Mass spectrometric identification and structural analysis of the third generation synthetic cannabinoids on the UK market since the 2013 legislative ban

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Abstract

To examine the impact of the second legal ban on synthetic cannabinoids (SCs) in the UK in February 2013, we surveyed the UK legal high market just before and after the change in legislation, looking for new SCs. The technique gas chromatography – mass spectrometry in electron ionization mode, most widely applied for analysis, was found to be insufficient for the identification of several SCs, and therefore liquid chromatography – high resolution-mass spectrometry (LC–HR-MS) was required. LC–HR-MS(/MS) measurements of the protonated molecule and product ions allowed the detection of up to 27 compounds as the third generation SCs in the samples analysed as part of this study, including two unknown compounds that were tentatively identified as F2201 and dealkyl-SDB006. Our results showed that banned compounds were removed from the market on the day when the ban was in place, and were replaced by other SCs immediately after the ban. In only one occasion, a banned compound (UR-144) was detected after the date when the new legislation came into place. It is also noteworthy that regardless of the change in legislation, new compounds continued to enter the market. Product ion spectral information on the third generation SCs at different collision energies given in this paper will be of help for forensic and clinical laboratories and will facilitate the detection and identification of new SCs by laboratories of control. This information is very valuable for law enforcement and policymakers and will be of help in future prevention programs.

Keywords

Synthetic cannabinoids (SCs), Legislation, Third generation SCs, LC–QTOF-MS/MS, F2201, Dealkyl-SDB-006

Introduction

Synthetic cannabinoids (SCs) have been introduced as drugs of abuse over the past years as a legal alternative to cannabis. They are mainly being sold mixed with herbal substances, but can also be bought in resin-like material, as powder, and in liquid e-cigarette refills. The existence of synthetic cannabinoid receptor agonists in the abuse market was first reported in 2009 by Japanese and German researchers [1-3]. In the UK, the first generation of SCs were banned in 2009. The rise of new compounds has made it more and more difficult for toxicologists to keep up to date with standard analytical techniques and consequently has put users at risk when abusing these substances. In addition, users often take new substances unknowingly, because branded products change their ingredients over time and, in particular, when new legislation is put into place that bans existing SCs.

Analysis of street samples containing SCs has been undertaken by mass spectrometry (MS), coupled to either gas chromatography (GC) or liquid chromatography (LC) [4-8]. GC–MS has the advantage of the use of libraries under electron ionization (EI) conditions, making it possible to tentatively identify a substance when no reference standard is available in the laboratory. However, there is little possibility of identifying SCs by match in standardized GC–EI-MS libraries when such compound has not been previously reported. In this study, high-resolution-mass spectrometry (HR-MS) has resulted in a valuable screening tool because it provides sensitive full spectrum MS data with high mass resolution and mass accuracy [9-11]. The information provided has made the tentative identification of the compounds detected feasible, with high degree of reliability, even without the use of reference standards.

New SCs often share a common structure made out of four basic parts: a hydrophobic chain, an aromatic ring structure, a linker and a hydrophobic end-group. This common structure makes it easier to market new compounds, because these parts are interchangeable; the

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported 30 new SCs in 2014, making them the second most abundant group among the new psychoactive substances (NPS) reported in Europe [12]. In February 2013, a new ban came into place in the UK. It banned the so-called second generation of synthetic cannabinoid receptor agonists [13]. This legislation banned five substances and also, contained a generic ban on compounds, being described as "structurally derived from" 14 different compounds.

In this work, the effect of the 2013 ban on the UK market has been assessed. For this purpose, 188 products were acquired in different periods, before and after the ban. The new synthetic cannabinoids that emerged have been analysed by both GC– EI-MS and LC–HR-MS with a hybrid quadrupole time-of-flight (QTOF) analyser. In many cases, GC–MS analysis was insufficient to reach the unequivocal identity of the compound, and therefore LC–HR-MS was required for identification. The different compounds identified before and after the ban are discussed, and accurate-mass spectral information of the third generation SCs using different collision energies, useful for future analysis by control laboratories, is given.

