



Fatal intoxication with new synthetic cannabinoids 5F-MDMB-PICA and 4F-MDMB-BINACA—parent compounds and metabolite identification in blood, urine and cerebrospinal fluid

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Abstract

Synthetic cannabinoids (SCs) remain one of the largest groups of new psychoactive substances. Recently, new synthetic cannabinoids 5F-MDMB-PICA and 4F-MDMB-BINACA are increasing in popularity. A 33-year-old man lost consciousness after smoking an unknown substance. A glass pipe and two lumps of substance that turned out to contain 5F-MDMB-PICA and 4F-MDMB-BINACA were found at the scene. Blood, urine and cerebrospinal fluid were collected during the examination of the body. The synthetic cannabinoids were isolated from autopsy materials by precipitation with acetonitrile and extraction with ethyl acetate. The screening and quantitative analyses were performed by liquid chromatography with tandem mass spectrometry (LC–MS/MS). The liquid chromatography–quadrupole/time of flight mass spectrometry (LC–Q/TOF) technique was used for metabolite identification. 5F-MDMB-PICA was detected and quantified in all analysed materials, whereas 4F-MDMB-BINACA was found only in cerebrospinal fluid. The determined concentrations of 5F-MDMB-PICA were 0.9 (blood), 0.1 (urine) and 3.2 ng/mL (cerebrospinal fluid). The concentration of 4F-MDMB-BINACA in cerebrospinal fluid was 0.1 ng/mL. The main metabolites of both compounds (hydrolysis and oxidative defluorination) were found in all analysed body fluids. Cerebrospinal fluid may be important alternative material in autopsy cases. Rapid elimination of 5F-MDMB-PICA and 4F-MDMB-BINACA compounds also means that the metabolite analysis can be crucial for the investigation. Laboratories must be made aware of their presence and incorporate these SCs and their metabolites into workflows for detection and confirmation. Ester hydrolysis and oxidative defluorination products can be found in blood, urine and cerebrospinal fluid making them useful biomarkers of intake.

Keywords Synthetic cannabinoids · 5F-MDMB-PICA · 4F-MDMB-BINACA · Intoxication · Metabolites

Introduction

By October 2020, 820 new psychoactive substances (NPS) were monitored by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA), and approximately 1004 NPS have been reported to the United Nations Office on Drugs and Crime (UNODC). Synthetic cannabinoids (SCs), also known as synthetic cannabinoid receptor agonists (SCRAs), remain one of the largest groups of NPS.

Moreover, SCs still dominate among NPS seizures and are found in many intoxication cases in Europe [1, 2]. SCs share the ability to affect the cannabinoid receptors (CB₁ and CB₂) in the body, mimicking the effects of tetrahydrocannabinol (THC), the main psychoactive component of cannabis. These substances are highly efficacious and act as agonists at cannabinoid receptors, while THC is only a partial agonist. The extreme potency of many SCs entails a high risk of life-threatening intoxications to users and the possibility of causing outbreaks of mass poisonings [3].

Recently, new synthetic cannabinoids 5F-MDMB-PICA and 4F-MDMB-BINACA (Figs. 1 and 2) hit the market [4]. 5F-MDMB-PICA (5F-MDMB-2201; IUPAC name: methyl (2S)-2-([1-(5-fluoropentyl)-1H-indole-3-carbonyl]amino)-3,3-dimethylbutanoate) appeared in Europe and the USA in 2016 [5–7]. As of early 2019, it was the most prevalent synthetic cannabinoid in the USA [8]. Some authors argue

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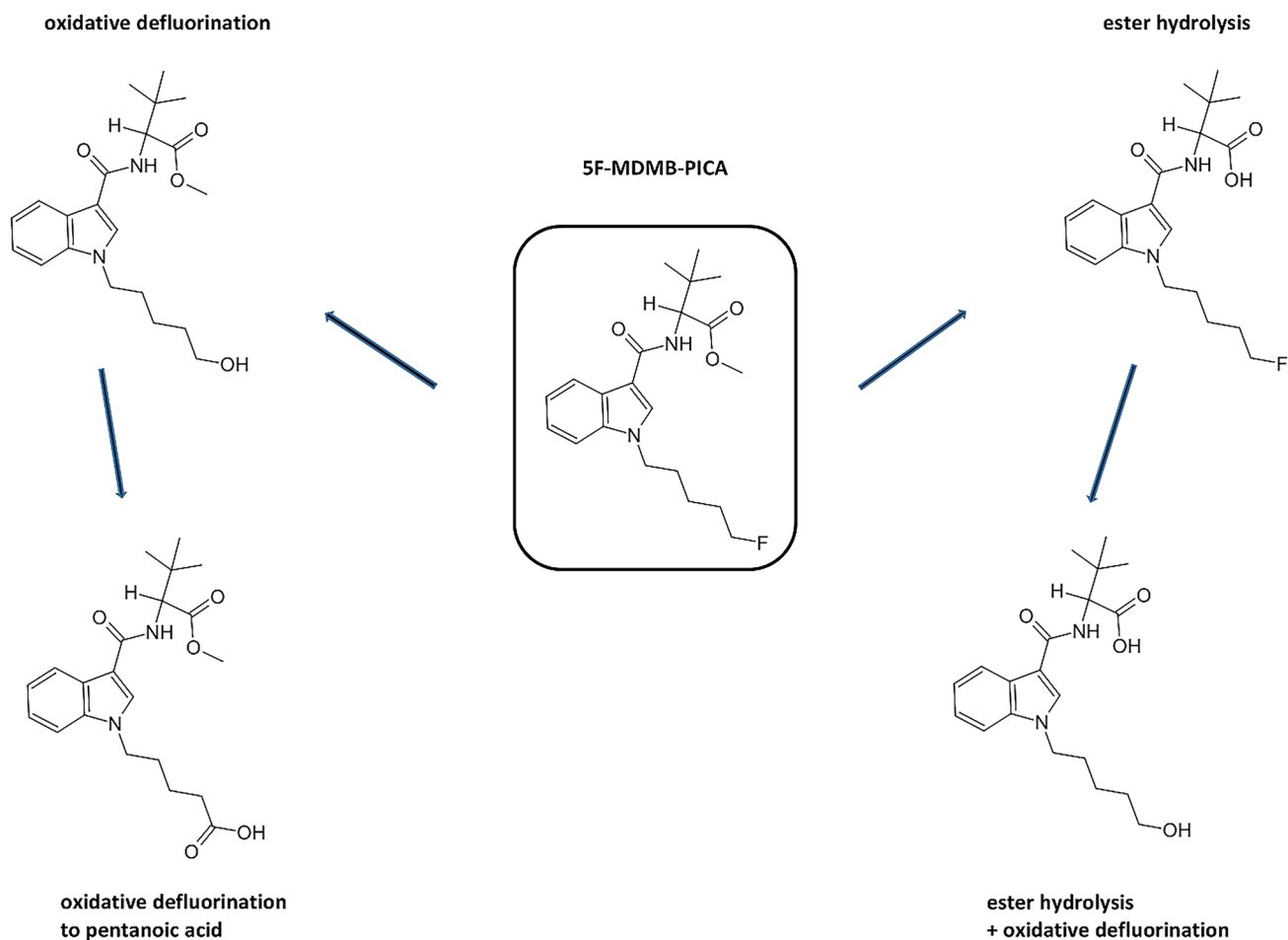


