28 of them are synthetic cannabinoid of class compounds.

The synthetic cannabinoid is the most popular NPS substance group among others drugs in Indonesia. It is available in powder and liquid form, but also contained in tobacco products by soaking or spraying the powder to the tobacco leaves. The primary route of administration is inhalation either by smoking the tobacco products directly or vaping a liquid through an e-cigarette [2]. Users reported experiences that similar to those produced by marijuana and in some cases the effects are even stronger than those of marijuana. moreover, the synthetic cannabinoid in tobacco products are being popular recently in Indonesia related to the number

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of tobacco smokers. Based on the Ministry of Health report in 2018, the smoker prevalence in the age population (10-18 years old) was 9.1%, a higher number than that in 2013 (7.2%) [3]. Thus, this ype of synthetic cannabinoid mixed to tobacco products rapidly changed following the trend of synthetic cannabinoid in global market.

A 5F-ADB belongs to synthetic cannabinoid receptor agonist (SCRA) with an indazole core. 5F-ADB is potent agonists of CB_1 and CB_2 receptors. Banister et al (2016) reported 5F-ADB has shown to be more potent than THC (almost 290x) [4]. The 5F-ADB was seized and analyzed for the first time in Indonesia in the year of 2016. And since then, it has been the most popular synthetic cannabinoid in Indonesia in just 2 years period.

The structure, spectra and predominate fragmentation patterns of 5F-ADB was reported by Richter et al (2019) using Liquid Chromatography-High Resolution tandem Mass Spectrometry (LC-HRMS/MS) [5]. Based on this study, MS2 spectrum of 5F-ADB had ion fragmentation at m/z 251.1184 (as a base peak), m/z 318.1973, m/z 378.2177, m/z 213.21022, m/z 233.1081 and m/z 145.0395 [5]. Moreover, Kusano et al. (2018) detected 3 metabolites of 5F-ADB in postmortem urine using LC/MS/MS and LC/Q-TOFMS. First ester hydrolysis (M1) was taken place, then, followed by oxidative defluorination (M2) and further oxidation process to produces the carboxylic acid (M3). Furthermore, Kusano et al also presented the data for accurate mass, exact mass and assigned formula of each metabolites of 5F-ADB [2,6].

In the previous study, our laboratory has identified several synthetic cannabinoids in urine sample with extrelute NT3 using GCMS. The synthetic cannabinoids could be detected as parent compound with no metabolites was identified. In the recent report, we developed a liquid chromatography–high resolution mass spectrometry (LC-HRMS) method for identifying the 5F-ADB and its metabolite in urine sample. The reference standard of 5F-ADB was used as comparison in qualitative analysis. In this report, the identified reference material which is powdered subtance found to contain 5F-ADB from the previous identification using FTIR and GCMS analythical technique was applied. A published MS2 spectrum of 5F-ADB by Richter et al. [5] was also used as comparison.

EXPERIMENTAL SECTION

Chemicals

Hydrochloric acid fuming 37%, chloroform and formic acid used were for analysis grade. Acetonitrile used was liquid chromatography grade while methanol and water used were hypergrade for LCMS. All solvents were purchased from Merck, Germany.

Instrumentation

Instrumentation applied for analysis is LC-HRMS. The LC-HRMS system was composed of a Thermo Scientific Dionex Ultimate 3000 RSLC system equipped with an analytical column Thermo Accucore Phenyl Hexyl 100 mm x 2.1 mm ID x 2.6 µm, coupled to Thermo Q Exactive Plus HRAM LC-MS/MS system. The whole equipment and the column were provided by Thermo Fisher Scientific (Milan, Italy).

The analytical column and sample manager temperatures were maintained at 40◦C and 5◦C, respectively. The mobile phases were composed of A (0.1% Formic Acid in Water) and B (0.1% Formic Acid in Acetonitrile). Separation was achieved using the following mobile phase gradient as follows: 0-1 min at 1% B and 1-18.5 min from 1% to 99% B. Each run was followed by 1.5 min wash with 99% B, decreasing to 1% B in 0.2 min and an equilibration period of 2.8 min with 1% B. Total run time was 23 min per analysis. Volume injections were 1, 3 and 6 µL and the flow rate was set at 0.5 mL/min.