Material and methods

Reagents and Chemicals

High-performance liquid chromatography (HPLC)-grade water was obtained by purifying demineralised water in a Milli-Q plus system from Millipore (Bedford, MA, USA); HPLC-grade methanol (MeOH), formic acid (HCOOH) and sodium hydroxide (NaOH > 99%) were acquired from Scharlau (Barcelona, Spain); leucine encephalin, methyl-*t*-butyl ether, quinoline and tripelenamine from Sigma-Aldrich (St. Louis, MO, USA); reference standards of SCs from Cayman Chemical (Ann Arbor, MI, USA), which has been dissolved in methanol at a concentration of 1 mg/mL.

Samples

Three periods in the sampling campaign can be distinguished: 1) December 1st, 2012 – February 26th, 2013, the date when the new ban in the UK came into place, 49 samples were bought just before the new legislation. 2) February 26th, 2013 – June 30th, 2013, 54 samples were acquired immediately after the ban was in place. Samples from these first two sampling campaigns were bought from websites and head-shops or acquired from police authorities. All samples were powders or herbal material sold as smoking mixtures. 3) July 1st, 2013 – January 31st, 2015, 85 samples were bought from the Internet regardless of the description. Among these samples were powders, herbal mixtures, one resin-like sample, and liquid e-cigarette refills, which we subject to detailed analysis by LC–QTOF-MS(/MS).

Sample preparation

Approximately 1 mg of powder was dissolved in 1 mL of methanol in a 1.5 mL polypropylene tube. Solutions were then vortexed for 1 min or subjected to ultrasonic-assisted extraction for 15 min, and afterwards centrifuged at 8,000 rpm (6,030 *g*) for 5 min. For herbal mixtures, approximately 50 mg was mixed in 1 mL of methanol and vortexed for 30 min and centrifuged

at 8,000 rpm (6,030 *g*) for 1 min. For LC–HR-MS, an aliquot of 100 µL of the methanol extract was ten-fold diluted with water. For GC–MS analysis, a 10µL aliquot of the supernatant was diluted with 1 mL of methyl-*t*-butyl ether, containing 100 µg/mL quinoline and tripelenamine.

Instrumentation

LC–QTOF-MS(/MS) analyses were performed using an Acquity Ultra-Performance Liquid Chromatography UPLC system (Waters, Milford, MA, USA), which was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (QTOF XEVO G2, Waters Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface operating in positive ion mode. The chromatographic separation was performed using an Acquity UPLC BEH C18 analytical column (100 × 2.1 mm with 1.7 µm particle size; Waters). The column temperature was set to 40 ºC. The mobile phases used were A = H_2O and B = MeOH, both with 0.01% HCOOH, at a flow rate of 300 µL/min [more details in supplementary material (SM) and [7]].

GC–MS analyses were done using an Agilent 7890A GC with 5975C VL MSD (Agilent, Santa Clara, CA, USA) equipped with a split-splitless injector and an HP5-MS column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) and running on Agilent ChemStation. 1 µL was injected using 5:1 split ratio. The column was held at 80°C for 4 min and then ramped up at 40°C/min to 290°C and held to a total run time of 40 min. A mass range of m/z 40 to 400 was scanned with scan-time 0.25 sec.

Results and discussion

LC–QTOF-MS(/MS) analysis of synthetic cannabinoids

In total, 27 new cannabinoids as the 3rd generation SCs were detected for the first time in products sold on the UK market during the period just before and after the new ban came into place (December 1st, 2012– January 31st, 2015). Analyses were first performed by GC– EI-MS. It allowed several cannabinoids to be confirmed by the use of reference standards or tentatively identified by comparison with the GC–MS spectra included in Cayman Chemical Web page. The same samples were also analyzed by LC–QTOF-MS(/MS) in order to gain more confidence in the tentatively identified compounds and to study the fragmentation pathways of these new cannabinoids. Data given in this paper refers only to LC–QTOF-MS(/MS) accurate-mass analysis, because this is the most relevant and new information considered of interest for the readers.

