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Toxic by design? Formation of thermal degradants and cyanide from carboxamide-type synthetic cannabinoids CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18 during exposure to high temperatures

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Abstract

Purpose—The use of novel synthetic cannabinoids as intoxicants continues in spite of associated health risks. These compounds are typically smoked or vaporized, but many synthetic cannabinoids contain thermally labile chemical moieties. This study investigated the thermal stability six carboxamide-type synthetic cannabinoids (CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18) in order to characterise potential user exposure to thermolysis products.

Methods—Compounds were heated sequentially to 200, 400, 600 and 800 °C using a thermolysis probe, and the resultant thermolysis products were analysed via GC–MS. A secondary analysis quantified thermolytically generated cyanide via LC–MS/MS.

Results—All six synthetic cannabinoids underwent thermal degradation when heated above 400 °C, and released a variety of potentially toxic products, including toluene, naphthalene, and 1-naphthalamine. Compound-specific degradants were tentatively identified together with a general degradative pathway for carboxamide-type synthetic cannabinoids, which proceeds via indole- or indazole-amide formation and subsequent dehydration to an indole- or indazole-carbonitrile. This degradative pathway culminated in the thermolytic liberation of cyanide, in amounts up to 27 µg per mg of starting material.

Conclusions—People who smoke carboxamide-type synthetic cannabinoids are likely to be exposed to range of potentially toxic thermal degradants, including cyanide. These degradants could have significant health impacts in human users.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Keywords

Synthetic cannabinoids; Toxicity; Carboxamides; Thermolytic products; Cyanide; Degradants

Introduction

Synthetic cannabinoids are an ongoing public health challenge, even with continuing efforts to identify these compounds, to characterise their pharmacological and toxicological properties, and to limit their manufacture, distribution and use. Comprised of several structural classes of cannabinoid receptor ligands, these compounds are being recreationally used and abused for their psychoactive effects. The psychoactive “cannabimimetic” effects are generally mediated by the cannabinoid 1 (CB₁) receptor [1, 2], but synthetic cannabinoids often retain affinity and activity at CB₂ receptors [3, 4]. Synthetic cannabinoids have been linked to a variety of toxic effects, including myocardial infarction, kidney injury, seizures and death [5–8], where the precise mechanisms underlying these effects are yet to be fully elucidated.

Synthetic cannabinoids are usually smoked [9, 10], but can also be vaporised or ingested [11, 12]. Smoking involves heating to high temperatures – burning a cigarette or “joint” produces temperatures of approximately 700 °C, which can increase up to 900 °C during puffs [13]. At these temperatures, some synthetic cannabinoids thermally decompose, forming a variety of thermolysis products. For example, UR-144 and XLR-11 contain a sterically-strained tetramethylcyclopropyl ring system that opens when heated [14, 15]. These ring-opened degradants retain efficacy at CB₁ receptors, and substitute for ⁹-THC in drug discrimination tests in rodents [16]. Thermal degradants are noteworthy for two reasons; first, thermolysis products may play a role in the net effect of synthetic cannabinoids (toxic or otherwise), either by action on cannabinoid receptors or other hitherto uncharacterised molecular targets, and second, thermal degradants may be useful analytical targets for detection and/or confirmation of synthetic cannabinoid exposure.

An alarming consequence of the thermal instability of some synthetic cannabinoids is that they can release potentially toxic thermal degradants. For example, a common constituent of synthetic cannabinoids is a naphthyl group, which is found in JWH-018, AM-2201, and THJ-018 among others. Naphthalene is released when JWH-018 is heated, which is potentially cytotoxic or carcinogenic [17, 18].

While the thermal degradants of several older compounds including JWH-018, UR-144, XLR-11, and PB-22 have been described [16, 19–21], relatively few synthetic cannabinoids belonging to subsequent generations of compounds have been assessed for thermal stability. Recently, the thermal degradants of carboxamide-type synthetic cannabinoids AB-CHMINACA, AB-PINACA, and AB-FUBINACA were characterized [18]. These compounds degraded into a wide range of products, including quinoline, indole, naphthalene, and 1-naphthol. However, the synthetic cannabinoid landscape is increasingly diverse, and now includes a range of indole-, indazole-, and 7-azaindole-carboxamides with pendant cumyl or methyl valinate and methyl *tert*-leucinate groups, among others [22–24].