Procedure

Preparation of identified reference material solutions

A 10 mg powder found to contain 5F-ADB was weighed and dissolved in 10 ml methanol, then it was diluted subsequently until 0.05 ppm with methanol. A 2.0 mL solution was filtered using PTFE filter prior to LC-HRMS analysis.

Sample preparation

A 5.0 ml of each blank urine and sample managed by the same preparation. Both of them made in acidic (pH 0–1) with concentrate hydrochloric acid. Then it was vortexed and incubated for 30 minutes at 70 °C. Allow the sample to reach room temperature, then simply applied to the EXtrelut® column NT3. Wait for 15-30 minutes to let the sample interacted with stationary phase. After the sample was dry, elution was taken place using 10 ml mixed of chloroform/methanol (85:15). The extracted sample fitted into vial and evaporated under stream of dry air of nitrogen. A dried residue was reconstituted with methanol and filtered using PTFE filter. Various volume (1, 3, and 6 µL) of sample subjected to LC-HRMS analysis.

RESULT AND DISCUSSION

Identification of 5F-ADB in urine sample

Qualitative analysis was carried out using LC-HRMS by comparing retention time and mass spectra between sample with the reference. Typical chromatograms are given, such in Figure 1. Both sample and identified reference material have similar retention time and mass spectra fragmentation. 5F-ADB and the reference was identified by LC-HRMS in the retention time 11.99 and 12.00 min, respectively. XCalibur and Free Style software with similarity to the library more than 90% was used to measure. Spectra contained fragment ion at m/z 251.12 (as base peak), 213.10; 145.04; 233.11 and 318.20 and it was similar to the mass spectra of 5F-ADB reported by Richter et al [5]. All the fragment ions except m/z 251.12 are the result of 5F-ADB fragmentation.

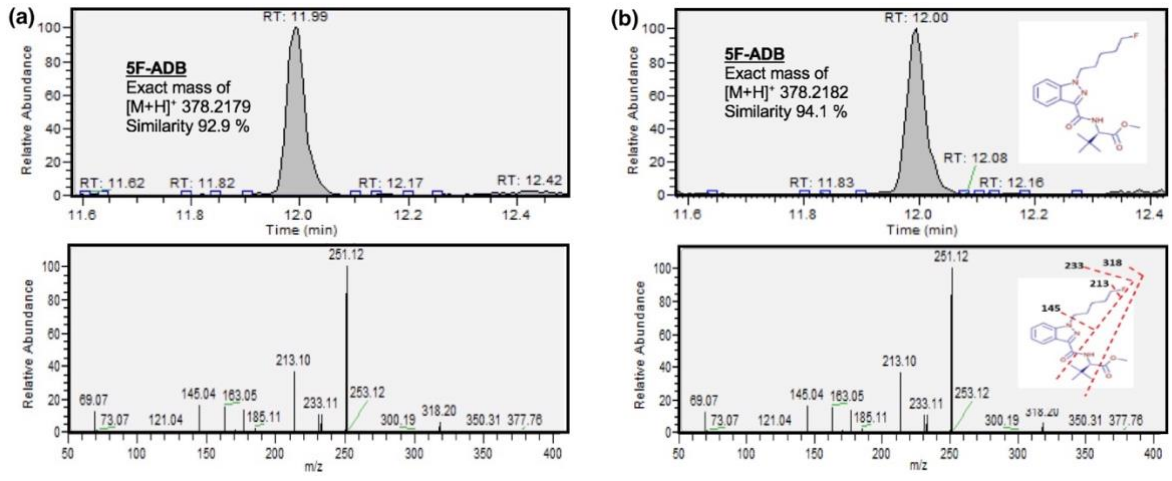

Figure 1. HRMS chromatogram and product ion spectra of (a) identified reference material of 5F-ADB and (b) urine sample.