Fig. 1 Metabolites of 5F-MDMB-PICA detected in the analysed fatal case

that this compound is very potent, with an activity that is a few hundred times higher compared to THC [9–11]. Recent research, however, indicates that it might be less potent than previously believed [12]. 4F-MDMB-BINACA (4F-MDMB-BUTINACA; IUPAC name: methyl (2S)-2-[[1-(4-fluorobutyl)-1H-indazole-3-carbonyl]amino]-3,3-dimethylbutanoate) began to emerge in 2018 both in Europe and the USA [13, 14]. It is also a potent and efficacious cannabinoid receptor agonist [15].

These cannabinoids have been encountered in powdered forms and liquids used for vaping but mostly as synthetic constituents added to a plant matrix for the purpose of smoking [7, 13]. 5F-MDMB-PICA and 4F-MDMB-BINACA were also found in infused papers in a prison setting where they are commonly vaped [16]. Synthetic cannabinoid products are often sold in mixtures. There were also 5F-MDMB-PICA and 4F-MDMB-BINACA products on the market containing additional SCs, e.g. APP-BINACA [6, 17]. In toxicology casework, 5F-MDMB-PICA and 4F-MDMB-BINACA were commonly found together [4, 17, 18].

Adverse effects that might be associated with 5F-MDMB-PICA were mood changes, aggression, confusion, erratic behaviour, mental lapses, disorientation, slowed reactions, logorrhoea, slurred speech, balance deficiencies and ocular effects, such as reddened conjunctivae and glassy eyes, as well as delayed or unresponsive pupil light reactions [18]. Effects documented for 4F-MDMB-BINACA included auditory and visual hallucinations, vomiting, paranoia, euphoria, relaxation, irregular heartbeat, agitation, confusion, insomnia and chest pain [13].

The use of 5F-MDMB-PICA and 4F-MDMB-BINACA is a threat to health and life and can lead to both non-fatal and fatal poisonings. “Mass-overdose” cases associated, among others, with the detection of 5F-MDMB-PICA have occurred in Connecticut and Washington, DC. Moreover, 33 case reports involving the detection of 5F-MDMB-PICA were recorded by the UNODC’s ToxPortal between 2017 and 2019 [7]. These cases concerned both deaths and clinical admissions, but most of them were poly-drug cases. Two case reports involving the detection of 4F-MDMB-BINACA