The present study continues the characterization of the thermal stability and thermal degradants of next-generation synthetic cannabinoids, focusing on CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18 (Fig. 1). These compounds were selected to encompass a variety of structural elements found in recently detected synthetic cannabinoids and all substitute for ⁹-THC in drug discrimination tests in mice [25]. Additionally, several of these compounds have been implicated in poisonings; AMB-FUBINACA was involved in a toxic outbreak in New York [24], MDMB-FUBINACA is associated with poisonings in Russia [26], while NNEI has been implicated in a fatal poisoning in Japan [27]. In the present study, all compounds were heated using a thermolysis probe and resultant thermal degradants were analysed via gas chromatography–mass spectrometry (GC–MS). Based on these thermolytic data, a second experiment quantified the release of cyanide from these compounds at high temperatures.

Materials and methods

Chemicals and reagents

Synthetic cannabinoids CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18 were obtained from Cayman Chemical (Ann Arbor, MI, USA); acetonitrile and methanol from Fisher (Pittsburgh, PA, USA); helium carrier gas from Airgas (Durham, NC, USA); boric acid, 2,3-naphthalenedicarboxaldehyde (NDA), potassium cyanide (and K¹³C¹⁵N internal standard), taurine (2-aminoethanesulfonic acid), and sodium hydroxide were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). All chemicals and solvents were at least ACS or high-performance liquid chromatography grade, respectively.

Thermolysis of synthetic cannabinoids and GC–MS analysis

CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18 were heated sequentially to 200, 400, 600, and 800 °C using a 5250T thermolysis/pyrolysis probe (CDS Analytical Inc., Oxford, PA, USA), using methodology similar to that reported previously [16]. The probe was equipped with an autosampler turret coupled to a GC–MS system (Agilent Technologies, Santa Clara, CA). Each synthetic cannabinoid was dissolved in acetonitrile at a concentration of 0.5 mg/mL, and 16 µL was added to quartz capillary tubes in triplicate. Each quartz tube was loaded into the thermolysis autosampler, which passed individual samples to the thermolysis/pyrolysis probe equilibrated at 50 °C, which was then rapidly heated (20 °C/s) to the desired temperature. Ambient (zero grade) airflow was passed through the probe and then through a charcoal trap/desorption tube held at 50 °C. The pyrolysis/thermolysis probe temperature was held for 20 s, then airflow was switched to helium for 1.18 min while the probe was returned to 50 °C. Subsequently, the charcoal trap was rapidly heated to 300 °C while the helium flow was diverted to the GC–MS inlet (held at 300 °C) for analysis.

The GC–MS was equipped with a DB-1 analytical column (30 m × 250 µm × 0.25 µm; Agilent Technologies), which was maintained at 40 °C during the transfer, then increased at 10 °C/min to 300 °C, held for 9 min, and then increased at 15 °C/min to 325 °C and held for 5 min. Helium carrier gas flow rate was 1 mL/min. Analyte detection was performed using

electron ionization (EI) at 70 eV, scanning between m/z 50–550. Compounds were identified using the NIST 2017 EI mass spectra reference library or tentatively identified via manual interpretation of the mass spectral data.

Quantification of the thermolytic liberation of cyanide

Most thermolytic degradants could be detected using the method detailed in the previous section; however cyanide (i.e., HCN) is a small volatile molecule with a mass similar to that of air (i.e., N_2 , O_2), and could not be detected via the GC–MS method specified above. Several studies concerning cyanide content in cigarette smoke detail derivitization procedures for the analysis of cyanide. Cyanide was trapped, derivatised, and analysed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) via the following procedure, adapted from one such study [28].