A cleavage of the carbonyl of the ester group led to fragment ion at m/z 318.20. Fragment ion at m/z 233.11 subsequently was obtained from the cleavage of amide bond. Elimination of the fluorine atom in the terminal of fluoropentyl side provide fragment ion at m/z 213.10.

Furthermore, the loss of fluoro-pentyl side led to the fragment ion at m/z 145.04 (Figure 4). Moreover, fragment ion at m/z 251.12 undergo rearrangement produce m/z 378.22. This characteristic fragment ion was only described by Richter et al. [13].

Identification of 5F-ADB metabolite in urine sample

In this study, 5F-ADB metabolites will be observed. Exact mass data of three metabolites of 5F-ADB reported by Kusano et al. [14] was used as key data to find the presence of 5F-ADB metabolites in sample. The result indicates no metabolite of 5F-ADB can be detected under 1.0 µL volume injection of sample. Adding volume injection to 3 µL and 6 µL showed the signal. It was found that 5F-ADB metabolite 7 ($C_{19}H_{27}FN_3O_3^+$) can be identified through FTMS +pESI with Full ms and Full MS2 scan mode using XCalibur and Free Style software in both volume injections. 5F-ADB metabolite 7 is an ester hydrolysis product from 5F-ADB. It can be obtained from the first step of metabolic pathway of 5F-ADB (Figure 2).

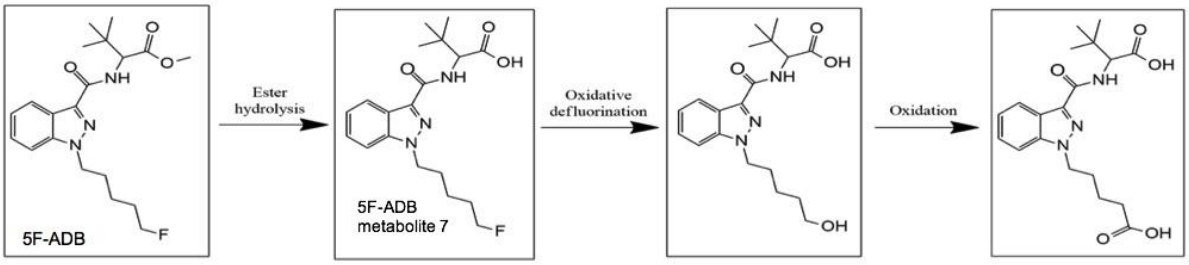

Figure 2. Proposed metabolic pathway of 5F-ADB in human (adapted from [14])

The 5F-ADB metabolic pathway is an oxidative defluorination reaction with the help of the CYP enzyme to obtain 5-hydroxypentyl metabolite ion, $[C_{19}H_{28}N_3O_4]^+$. However, this reaction must be preceded by ester hydrolysis reaction to produce intermediate metabolites, namely 5F-ADB methyl ester hydrolysis metabolite [14]. This intermediate metabolite could be detected as 5F-ADB metabolite 7 (read by mzCloud database), while the 5-hydroxypentyl metabolite could not be detected.