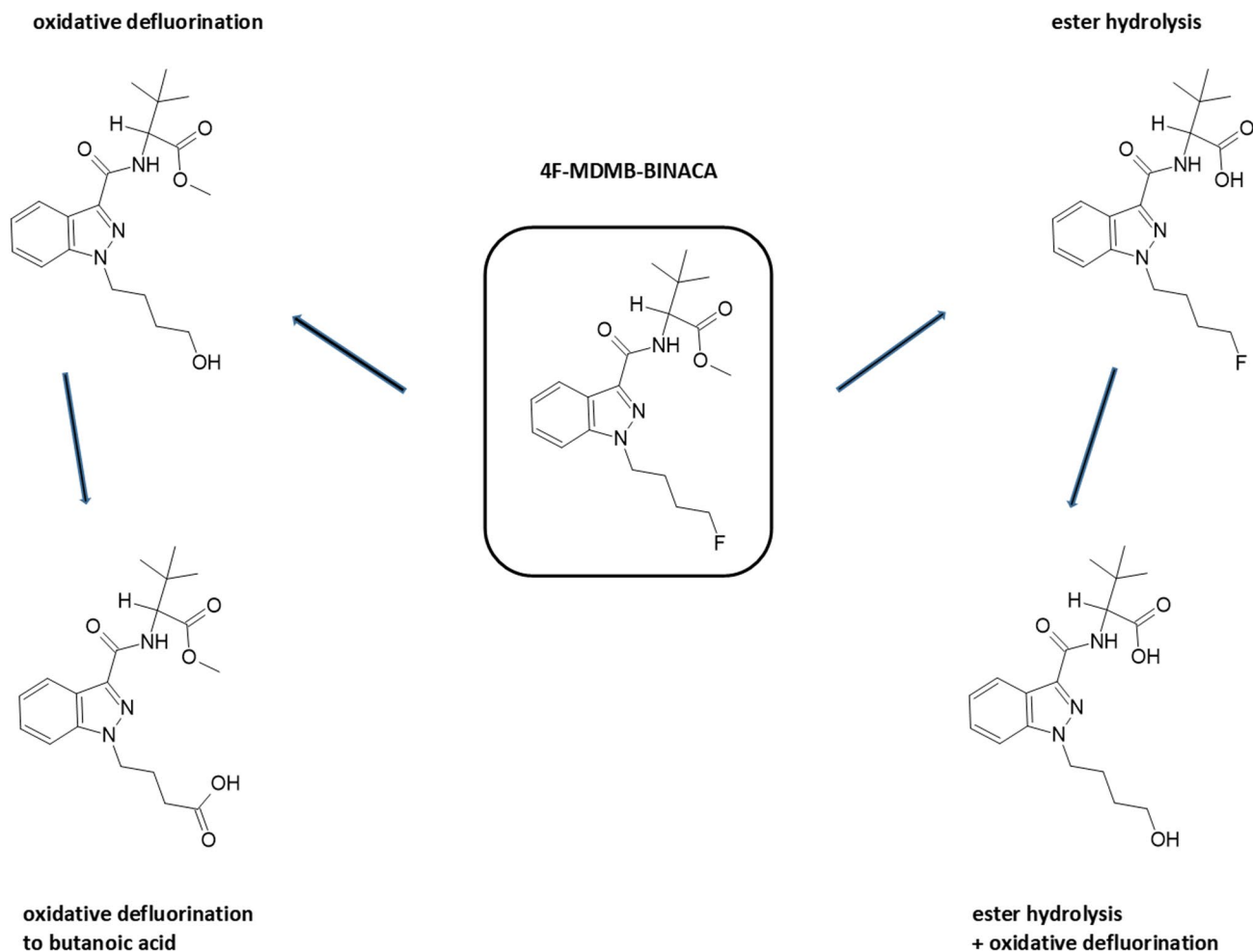


Fig. 2 Metabolites of 4F-MDMB-BINACA detected in the analysed fatal case

were reported by the UNODC's ToxPortal in 2019 [13]. EMCDDA also recorded several death cases associated with the use of 4F-MDMB-BINACA in the USA [14].

As both 5F-MDMB-PICA and 4F-MDMB-BINACA are new SCs, not many works have analytically confirmed these substances in biological materials. This is not surprising as the detection, identification and determination of new SCs in biological fluids are extremely difficult tasks. Due to their high potency, the doses of these substances are very low, which in turn translates into very low concentrations in the blood, urine or other biological materials. The metabolism of SCs is often rapid, which causes the parent substances to disappear quickly. In turn, among the large number of metabolites, there are also those that are active and can act on the body as well as manifest toxic effects. Therefore, the determination of metabolites is important and provides additional benefits.

The aim of this paper was to present the toxicological findings in a fatal case, in which both 5F-MDMB-PICA

and 4F-MDMB-BINACA were detected and quantified in post-mortem fluids. Metabolite analysis, which can facilitate identification, is also presented.

Case history

A 33-year-old man lost consciousness after smoking an unknown substance in an apartment. A medical emergency team was called out, but despite resuscitation operations, the man died. A glass pipe and two lumps of unknown substance were found at the scene. The forensic doctor who arrived at the scene did not reveal any injuries on the body and stated that the circumstances of the disclosure of the body indicated that death could have occurred as a result of intoxication. As the incident took place at the beginnings of the COVID-19 pandemic, an autopsy conducted 10 days after death was limited only to forensic external examinations and the collection of biological material for further toxicological

studies. The performed activities did not allow the determination of the cause of death. The man was found to be underweight (height 171 cm, weight 52 kg, BMI 17.78). Blood and urine analyses for ethyl alcohol were carried out by gas chromatography with a flame ionization detector (GC–FID) using an Agilent Technologies 7890A apparatus equipped with DB-ALC1 and DB-ALC2 columns (Agilent Technologies, Santa Clara, CA, USA). The determined concentrations of ethyl alcohol in blood and urine were 0.83 and 1.27 g/L, respectively. Blood was also analysed for commonly abused drugs with enzyme-linked immunosorbent assay (ELISA) and the use of Immunalysis reagents (Immunalysis Corporation, Pomona, CA, USA). The presence of substances from the following groups, amphetamines, benzodiazepines, cannabinoids (tetrahydrocannabinol and its metabolites), opioids and cocaine, was excluded. Further analyses showed the presence of synthetic cannabinoids 5F-MDMB-PICA and 4F-MDMB-BINACA in the glass pipe. Blood (femoral), urine and cerebrospinal fluid were collected during the examination of the body. It should be clarified here that recent studies indicate that cerebrospinal fluid shows potential in the toxicological analysis in the cases of new psychoactive compounds poisoning [19]. The collected biological fluids were sent to the Institute of Forensic Research for further, more specific toxicological analyses.