A trapping apparatus was constructed in house, closely based on previous reports [18, 28]. A small air pump was used to direct air through a carbon filter before passing through a glass sample chamber containing 1 mg of the relevant synthetic cannabinoid, at a rate of 1 L per min, measured by an electronic flow meter, approximating average flow rates through a conventional cigarette [29]. Each compound was used as a neat solid, or an oil in the case of AMB-FUBINACA, to exclude the potential release of cyanide from acetonitrile or other solvents. The chamber was heated rapidly using a propane torch to 800 ± 25 °C, identical to a recently reported apparatus for the thermolysis of synthetic cannabinoids [18]. The temperature of the sample chamber was monitored with a thermocouple. The resultant smoke was carried by the airstream through a clean glass pasture pipette which was immersed in 3 mL of 1M NaOH, which acted as a cyanide trap.

Solutions of 6.2 mg/mL taurine in water, 1.8 mg/mL NDA in methanol, and 4 mg/mL boric acid in water were prepared on the same day as the analysis. These solutions were combined in a 1:1:17.6 (v/v/v) ratio of the taurine, NDA, and boric acid to produce the cyanide derivitization solution [28]. A 100 ng/mL $K^{13}C^{15}N$ internal standard solution was prepared in 1 M NaOH. Once trapped in 1 M NaOH, cyanide was derivitized by adding 10 μ L of the NaOH solution and 10 μ L internal standard solution to 980 μ L of the derivitization solution. Derivitized samples were transferred into 2 mL amber autosampler vials and immediately analysed via LC–MS/MS. To produce a standard curve, appropriate amounts of a KCN reference standard were added to 1 M NaOH. 10 μ L of the resultant KCN solutions and 10 μ L internal standard solution were added to 980 μ L aliquots of the derivitization solution and immediately analysed.

Three control experiments were performed. First, the empty sample chamber was heated to 800 ± 25 °C with air passed through the apparatus as specified above, and 10 μ L of the NaOH solution was derivitized and analysed, to exclude the possibility of formation of cyanide from the apparatus. In the second control experiment, the CUMYL-PICA was dissolved directly in the NaOH solution (1 mg/mL), and 10 μ L of this solution was derivitized and analysed, in order to exclude the possibility of cyanide formation from a direct chemical reaction with NaOH. Finally, JWH-018, which lacks a carboxamide linking group, was analysed to determine whether the carboxamide group is necessary for cyanide generation.

A Shimadzu Nexera® liquid chromatograph equipped with a Kinetex XB-C18 column (100 mm × 2.1 mm i.d, particle size 1.7 µm; Shimadzu, Kyoto, Japan) was used for chromatographic separation. Isocratic elution was performed with a 40:60 mixture of 2 mM ammonium acetate buffer (pH 3.0) and methanol. This was coupled to a LCMS-8030 triple quadrupole mass spectrometer (Shimadzu), which was operated in negative electrospray ionization mode with multiple reaction monitoring for analyte identification and quantification. The transitions used for quantification were m/z 299.1 → 191.1 for cyanide and m/z 301.1 → 193.1 for the internal standard, with collision energy 23 eV. The retention time for the NDA-derivitized cyanide and internal standard was 3.0 min.

Method validation for cyanide quantification

Selectivity, linearity, accuracy, precision, the lower limit of quantification (LLOQ), and limit of detection (LOD) for the cyanide quantification assay were determined as follows. Selectivity was examined by analysing blank samples (10 µL 1 M NaOH in 990 µL derivitization solution) for interfering background signals. Linearity was assessed using calibrators at ten ascending concentrations. Accuracy (bias) was determined using quality control (QC) samples at low and high concentrations relative to the calibration range, with bias calculated as percent deviation from the nominal analyte concentration. Precision was calculated using the same QC samples, with percent relative standard deviation (RSD) calculated from three runs on the same day (intraday precision) and on three separate days (interday precision). LLOQ was selected on the basis of accuracy ($\pm 20\%$) and precision ($< 20\%$ RSD) of QC samples, while LOD was set at a signal-to-noise ratio of 3.