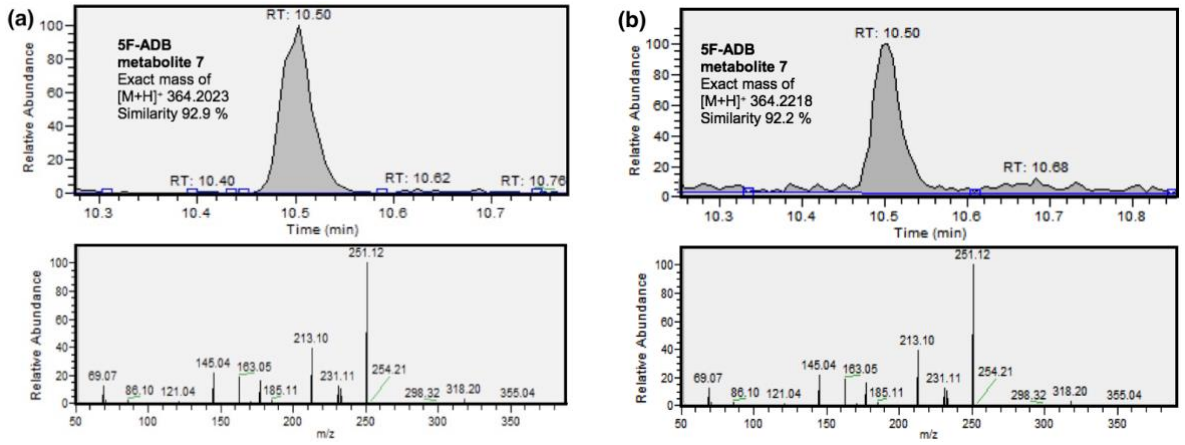

Figure 3. HRMS chromatogram and product ion spectra of 5F-ADB metabolite 7 in urine sample obtained from (a) 3 µL and (b) 6 µL volume injection

Peak of 5F-ADB metabolite 7 was observed in 10.50 min with similarity to the library more than 90%. The MS2 spectrum of 5F-ADB metabolite 7 (Figure 3) which was originated from ester hydrolysis contain the same fragment ions with the spectrum of the parent compound, 5F-ADB. The 5F-ADB and 5F-ADB metabolite 7 differ by ester and carboxylate group in the head of the structure. So that pose a different molecular weight which are 377.46 and 364.20, respectively. Further cleavage of carbonyl C atom from the ester and carboxylate, led to produce the same fragment ion at m/z 318.20 as well as the subsequent fragmentation (Figure 4). 5F-ADB metabolite 7 also experienced rearrangement reaction and obtained ion m/z 251.12 from ion m/z 364.20.