Materials and methods

The autopsy materials were analysed for the presence of a wide range of drugs and toxic substances including new psychoactive substances. The screening analyses were performed by liquid chromatography with tandem mass spectrometry (LC–MS/MS) and liquid chromatography–quadrupole/time of flight mass spectrometry (LC–Q/TOF). LC–MS/MS screening analyses were performed on an Agilent Technologies 1200 series liquid chromatograph connected to a 6460 Triple Quad mass spectrometer. The screening analyses for the presence of NPS were carried out using an updated previously published method [20]. The analytes were isolated by precipitation with acetonitrile. The following precursor ions and the fragment ions were monitored for mentioned compounds: 377.2 → 232.1, 377.2 → 144.1, 377.2 → 116.1 for 5F-MDMB-PICA and 364.2 → 219.1, 364.2 → 145.1, 364.2 → 90.1 for 4F-MDMB-BINACA. Fragmentor voltages were 83 V for 5F-MDMB-PICA and 93 V for 4F-MDMB-BINACA. Collision energies (V) were (in the order of the abovementioned transitions) 16, 44, 68, and 24, 48, 80, respectively. Certified reference standards were from Cayman Chemicals.

The quantitative analyses of 5F-MDMB-PICA and 4F-MDMB-BINACA were conducted with the LC–MS/MS method. Liquid–liquid extraction with ethyl acetate

(pH 7.4) was applied for the isolation. Targeted analyses were performed on a Shimadzu Nexera XR liquid chromatograph connected to a Shimadzu LCMS-8045 triple quadrupole mass spectrometer. Separation was achieved on a Kinetex 2.6u C18 (100 Å, 100 × 4.6 mm) column (Phenomenex), thermostated at 32 °C. The mobile phase was composed of a mixture of 0.1% formic acid in water (A) and acetonitrile (B). The flow rate was 0.5 mL/min. Analyses were conducted in gradient mode (shown in relation to B content): 0 min, 5%; 2 min, 15%; 6 min, 98%; 6.5 min, 98%; 7 min, 5%; and 12 min, 5%. Multiple reaction monitoring with positive ion detection was applied. The abovementioned precursor ions and three fragment ions were monitored (quantifiers: 377.1 → 232.1 and 364.2 → 219.1) with mephedrone-D₃ (181.1 → 163.1, 181.1 → 148.1) used as internal standard. The mass detector parameters were as follows: nebulizing, heating and drying gas flows 2.4, 10 and 10 L/min, interface, desolvation line and heating block temperatures 300, 100 and 400 °C, respectively. Collision energies [V] for successive transitions were –17, –42, –55 (5F-MDMB-PICA); –26, –17, –44 (4F-MDMB-BINACA); and –14, –22 (mephedrone-D₃). The developed method was linear in the tested range (0.1–4 ng/mL; six point), and the determination coefficients R² for 5F-MDMB-PICA and 4F-MDMB-BINACA were 0.9793 and 0.9905, respectively. Limits of detection (LOD) for abovementioned compounds were 0.07 and 0.04 ng/mL, while the limit of quantitation (LOQ) was 0.1 ng/mL for both compounds. The accuracy (at 1 ng/mL) was 81.1 and 94.7%, and the intraday and interday precision varied from 4.6 to 13.8% and from 7.9 to 11.5% for 5F-MDMB-PICA and 4F-MDMB-BINACA, respectively.

Metabolite analyses were carried out using an Agilent Technologies 1260 Infinity II liquid chromatograph connected to a 6546 Accurate-Mass LC-Q/TOF. Separation was carried out on a Kinetex C18 column (Phenomenex). The mobile phase flowing through the column in a phase composition gradient system at a rate of 0.5 mL/min was a mixture of 0.1% formic acid in water (A) and acetonitrile (B). The following gradient programme was used (in relation to component B): 0 min, 10%; 1 min, 20%; 13 min, 90%; 16 min, 90%; 16.5 min, 10%; and 22 min, 10%. The ionization method applied was electrospray ionization with positive ion monitoring in “all-ion” mode. Nitrogen at a temperature of 325 °C and a flow rate of 12 L/min was used as a sheath gas and as a nebulizing gas at 45 psi. The capillary voltage was 3000 V. The fragmentor voltage was 100 V and the collision energies 0 and 20 V. In order to minimize the mass determination error, in the course of analysis, spectra were automatically corrected by measurement of a reference mixture containing two compounds: purine ([M + H]⁺ = 121.0509 Da) and HP-921 – hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine ([M + H]⁺ = 922.0098 Da).

Table 1 Determined concentrations and detected metabolites of 5F-MDMB-PICA and 4F-MDMB-BINACA in analysed body fluids

| | Whole blood | Urine | Cerebrospinal fluid |
|--|------------------|------------------|---------------------|
| 5F-MDMB-PICA | 0.9 ng/mL | 0.1 ng/mL | 3.2 ng/mL |
| Ester hydrolysis | + | + | + |
| Ester hydrolysis + oxidative defluorination | + | + | + |
| Oxidative defluorination | + | + | + |
| Oxidative defluorination to pentanoic acid | + | – | + |
| 4F-MDMB-BINACA | – | – | 0.1 ng/ml |
| Ester hydrolysis | + | + | + |
| Ester hydrolysis + oxidative defluorination | – | – | ? |
| Oxidative defluorination | – | – | + |
| Oxidative defluorination to butanoic acid | – | – | + |

“+” – present, “–” – not detected, “?” – tentative

Results

As a result of the performed analyses, 5F-MDMB-PICA and 4F-MDMB-BINACA were discovered in the concentrations presented in Table 1. The detected metabolites of both compounds are shown on Figs. 1 and 2. LC–Q/TOF chromatograms of parent compounds and metabolites in all analysed materials are presented in Figs. 3 and 4.