Results and discussion

Thermal stability of carboxamide-type synthetic cannabinoids

The six synthetic cannabinoids exhibited a wide range of chemical stability across the experimental temperature range (Table 1). Most degradants were first detected at 400 or 600 °C whereas 200 °C was generally not sufficient to produce degradants or to volatilize the parent compounds. Based on total ion chromatogram peak areas, CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, and NNEI underwent extensive degradation at lower temperatures (400 – 600 °C), while MN-18 and MDMB-FUBINACA were considerably more stable. The additional nitrogen in the indazole of MN-18, as compared to the indole of NNEI, appears to confer improved thermal stability up to 600 °C.

To our knowledge, there are no controlled studies of the temperature of authentic synthetic cannabinoid products when smoked or vaporized. Most available data concerns conventional cigarettes, where the temperature ranges from 400 – 900 °C, dependent on the location within the cigarette measured (for the gas phase, the temperature is highest along the central axis of the cigarette) [13]. Synthetic cannabinoids can be used as a powder or oil, or can be dissolved in a volatile solvent and sprayed onto herbal material. The burning temperatures of these products are likely to vary as a function of the material used, any residual solvent, the specific smoking method, the synthetic cannabinoid(s) present, and the amount used. Nevertheless, because many degradants were observed at relatively low temperatures

(400 °C), the present data are strong evidence that users of a wide range of carboxamide-type synthetic cannabinoids will be exposed to a variety of thermal degradants.

Thermolytic degradants and degradative pathways

Across the six compounds tested, 28 discrete thermolytic products were identified (Table 1). Both CUMYL-PICA and 5F-CUMYL-PICA released α -methylstyrene at 400 °C, and toluene at 600 °C and 800 °C, respectively (Fig. 2a). AMB-FUBINACA and MDMB-FUBINACA thermolytically lost the methyl-ester substituent at 400 °C (Table 1; Fig. 2b). NNEI and MN-18 produced quinoline and cinnoline, respectively, and naphthalene was liberated from both compounds. NNEI also produced 1-pentylindoline-2,3-dione (Fig. 2c), previously detected from thermolysis of JWH-018, UR-144, and XLR-11 [18, 20]. 1-Naphthalamine was also observed when NNEI was heated to 400 °C.

In addition to the specific degradants noted above, a general degradative pathway was observed for all compounds except NNEI (Fig. 3). At 400 °C, the pendant group (i.e., naphthyl, cumyl groups) was lost, leaving an amide bound to the indole or indazole substituent. This amide was dehydrated to the corresponding nitrile, which was lost at temperatures of 400 °C for CUMYL-PICA, 5F-CUMYL-PICA, and AMB-FUBINACA, or at 600 °C for MDMB-FUBINACA and MN-18. Cyanide formation is quantified in the following sections. Interestingly, NNEI followed an alternate pathway, where the carboxamide group appeared to preferentially associate with the pendant naphthyl group, and was subsequently dehydrated and rearranged to 1-naphthalenecarbonitrile (Fig. 2c).

Thermal degradants are potential analytical targets for synthetic cannabinoid detection. Smoking residues could be analysed for thermal degradants, which could be more abundant than the parent compound after heating. Identification of specific synthetic cannabinoids using this approach may be difficult, because structurally similar synthetic cannabinoids can produce identical degradants. However, this fact could be leveraged for broad screening for entire compound classes. For example, the presence of 1-pentyl-1*H*-indole or 1-pentyl-1*H*-indazole could provide preliminary indication of the presence of a wide range of synthetic cannabinoids.

It is important to note that any effects of synthetic cannabinoid thermal degradants will not be detected in studies using other routes of administration (e.g., injections). Some degradants such as naphthalene, 1-naphthylamine, and toluene have known toxic effects or potential carcinogenicity [30, 31]. Other degradants could retain cannabimimetic activities. In addition to in vitro and in vivo screening of synthetic cannabinoids, ongoing investigation of their thermal stabilities is required for a complete characterization of potential effects in human populations.