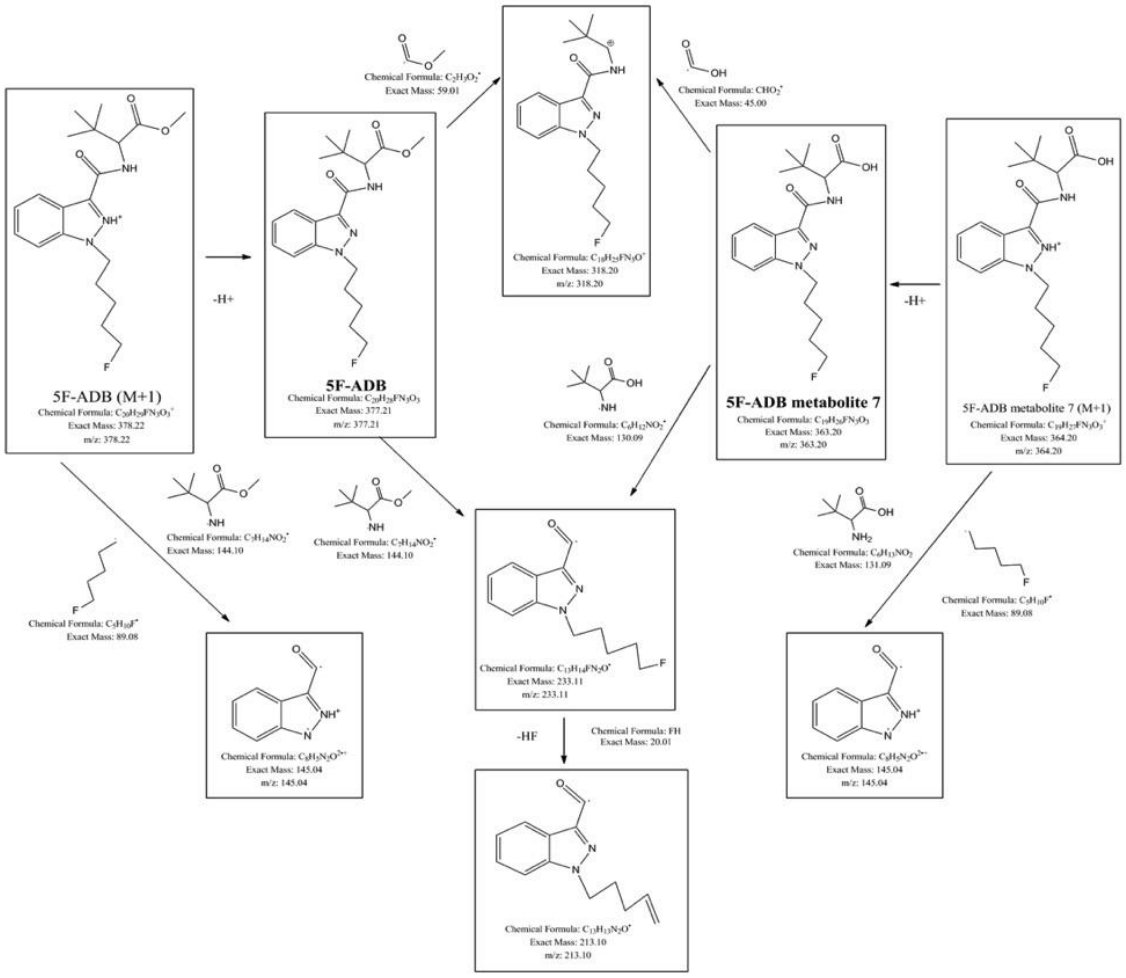

Figure 4. Proposed fragmentation pattern of 5F-ADB and 5F-ADB metabolite 7

Selection of volume injection

In this study, sample was subjected to LC-HRMS in three different volume injections (1, 3 and 6 µL) with 3 times repetitions for each volume injection. It was applied in this project to understand the impact of various volume injections to the result of analysis. Moreover, analysis was performed by calculating the relative standard deviation of area (RSD_{area}) obtained in each volume injection. Limit of detection (LOD) and limit of quantitation (LOQ) of 5F-ADB using LC-HRMS method was estimated 25 ppb and 50 ppb, respectively.

Table 1 is showed the quantification of each peak from chromatogram of 5F-ADB and 5F-ADB metabolite 7 in three difference volume injection. In all volume injection give a good acceptance with relative standard deviation (RSD_{area}) less than 5% in 3 µL and 6 µL volume injections. However, 5F-ADB metabolite 7 was not detected in all repetition of 1 µL volume injection. RSD_{area} for 5F-ADB metabolite 7 from three times repetition of 6 µL volume injection was higher than 5%. Thus, it is recommended to inject the sample in 3 µL volume injection to identify 5F-ADB and its metabolite in urine sample. Furthermore, 3 µL is also suggested to avoid carry over from higher volume injection.

Table 1. Area under curve of 5F-ADB and 5F-ADB metabolite 7 in each volume injection

Repetition	Area of 5F-ADB			Area of 5F-ADB metabolite 7		
	1 µL	3 µL	6 µL	1 µL	3 µL	6 µL
1	19583989	58299279	120623604	ND	22787196	47886845
2	19589418	58558922	117569150	ND	23090754	42428939
3	17609693	59679569	119142295	ND	23318079	46573304
Mean	18927700.0	58845923.3	119111683.0	-	23065343.0	45629696.0
SD	1141431.00	733537.62	1527457.08	-	266352.17	2848680.66
%RSD	**6.0305**	**1.2465**	**1.2824**	**-**	**1.1548**	**6.2430**

Note: Acceptance criteria of %RSD for precision parameter was adopted from UNODC [15] with modification by The Center of Narcotics Laboratory to the more stringent range of acceptance which is ≤5%. ND means not detected in the sample.

CONCLUSION

LC-HRMS method was able to detect 5F-ADB and its metabolite in urine sample. Metabolite detected was 5F-ADB metabolite 7 ($C_{19}H_{27}FN_3O_3^+$) which had undergone ester hydrolysis pathway. Volume injection 3 µl presented a good acceptance in relative standard deviation of area (RSD_{area} under 5%) in the 5F-ADB and 5F-ADB metabolite 7 identification.

CONFLICT OF INTEREST

Authors declare no any conflict of interest.

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