Discussion

In the case in question, a glass pipe and lumps of substance were found near the body that revealed the presence of 5F-MDMB-PICA and 4F-MDMB-BINACA. This considerably facilitated subsequent toxicological analyses. Lack of such information very often leads to negative results in further toxicological analyses towards NPS, as there is a

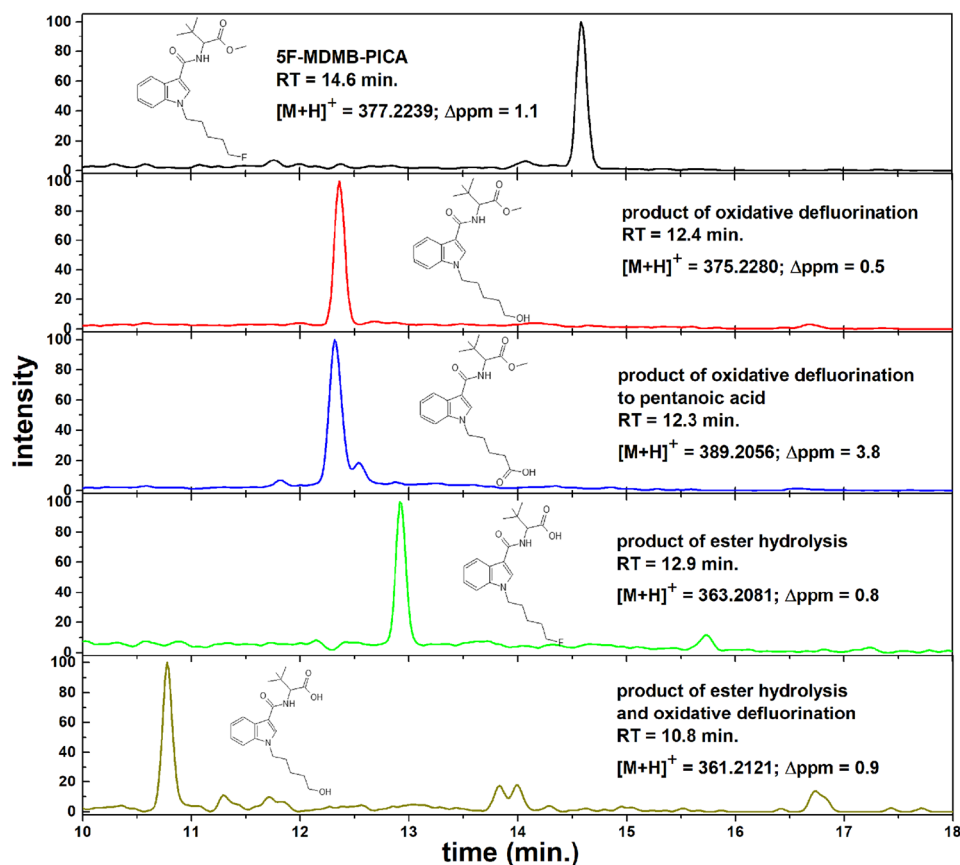


Fig. 3 Extracted-ion chromatograms (EIC) of 5F-MDMB-PICA and its detected metabolites