Cyanide derivitization method validation

Method validation showed that quantification of cyanide via derivitization and LC–MS/MS analysis was accurate and precise (supplementary material, Table S1). The standard curve was linear from 25 ng to 30 μ g CN[−] per mL of NaOH. The method was highly sensitive; in fact, derivitization of NaOH without any added cyanide produced a small but distinct derivitized-cyanide peak. However, this interfering peak was consistent between samples,

and was well below ($\sim 50 \times$) the 25 ng/mL lower limit of quantification (LLOQ) in peak area. Presumably, this occurred as a result of low-level environmental cyanide that is present at concentrations between 180 – 190 ng/m³ in ambient air [32]. Moreover, although prior reports used acetonitrile to dissolve the NDA and as mobile phase [28], we found that use of HPLC grade acetonitrile in either capacity slightly increased the background derivitized-cyanide peak, possibly due to minor HCN contamination from the acetonitrile manufacturing process. For this reason, we substituted acetonitrile with methanol in our analysis, which did not add to the background peak. The empty apparatus did not produce cyanide above LLOQ, nor did CUMYL-PICA when dissolved directly in 1M NaOH, excluding both the apparatus and a direct reaction with NaOH as a source of cyanide in subsequent tests.

Thermolytic release of cyanide

Table 2 shows the amount of cyanide thermolytically liberated from a milligram of each synthetic cannabinoid at 800 ± 25 °C. To our knowledge, this is the first report of this phenomenon for synthetic cannabinoids of any type. Indazole carboxamides AMB-FUBINACA, MDMB-FUBINACA, and MN-18 produced the most cyanide, releasing 25.51, 27.44, and 25.45 µg/mg, respectively. Indole carboxamides CUMYL-PICA, 5F-CUMYL-PICA, and NNEI produced 7.21, 1.19, and 0.04 µg/mg, respectively, while indole ketone JWH-018 produced 0.52 µg/mg, despite the lack of the carboxamide linker. By comparison, mainstream cigarette smoke usually contains between 50 – 150 µg cyanide per whole cigarette [28, 33].

In general, the indazole carboxamides produced more cyanide than indole carboxamides. Continued testing with a wider range of compounds would be useful for confirmation of this trend. We expected that JWH-018 would produce little to no cyanide, due to the lack of a carboxamide group. This was partially confirmed; JWH-018 produced less cyanide than all carboxamide-type compounds except NNEI, which followed an alternate degradative pathway that appears to greatly reduce cyanide release (Fig. 2c). The small amount of cyanide produced by JWH-018 could originate from breakdown of the indole moiety, or via formation of 1-naphthalenecarbonitrile, which has been identified as a thermolytic product of JWH-018 [34].

The symptoms of exposure to high levels of cyanide include confusion, loss of consciousness, seizures, and cardiac arrest [35]. These symptoms have been associated with synthetic cannabinoid toxicity [5, 7, 36]; however not all synthetic cannabinoids associated with human toxicity contain a carboxamide moiety, thereby excluding thermolytic liberation of cyanide via the mechanism identified in this study as a potential cause. In the case of carboxamide-type synthetic cannabinoids, it is also difficult to draw a causal link given the paucity of data concerning doses used recreationally. In the 2016 New York outbreak, AMB-FUBINACA was found at a mean concentration of 16 mg per gram of herbal product (ranging from 12.5 mg/g to 25.2 mg/g), but no data regarding the amount of herbal product used by each individual are available [24]. On online forums, users report taking relatively low doses (~ 5 mg) of carboxamide-type synthetic cannabinoids [37], but these data are anecdotal and reported measurement techniques are imprecise (for example, measurement is

often performed by eye and purity is unknown). Therefore, confirmation via blood testing or urinalysis of cyanide and metabolites (e.g., thiocyanate) in persons with recent exposure to indazole carboxamide-type synthetic cannabinoids is required to determine whether the amount of cyanide produced by these synthetic cannabinoids is likely to be acutely dangerous.