Making use of LC–QTOF-MS(/MS), the sample extracts were injected in full-acquisition mode working under MS^E mode, acquiring the low and high collision energy spectra during the same injection[7]. Narrow-window extracted ion chromatograms were then obtained (±100 ppm mass window) at the theoretical mass of the expected protonated molecules. In all cases, mass errors obtained were lower than 5 ppm for the protonated molecule. The sodium and potassium adducts were also commonly found. In a second step, MS/MS experiments were performed in an additional injection, obtaining the accurate-mass product ion spectra after isolation of the precursor ion selected taking into account the structure of the cannabinoids. MS/MS experiments were much useful to justify the product ions obtained and to propose the fragmentation pathway of the compounds. Variation in the amount of SC present was not tested, as analysis was purely qualitative.

Below, our results are briefly commented, emphasizing the major product ions observed. The exact masses, as shown in Tables 1-5, were used for the discussion of the product

ions observed and to facilitate the reading. Furthermore, to help the discussion on the chemical structures of cannabinoids identified, the compounds were classified in different groups considering their fragmentation pattern (Fig. 1). For those interested in more details regarding fragmentation, we recommend reading the information given in the supplementary material (SM). Figures included in SM (Figures S.1-S.25) show the accurate/experimental masses provided by LC–QTOF-MS(/MS).

Cannabinoids containing an adamantyl group linked by an amide and SDB-006

This group of cannabinoids includes four compounds: APICA, 5F-APICA, APINACA and 5F-APINACA, all of which have an adamantyl group linked to the core by an amide bond. The core can be an indazole (APINACA and 5F-APINACA) or indole (APICA and 5F-APICA) and the tail a pentyl (APINACA and APICA) or a 5-fluoropentyl (5F-APICA and 5F-APINACA) chain (Fig. 1).

In all four compounds identified, the most abundant product ion at 30 eV corresponded to the adamantyl group (ion C, *m/z* 135.1174, $C_{10}H_{15}$) (Fig. 2a). Table 1 shows the product ions as well as the corresponding elemental compositions for all cannabinoids included in this group. Regarding SDB-006 (*m/z* 321.1967), the product ion resulting from the breaking of the central amide (*m/z* 214.1232) and that corresponding to the pentyl indole group (*m/z* 188.1439) are the most abundant ones (Fig. 2b; Table 1). LC–QTOF-MS(/MS)spectra at different collision energies for all cannabinoids in this section are included in SM (Figs. S.1-S.5).

Cannabinoids with a quinolinyl ester, NM-2201 and 5F-MN-18

Four cannabinoids belong to this group of compounds containing a quinolinyl ester: PB-22, 5F-PB-22, BB-22 and 5F-NPB-22 (Table 2). In addition, two related compounds were also identified and are discussed here. NM-2201 is structurally similar to these cannabinoids; the

only difference is the naphthalene group instead of a quinolinyl group. 5F-MN-18, which is closely related to NM-2201, has an amide linkage. This resulted in a similar fragmentation pattern. The compound FUB-PB-22 also contained a quinolinyl ester; however it will be discussed below as a cannabinoid with a *para*-fluorotoluene chain because the mass spectra were quite similar to other cannabinoids containing this moiety.

For PB-22, 5F-PB-22, BB-22, 5F-NPB-22 and NM-2201, the main product ion (B) was formed by cleavage of the ester bond (Table 2; Fig. 3). Another important product ion (E) was related to presence of an indole or indazole in the structure. For indole-based structures, PB-22, 5F-PB-22, BB-22 and NM-2201, ion E at *m/z* 144.0449 (C_9H_6NO) was observed, whereas for indazole-based structures and as 5F-NPB-22 and 5F-MN-18, the product ions E corresponded to *m/z* 145.0402 ($C_8H_5N_2O$). Additionally, for 5F-NPB-22 and 5F-MN-18, the *m/z* 213.1028 (ion C) was observed, corresponding to the loss of hydrogen fluoride (HF) from *m/z* 233 (Figs. S.6-S.11).

Cannabinoids with a branched end group

Most cannabinoids have a ring structure as end group (naphthalene, quilolinyl, adamantyl, etc.), but nine new cannabinoids from this study have a branched side chain instead: ADB-PINACA, AB-PINACA, 5F-AB-PINACA, 5F-Cumyl-PINACA, AB-CHMINACA, MDMB-CHMICA, and 5F-AMB as well as AB-FUBINACA and ADB-FUBINACA (Fig. 1). The latter two contain a *para*-fluorotoluene chain and will be discussed in the next section.