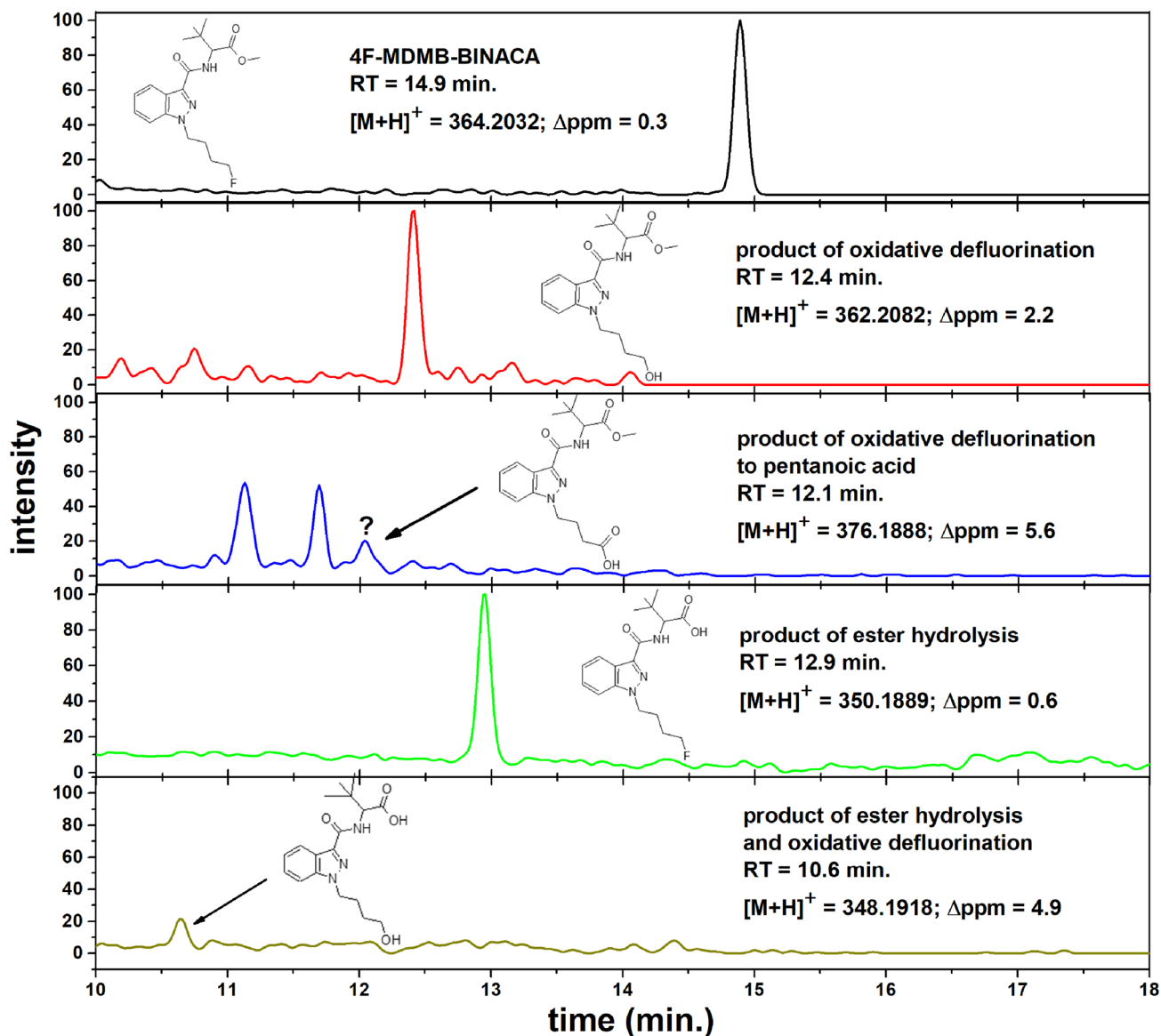


Fig. 4 Extracted-ion chromatograms (EIC) of 4F-MDMB-BINACA and its detected metabolites

high probability that the low concentrations of new drugs that may have contributed to intoxication will not be identified during routine systematic toxicological analysis (STA). If there are no indications of which substances may have been consumed, proper screening methods and expert analyst knowledge are essential.

In the case of substances with low concentrations in biological material, liquid or gas chromatography with tandem mass spectrometry techniques have the necessary sensitivity. In the discussed case, a liquid chromatograph with a triple quadrupole mass spectrometer operating in multiple reaction monitoring (MRM) mode was used for NPS screening. Such a methodology, however, requires new compounds to be included (updated) in order to

detect them. It is worth noting here the importance of both monitored MRM pairs and the retention time parameters. In the presented case, MRM transitions for cannabinoid 5F-PB-22 were also observed, but the retention time was slightly shifted in relation to the standard for this substance. This was a consequence of the similar nominal masses of 5F-MDMB-PICA and 5F-PB-22, as well as their product ions, that cannot be resolved by unit-mass instruments such as triple quadrupole. Such a phenomenon had already been observed during the analysis of authentic biological material [6] and shows how vigilant a toxicologist needs to be during the analysis and how important it is to update the methodology based on NPS standards.

Once a substance is detected and identified, it must be quantified. The concentrations of detected substances are an important part of the subsequent determination of the cause of death; however, conclusions regarding the cause of death cannot be based upon concentrations alone and must include wide-ranging knowledge of the situation in a given case. An analysis of previously published cases involving 5F-MDMB-PICA and 4F-MDMB-BINACA can be beneficial; however, most of them are complex intoxications. Two cases of fatal intoxications with 5F-MDMB-PICA were presented in Poland. The determined blood concentrations of this compound in these cases were 3.7 ng/mL and 1.8 ng/mL, respectively. In both cases, ethyl alcohol was also found in concentrations of 1.2‰ and 2.45‰, respectively [21, 22]. Kleis et al. reported twelve cases with proven 5F-MDMB-PICA consumption, including three fatalities, four cases of driving under the influence of drugs and five other criminal acts. Blood or serum concentrations ranged from 0.1 to 16 ng/mL. Co-consumption of other drugs occurred in nine out of the 12 cases, including all fatal cases. In five of these cases, 4F-MDMB-BINACA was also detected (concentrations in the range of 0.25–6.6 ng/mL) [18]. It is worth noting that in other case series (20 post-mortem and six drug-driving investigations), 4F-MDMB-BINACA was also commonly found in conjunction with other SCs, especially 5F-MDMB-PICA ($n=12$) [4]. 5F-MDMB-PICA as the only exogenous compound was found in few cases. The determined concentrations in femoral blood in two cases, however, were 0.28 and 0.32 ng/g. The cause of death was considered due to ketoacidosis possibly with a contribution from drug use [12]. In our case, the blood concentration of 5F-MDMB-PICA was higher (0.9 ng/ml), but history of diabetes is unknown. Ethyl alcohol was also present in the blood (0.83 g/L).

This is the first study presenting the detection of 5F-MDMB-PICA and 4F-MDMB-BINACA with main metabolites of both compounds in cerebrospinal fluid. Interestingly, the concentration of 5F-MDMB-PICA in cerebrospinal fluid was 3.55 times higher than in blood, and 4F-MDMB-BINACA was only detected in this material. This suggests that SC concentrations in cerebrospinal fluid are much higher than in blood, indicating that it may be a very important alternative material in autopsy cases. Cannabinoids are lipophilic drugs, and, therefore, one can expect them to cross into the cerebrospinal fluid from blood easily. This is not so obvious because, in general, cerebrospinal fluid drug concentrations are significantly lower than in their corresponding blood concentrations. For the majority of drugs, even those that are lipophilic, the cerebrospinal fluid/blood ratios are in the range of 0.05–0.50 [23]. An atypical drug in this context is 6-acetylmorphine with determined ratios of 5.13 and 2.37 in two autopsy cases [24]. Some authors suggest that higher ratios were indicative of a longer survival time or chronic ingestion [23].