Importantly, the dose-effect curve for acute cyanide toxicity in humans is very steep; there is only a small difference between a tolerable dose (50 mg/m³ in air for up to an hour, blood concentration of 0.5 mg/L), a dangerous dose that may lead to death (120 mg/m³ in air for less than an hour, blood concentration of 2–3 mg/L), and an immediately fatal dose (300 mg/m³ in air, > 3 mg/L in blood) [32]. The half-life of cyanide in blood is only about 4 min [38], such that a large single exposure can be more dangerous than repeated small exposures which can be rapidly eliminated from blood. Accordingly minor differences in synthetic cannabinoid use such as the amount used, method of use (e.g., small puffs over time versus one large “hit”), smoking/vaporization apparatus, or individual differences in blood volume or metabolism could produce substantially variable blood cyanide concentrations. Another complicating factor is the plant material used in herbal products, which could release cyanide independently, or metabolic liberation of cyanide from some synthetic cannabinoids [39].

Conclusions

The synthetic cannabinoids CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18 produced thermolytic degradants when heated above 400 °C, suggesting that users will be exposed to a range of degradants when these compounds are smoked. Several compound-specific degradants were tentatively identified, in addition to a general pathway that may prove useful for predicting degradants of novel carboxamide-type synthetic cannabinoids. Potentially toxic compounds including naphthalene, 1-naphthylamine, and toluene were also observed. Moreover, at high temperatures, cyanide was liberated from carboxamide-type synthetic cannabinoids. We recommend measurement of cyanide and thiocyanate in biological samples taken from people recently exposed to carboxamide-type synthetic cannabinoids in order to ascertain whether this exposure could produce toxicity in vivo.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Structures of carboxamide-type synthetic cannabinoids CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18. Older generation synthetic cannabinoids JWH-018 and UR-144 are shown for comparison

Fig. 2. Proposed major thermolytic pathways for **a** CUMYL-PICA and 5F-CUMYL-PICA; **b** AMB-FUBINACA and MDMB-FUBINACA; **c** NNEI and MN-18. See Table 1 for further detail

Fig. 3.

Proposed general pathway for thermolytic degradation of carboxamide-type synthetic cannabinoids

Table 1

Estimated thermal degradants of selected carboxamide-type synthetic cannabinoids

Parent compound	Degradant	RT	Major diagnostic ions (<i>m/z</i>)	Total ion chromatogram peak area ($\times 10^6$)			
				200 °C	400 °C	600 °C	800 °C
<i>CUMYL-PICA</i>	-	28.45	348, 230, 144	-	144.5	8.351	-
	Toluene	3.84	91	-	-	1.406	-
	α -Methylstyrene	7.14	118, 117, 91	-	4338	15.58	-
	1-Pentyl-1 <i>H</i> -indole	16.04	187, 130	-	660.6	14.65	3.631
	1-Pentyl-1 <i>H</i> -indole-3-carbonitrile	20.60	212, 197, 155, 142, 128	-	5284	7.395	3.234
	1-Pentyl-1 <i>H</i> -indole-3-carboxamide	23.38	230, 214, 144	-	1257	7.069	2.195
<i>5F-CUMYL-PICA</i>	-	29.17	366, 248, 144	-	42.03	8.871	-
	Toluene	3.84	91	-	-	-	0.8174
	α -Methylstyrene	7.14	118, 117, 91	-	5099	52.41	1.132
	1-(Penten-4-yl)-1 <i>H</i> -indole	15.95	185, 130	-	60.49	10.03	-
	1-(5-Fluoropentyl)-1 <i>H</i> -indole	17.30	205, 130	-	227.9	2.746	-
	1-(Penten-4-yl)-1 <i>H</i> -indole-3-carbonitrile	20.30	210, 155, 128	-	427.0	6.820	-
<i>AMB-FUBINACA</i>	1-(5-Fluoropentyl)-1 <i>H</i> -indole-3-carbonitrile	21.72	229, 210, 155	-	5834	7.524	3.065
	1-(5-Fluoropentyl)-1 <i>H</i> -indole-3-carboxamide	24.35	248, 232, 173, 155, 144	-	787.7	12.30	-
	-	27.37	383, 324, 253, 145, 109	-	6832	2.021	-
	1-(4-Fluorobenzyl)-1 <i>H</i> -indazole	19.68	226, 109	-	4.806	-	-
	1-(4-Fluorobenzyl)-1 <i>H</i> -indazole-3-carbonitrile	21.73	251, 109	-	183.5	6.303	-
	1-(4-Fluorobenzyl)-1 <i>H</i> -indazole-3-carboxamide	24.05	269, 225, 109	-	16.00	-	-
<i>MDMB-FUBINACA</i>	1-(4-Fluorobenzyl)- <i>N</i> -isobutyl-1 <i>H</i> -indazole-3-carboxamide	25.54	325, 253, 216, 145, 109	-	32.20	-	-
	Methyl (1-(4-fluorobenzyl)-1 <i>H</i> -indazole-3-carbonyl)glycinate	26.50	341, 323, 253, 145, 109	-	5.00	-	-
	-	27.55	341, 253, 145, 109	-	3017	1718	293.5
	1-(4-Fluorobenzyl)-1 <i>H</i> -indazole	19.73	226, 109	-	-	12.46	-
	1-(4-Fluorobenzyl)-1 <i>H</i> -indazole-3-carbonitrile	21.85	251, 109	-	162.6	5.976	-
	1-(4-Fluorobenzyl)-1 <i>H</i> -indazole-3-carboxamide	24.09	269, 225, 109	-	39.52	.8230	-
<i>NNEI</i>	1-(4-Fluorobenzyl)- <i>N</i> -neopentyl-1 <i>H</i> -indazole-3-carboxamide	25.55	339, 230, 145, 109	-	129.4	-	-
	-	32.31	214, 144	-	9.662	7.106	-