The most prominent product ion (ion D) in all spectra was the result of the cleavage of the central amide bond (Table 3; Fig. 4). The *m/z* 145.0398 (G) was also abundant in all spectra ($C_8H_5N_2O$), and resulted from the carbonyl-indazole group (except *m/z* 144.0441 for MDMB-CHMINACA due to the indole group, C_9H_6NO) after double cleavage at the central amide bond and at the root of the pentyl or 5-fluoropentyl side chain. (Figs. S.12-S.18).

Cannabinoids with a *para*-fluorotoluene chain

AB-FUBINACA, ADB-FUBINACA and FUB-PB-22 all have a *para*-fluorotoluene side chain (Figs. 1, 5) and shared common fragmentation pathways. At higher collision energies, where fragmentation is promoted, these three compounds showed two abundant product ions. The first (*m/z* 253.0777) was the result of the cleavage of the central amide bond (ion D), for AB-FUBINACA and ADB-FUBINACA, or of the ester (*m/z* 252.0825) for FUB-PB-22. The second, at *m/z* 109.0454 (ion E, C_7H_6F), was due to the presence of the *para*-fluorotoluene side chain (Table 4; Figs. S.19-S.21).

Cannabinoids with two chromatographic peaks

Two chromatographic peaks were observed in the LC–QTOF-MS chromatograms at the expected *m/z* for five compounds, concretely AB-FUBINACA, ADB-PINACA,AB-PINACA, 5F-AB-PINACA, and AB-CHMINACA (Table 3; Fig. 5a). For these compounds, the two chromatographic peaks presented different fragmentation, being all compatible with the structure of the corresponding cannabinoid. All of them possess a terminal amino group and an enantiomeric carbon at the linker part. Moreover, some common product ions were also observed, but with different relative intensities. In all cases, the first chromatographic peak presented an abundant protonated molecule, whereas the second presented as peak base at 10 eV with the product ion corresponding to the loss of NH_3. This did not happen in GC–MS, where only one chromatographic peak was observed. This might be explained by the occurrence of rotamers. However, isolation and further spectroscopic experiments is needed to confirm and support this hypothesis.

Cannabinoids with a carbonyl link

THJ-018 and THJ-2201 have similar structures, differing only in the absence and presence of a fluorine atom at the end of the chain, respectively. The main product ions were ion F, at *m/z* 145.0402 ($C_8H_5N_2O$), due to the carbonyl-indazole group, and ion B, which corresponded to the loss of the naphthalene group ($C_{10}H_8$) (Table 5). In the case of THJ-2201 (Fig. 6a), a subsequent loss of HF was also observed (ion C). (Figs. S.22-S.23).

Other two cannabinoids were included in the same group, EG-018 (Fig. 6b) and BZ-2201. They present similar fragmentation (Table 5), with the major product ions being *m/z* 155.0497 ($C_{11}H_7O$, corresponding to the carbonyl-naphthalene group) and 127.0548 (corresponding to the naphthalene group) (Figs S.24-S.25).

Unidentified novel synthetic cannabinoids

In addition to the SCs identified and discussed above, two samples contained unknown cannabinoids. After initial GC–MS experiments, their identification was not possible at the time of analysis. Therefore, subsequent analysis by LC–HR-MS was compulsory to elucidate the chemical structures of these compounds (for details see SM).

The unidentified compound **1** was found in an herbal sample. After studying its fragmentation by both GC– EI-MS and LC–QTOF-MS(/MS) and taking into account the fragmentation observed for other cannabinoids, we suggest it to be F2201 (Fig. 7). This compound is not new actually (already administered as CAS 1391485-39-4), but it had never been seen on the drug market up to the moment of the analysis.

GC–MS analysis of another herbal sample showed the presence of two compounds: SDB-006 and an unknown compound **2**. After studying the MS/MS accurate-mass spectra obtained by LC–QTOF-MS(/MS) and comparing its fragmentation with that observed for the other cannabinoids, we suggest the compound to be dealkyl-SDB-006 (Fig. 8).