The rapid metabolism of SCs and the short half-lives of these compounds are why concentrations of parent compounds are very low and can go undetected in the blood of even fatally intoxicated people [25]. The rapid elimination of parent 5F-MDMB-PINACA and 4F-MDMB-BINACA and the prolonged circulation of their ester hydrolysis metabolites were proven [26]. This shows that metabolite analysis can be crucial for the investigation. Krotulski et al. presented a case of fatal intoxication where metabolite 5F-MDMB-PINACA 3,3-dimethylbutanoic acid was identified in the blood in the absence of parent 5F-MDMB-PINACA [27]. In most clinical cases, parent 5F-MDMB-PINACA and 4F-MDMB-BINACA were also undetectable in urine samples [26]. It should be pointed out here that active metabolites could potentially prolong the effects elicited by parent cannabinoids, contributing to toxicity.

Truver et al. identified fourteen 5F-MDMB-PICA metabolites in ante-mortem urine, but in post-mortem urine samples, only two metabolites were tentatively identified. In both post-mortem cases, the ester hydrolysis metabolite was found. Additionally, the conversion of ester hydrolysis with oxidative defluorination to pentanoic acid was identified in one case [12]. Mogler et al. analysed authentic urine samples obtained from 23 individuals. The ester hydrolysis product was the most abundant metabolite in all urine samples. The rest of the most abundant metabolites present in all analysed authentic urine samples were formed via degradation of the 5-fluoropentyl side chain to a propionic acid chain, mono-hydroxylation of the indole core and ester hydrolysis with hydroxylation [6]. From the 12 metabolites identified by these authors, we found only three in our samples: ester hydrolysis, oxidative defluorination and degradation of the 5-fluoropentyl side chain to pentanoic acid products. The metabolic pathways of 4F-MDMB-BINACA are expected to be similar, with ester hydrolysis being the major one [26]. Haschimi et al. analysed urine samples obtained from 17 individuals for the purpose of phase-I main metabolite identification. This analysis led to the detection of 13 metabolites of 4F-MDMB-BINACA. Ester hydrolysis was the most abundant metabolite detected [14].

Krotulski et al. analysed blood ($n=4$) and urine ($n=4$) positive cases for 4F-MDMB-BINACA. They found nine metabolites, two of which proved to be valuable biomarkers for monitoring 4F-MDMB-BINACA ingestion (4F-MDMB-BINACA 3,3-dimethylbutanoic acid and 4-OH-MDMB-BINACA) [4]. Authentic blood case samples (more than 3487) were analysed in other studies by Krotulski et al. 5F-MDMB-PICA was found in 2.6% of samples submitted to NMS Labs (USA) from March 2018 to March 2019. The hydrolysis metabolite (5F-MDMB-PICA 3,3-dimethylbutanoic acid) was not detected in blood without 5F-MDMB-PICA in this sample subset [27]. Interestingly, in other studies conducted in New Zealand, 5F-MDMB-PICA acid was

found in eight (1.4%) of 564 ante-mortem and post-mortem blood samples; however, the method used did not include the parent compound [28]. Kleis et al. reported 12 cases with proven 5F-MDMB-PICA consumption, including three fatalities, four cases of driving under the influence of drugs and five other criminal acts. In these cases, 5F-MDMB-PICA was detected in post-mortem blood (two cases) or serum (ten cases). Moreover, the hydrolysis metabolite was detected in six cases. In one fatal case, a urine sample was analysed in which only the abovementioned metabolite was found. In five out of these 12 cases, 4F-MDMB-BINACA was also identified (in one case along with the hydrolysis metabolite) [18].

The number of detected metabolites of 5F-MDMB-PICA in the fatal case in question was significantly lower than that determined in previous *in vitro* as well as *in vivo* studies concerning material collected from live individuals. A lower number of metabolites in the post-mortem samples, however, was previously observed [12]. The detection of only ester hydrolysis and oxidative defluorination metabolites suggests administration that took place shortly prior to death or the administration of

low doses resulting in low concentrations of both parent compound and metabolites. Therefore, these metabolites would be potentially good biomarkers of intake of 5F-MDMB-PICA or 4F-MDMB-BINACA even in post-mortem cases and should be considered for inclusion in toxicology testing protocols. The identification of metabolites can be enhanced by observation masses of both protonated molecular and fragment ions. At the same retention times where the peaks from parent 5F-MDMB-PINACA and its metabolites are present, the peaks from the core with tail and link fragments of the respective compounds were also found (Figs. 3 and 5). The same masses would be observed for synthetic cannabinoids with identical structures within the core, tail and link, i.e. MMB-2201, F-2201, 5F-AMB, 5F-PB-22, 5F-ADBICA, 5F-APP-PICA, 5F-MPP-PICA, 5F-PY-PICA and 5F-CUMYL-PICA, which can therefore be a useful tool to aid identification.