Parent compound	Degradant	RT	Major diagnostic ions (<i>m/z</i>)	Total ion chromatogram peak area ($\times 10^6$)			
				200 °C	400 °C	600 °C	800 °C
<i>MN-18</i>	Naphthalene	10.40	128	-	0.8419	-	-
	Quinoline	11.26	129, 102	-	-	11.74	-
	1 <i>H</i> -Indole	12.11	117, 90	-	-	105.4	-
	1-Naphthalenecarbonitrile	14.74	153, 126	-	-	39.23	-
	1-Naphthalamine	15.68	143, 115	-	380.5	174.0	-
	1-Pentyl-1 <i>H</i> -indole	15.99	187, 130	-	820.5	1841	27.34
	<i>N</i> -1-Naphthylformamide	18.66	171, 143, 116	-	10.35	-	-
	1-Pentylindoline-2,3-dione	19.79	217, 161, 146, 132, 119	-	48.32	47.16	-
	-	31.07	357, 215, 145	6.051	1033	985.6	752.5
	Naphthalene	10.40	128	-	0.2107	-	-
	Cinnoline	10.47	130, 102	-	-	5.683	-
	1 <i>H</i> -Indazole	11.61	118, 91	-	-	128.6	-
	1-Pentyl-1 <i>H</i> -indazole	15.74	188, 131	-	-	271.3	-
	1-Pentyl-1 <i>H</i> -indazole-3-carbonitrile	18.91	213, 156, 143	-	10.11	136.8	-
	1-Pentyl-1 <i>H</i> -indazole-3-carboxamide	21.53	231, 187, 174, 161, 131	-	3.222	-	-

RT retention time

Table 2
Quantification of cyanide thermally liberated from synthetic cannabinoids at 800 ± 25 °C

Compound (1 mg)	Molar mass (g/mol)	CN ⁻ (µg/mg compound)			CN ⁻ yield (% theoretical max.)
		Duplicate I	Duplicate II	Mean	
CUMYL-PICA	348.49	7.125	7.302	7.214	9.66
5F-CUMYL-PICA	367.50	1.184	1.175	1.190	1.67
AMB-FUBINACA	383.42	25.91	25.11	25.51	37.6
MDMB-FUBINACA	397.40	27.76	27.12	27.44	41.9
NNEI	356.50	0.032	0.045	0.039	0.05
MN-18	357.50	26.30	24.59	25.45	35.0
JWH-018	341.45	0.519	0.526	0.522	N/A
None (empty apparatus)	-	<LLOQ	<LLOQ	-	-
CUMYL-PICA dissolved in 1 M NaOH	348.49	<LLOQ	<LLOQ	-	-

N/A not applicable, *LLOQ* lower limit of quantification