Third generation synthetic cannabinoids on the UK market detected during the overall study

As most synthetic cannabinoids on the UK market were banned by the legislation coming into place in February 2013[13], it is not surprising that new SCs entered the UK market just before or mostly after the ban. As shown in the previous sections, a total of 25 cannabinoids (see Fig. 1) plus two unknown compounds (third generation SCs), new to the UK market, were detected in this work.

Three out of 25 compounds were found to be not previously reported cannabinoids and were identified in samples collected before the ban was in place (sampling period 1): APINACA (also known as AKB48), 5F-APINACA (5F-AKB48) and 5F-APICA (STS-135). These three compounds are closely related, because 5F-APINACA replaces a hydrogen atom by a fluoride atom in APINACA, and 5F-APICA is the indole analogue of 5F-APINACA.

Three other non-reported cannabinoids were detected in the four months immediately after the ban (sampling period 2): BB-22, PB-22 and 5F-PB-22. Again, these compounds are closely related; as only the side chain was different; BB-22 has a methylcyclohexyl side chain, PB-22 a pentyl side chain, and 5F-PB-22 a 5-fluoropentyl side chain.

Up to January 2015 (sampling period 3), a further 21 SCs, not previously reported in the UK market, were identified: 5F-Cumyl-PINACA, FUB-PB-22, 5F-NPB-22, EG-018, THJ-018, THJ-2201, NM-2201, BZ-2201, F2201, SDB-006, dealkyl-SDB-006, 5F-MN-18, APICA, MDMB-CHMICA (also incorrectly known as MMB-CHMINACA), AB-CHMINACA, AB-PINACA, 5F-AB-PINACA, ADB-PINACA, 5F-AMB, AB-FUBINACA and ADB-FUBINACA. As it can be seen, some of these compounds were structurally-related to earlier found cannabinoids. For example, in 5F-Cumyl-PINACA, the adamantyl group of 5F-APINACA is replaced by a cumyl group. FUB-PB-22 replaces the fluoropentyl chain of 5F-

PB-22 with a *para*-fluorotoluene group. Two additional cannabinoids identified, EG-018, and SDB-006, were not related to the previously reported findings. Finally, the two compounds, not identified after initial GC–MS analysis, could be tentatively reported as F2201 and dealkyl-SDB-006 in this study.

It remains a question how effective the new legislation has been. Several compounds disappeared from the market, and as such, the ban already worked, but these products have been replaced rapidly by new compounds. However, the emergence of new compounds is not solely due the legislative change, as many new cannabinoids emerged on the UK market without new laws. Similar to what occurred in Japan, where new cannabinoids entered the market without a ban[14]. Other driving factors could be a legislative change elsewhere, commercial purposes and/or supply problems. In any case, it seems clear that rapid replacements exist in the market of SCs, with continuous appearance of new compounds, making their control troublesome for analytical laboratories.

Sampling period immediately before and after the ban

In the first sampling period, 49 samples were acquired between December 1st, 2012 and February 26th, 2013, when the date the new ban in the UK came into place. Another 54 samples were acquired in the second sampling period after the ban and before June 30th, 2013. Data obtained in the analysis of these 103 samples were used to evaluate the immediate effect of the ban on the market of SCs. Identification of the compounds found in all these samples was supported by the use of reference standards or known samples. Ten different SCs were identified in 87 samples, while the remaining 16 did not contain SCs.

Before the ban, 88% of SCs found corresponded to compounds that were subsequently prohibited by the 2013 legislation. After the ban, 98% of the occurrences were of new (legal) SCs, *i.e.*, compounds not controlled within the 2013 legislation. The only sample to contain a

banned substance after the prohibition was a powder labelled LY2183240, which contained UR-144 (Fig. 9). It is possible that the person selling this sample was simply stuck with the leftover UR-144 when the ban came into place, and decided to sell it as a non-controlled substance, thus still making money for an otherwise worthless amount of the SC. This would mean that the user is not aware that they are buying an illegal substance and is not informed as to what drug they are taking, putting them at more risk. According to these data, the change in legislation seemed to have the desired effect of clearing the market of the banned products. This is not a surprising observation, as SCs are sold as legal highs, and it is within the vendors' interest not to sell illegal substances. This was also seen in a study by Kikura-Hanajiri et al. [14], who investigated the cannabinoid market in Japan following a change in legislation.