The analysis of the presented evidence together with the results of the toxicological tests of biological material collected during the autopsy led to the conclusion that the 33-year-old man died as a result of compound poisoning with two of the above-described new psychotic substances:

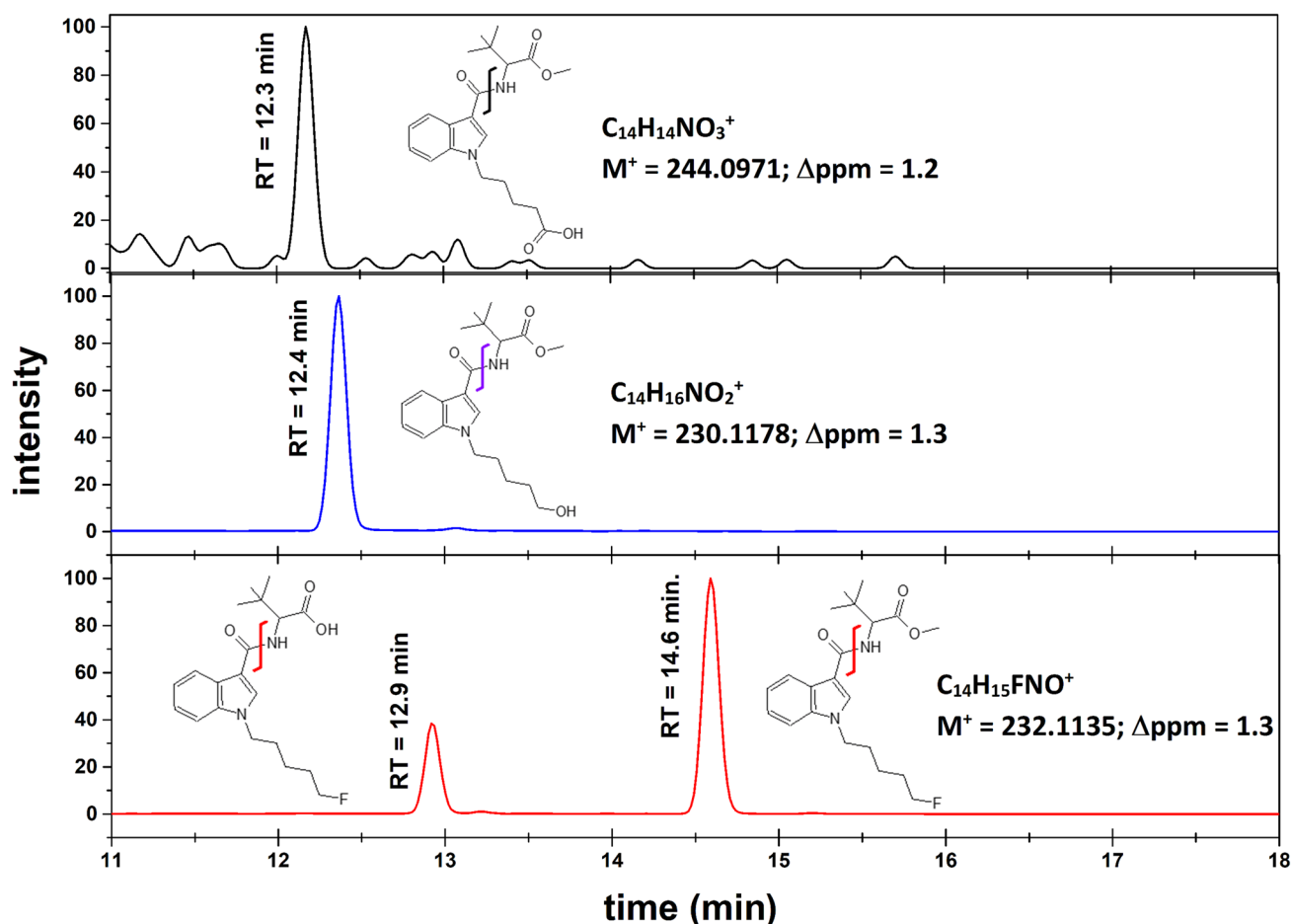


Fig. 5 Extracted-ion chromatograms (EIC) of 5F-MDMB-PICA and its metabolite fragment ions enhancing identification

5F-MDMB-PICA and 4F-MDMB-BINACA in combination with ethyl alcohol. However, since no autopsy was conducted, it is not possible to rule out some contribution from disease. There is evidence that drug use increases the risk of sudden cardiac death in young people and positive toxicology is frequent among young victims of sudden cardiac death. Cardiotoxic substances (including synthetic cannabinoids) can induce structural alterations in heart, which also increase the risk of cardiac death [29, 30].

Conclusions

5F-MDMB-PICA and 4F-MDMB-BINACA appear with increasing frequency bringing morbidity and mortality risks for drug users. Laboratories must be made aware of their presence and incorporate these SCs and their metabolites into workflows for detection and confirmation. The rapid elimination of 5F-MDMB-PICA and 4F-MDMB-BINACA compounds means that parent compounds can be undetectable in biological samples; therefore, metabolite analysis can be crucial for the investigation. Ester hydrolysis and oxidative defluorination products can be found in blood, urine and cerebrospinal fluid making them useful biomarkers of intake even in post-mortem cases. Therefore, we recommend these metabolites as targets for comprehensive screening procedures. The detection of 5F-MDMB-PICA and 4F-MDMB-BINACA with main metabolites of both compounds in cerebrospinal fluid was reported for the first time. The concentrations in cerebrospinal fluid are much higher than in blood, indicating that it may be a very important alternative material in autopsy cases.

Key points

1. New synthetic cannabinoids 5F-MDMB-PICA and 4F-MDMB-BINACA appear with increasing frequency bringing morbidity and mortality risks for drug users.
2. Cerebrospinal fluid may be important alternative material in autopsy cases.
3. Metabolite analysis can be crucial for the investigation.
4. Ester hydrolysis and oxidative defluorination products can be useful biomarkers of intake even in post-mortem cases.

Declarations

Conflict of interest The authors declare no competing interests.

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