When comparing the number of active ingredients per sample, there is a clear distinction to be made between powders (advertised as a pure compound) and herbal smoking mixtures. All the 16 powders analysed during this period contained one active ingredient. However, in herbal mixtures, the number of SCs that were present was variable. Before the ban, 33% of the herbal blends analysed did not contain any drugs, 45% contained one active ingredient, while 22% contained two SCs. After the ban, the great majority of samples (83%) contained only one active ingredient, while 15% contained no drugs; just 2% of the samples contained two SCs. It seems that immediately after the ban, manufacturers were using only one ingredient per sample. It might be due to a fear of mixing compounds that were relatively unknown for them.

In this sampling round, several products with the same brand name were sampled more than once, because they came from a different source or from different times. The results showed that an important number of them changed ingredients and this was not always due to the change in legislation. Before the ban, three brands were sampled more than once, and for all of them, different compositions were found for the analyzed samples. "Mary Joy Evolution"

contained UR-144 and MAM2201 or only MAM2201; "Blue cheese" contained XLR11 or XLR11 and MAM2201; and "Abyss" contained either a mixture of UR-144 and MAM2201 or only MAM2201. Only one brand, "Doob" was available before and after the change in legislation. Before the ban, it contained AM2201 or a combination of AM2201 and UR-144. It is unclear why manufacturer decided to change the product, but it may be due to a supply problem with one of the ingredients or simply due to profit. However, the sample of "Doob" we obtained after the ban contained only 5F-APINACA, a different SC. As UR-144 and AM2201 were both banned, it is likely that the manufacturer switched to another SC. Changing of active ingredients can put users at risk as other ingredients may have different pharmacokinetic or dynamic properties.

After the ban, four brands were sampled more than once and two of them did not change their ingredients, while two did. "Clockwork Orange" and "Chillem Blue" always contained 5F-AKB48 as the only active ingredient, while "Dutchy" contained either 5F-AKB48 or AKB48 and "Magic Dragon" contained either 5F-AKB48 or 5F-PB-22. Hence, it seems that it was not only due to the ban that manufacturers decidde to switch to other ingredients.

New physical forms for synthetic cannabinoids

During the three sampling campaigns (December 2012-January 2015), most SCs found on the abuse market were sold as herbal smoking mixtures (*i.e.*, dried herbs sprayed or mixed with SCs) or as powders. In the latter case, it is believed that the user mixes it with herbs before consumption. However, during this period, two other forms were found on the UK market. E-cigarette refills (Fig. 10a) are meant to be loaded into an electronic cigarette; they are present as solutions in a volatile solvent, such as propylene glycol. However, the refill purchased from a UK website contained the SC, 5F-Cumyl-PINACA. It is unknown for us whether this method of drug consumption is less or more harmful than the traditional smoking of dried herbs.

Another form that was encountered was a resin-like material laced with SCs, such as “Squidgy” (Fig. 10b). This sample contained 5F-AB-PINACA. It is unclear what the resin itself is made of, but it seems to be marketed to resemble hashish (cannabis resin).

Conclusions

In this work, we have surveyed the UK legal high market between December 2012 and January 2015. Our results reveal that the legislative ban succeeded in pushing the corresponding compounds from the UK market, but only one of the banned compounds (UR-144) detected after the date when the ban came into place (February 26th, 2013). However, a risk of banning existing compounds is the emergence of new compounds (which as our result show, did happen), with unknown and potentially more dangerous effects. Another problem associated with banning compounds is the lack of information for both drug users and healthcare workers. Drug users do not know what they are taking after a ban, because branded products change ingredients or vendors mislabel products to be able to sell leftover stock. For healthcare and forensic professionals, there is little knowledge on new compounds, and they might be missed in drug screenings.

In the face of the continuous changes in the products, it is necessary to reinforce analytical measurements for the monitoring of SCs to be able of efficiently detect and identify the new products that are substituting the already known compounds present in the market. Data presented for the third generation SCs in this work are useful not only for the monitoring of the SCs that we have found in the samples, but also to facilitate the detection and tentative identification of chemically-related compounds that share common product ions, which have been exemplified in tentative identification of unknown compound **1** and **2** to be F2201 and dealkyl-SDB-006, respectively, in this study. Product ion spectra for 27 SCs obtained from the third generation SC products using five different collision energies have been presented for such purpose. Such detailed data have not been reported to our knowledge. In addition, the appearance of two chromatographic (total ion current chromatograms or extracted ion chromatograms) peaks with a common octadecyl column appeared for AB-FUBINACA, ADB-PINACA, AB-PINACA, 5F-AB-PINACA and AB- CHMINACA all with the presence of a

terminal amino group together with an enantiomeric carbon at the linker part merits mentioning again in this study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

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

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14. Kikura-Hanajiri R, Uchijama N, Kawamura M, Goda Y (2014) Changes in the prevalence of new psychoactive substances before and after the introduction of generic scheduling of synthetic cannabinoids in Japan. Drug Test Anal 6: 832-839

TABLES

Table 1 Product ions obtained by liquid chromatography – quadrupole time-of-flight- tandem mass spectrometry (LC–QTOF-MS/MS) for synthetic cannabinoids (SCs) with adamantyl amide groups, showing their exact mass and elemental compositions

Compound	**A=[M+H]$^+$**	**B**	**C**	**D**	**E**	**F**	**RT**
APICA (2NE1)	365.2564 $C_{24}H_{33}N_2O$	214.1232 $C_{14}H_{16}NO$	135.1174 $C_{10}H_{15}$	107.0861 C_8H_{11}	93.0704 C_7H_9	79.0548 C_6H_7	14.9
5F-APICA (STS-135)	383.2499 $C_{24}H_{32}N_2OF$	232.1138 $C_{14}H_{15}NOF$	135.1174 $C_{10}H_{15}$	107.0861 C_8H_{11}	93.0704 C_7H_9	79.0548 C_6H_7	14.1
APINACA (AKB48)	366.2545 $C_{23}H_{32}N_3O$		135.1174 $C_{10}H_{15}$	107.0861 C_8H_{11}	93.0704 C_7H_9	79.0548 C_6H_7	15.5
5F-APINACA (5F-AKB48)	384.2451 $C_{23}H_{31}N_3OF$		135.1174 $C_{10}H_{15}$	107.0861 C_8H_{11}	93.0704 C_7H_9	79.0548 C_6H_7	14.5
SDB-006	321.1967 $C_{21}H_{25}N_2O$	214.1232 $C_{14}H_{16}NO$	188.1439 $C_{13}H_{18}N$	144.0449 C_9H_6NO	132.0813 $C_9H_{10}N$	91.0548 C_7H_7	12.9

RT retention time in minutes

Table 2. Product ions obtained by LC–QTOF-MS/MS for SCs with quinolyl esters, showing their exact mass and elemental compositions

Compound	A=[M+H]$^+$	B	C	D	E	F	G	RT
PB-22	359.1770 $C_{23}H_{23}N_2O_2$	214.1232 $C_{14}H_{16}NO$			144.0449 C_9H_6NO	116.0500 C_8H_6N		13.7
5F-PB-22	377.1665 $C_{23}H_{22}N_2O_2F$	232.1138 $C_{14}H_{15}NOF$			144.0449 C_9H_6N	116.0500 C_8H_6N		12.5
BB-22	385.1916 $C_{25}H_{25}N_2O_2$	240.1388 $C_{16}H_{18}NO$			144.0449 C_9H_6NO	116.0500 C_8H_6N		14.2
NM-2201	376.1711 $C_{24}H_{23}NO_2F$	232.1138 $C_{14}H_{15}NOF$			144.0449 C_9H_6NO	116.0500 C_8H_6N		14.1
5F-NPB-22	378.1618 $C_{22}H_{21}N_3O_2F$	233.1090 $C_{13}H_{14}N_2OF$	213.1028 $C_{13}H_{13}N_2O$	177.0453 $C_{12}H_5N_2$	145.0402 $C_8H_5N_2O$	117.0453 $C_7H_5N_2$	90.0344 C_6H_4N	11.9
5F-MN-18	376.1825 $C_{23}H_{22}N_3OF$	233.1090 $C_{13}H_{14}N_2OF$	213.1028 $C_{13}H_{13}N_2O$	177.0453 $C_{12}H_5N_2$	145.0402 $C_8H_5N_2O$	117.0453 $C_7H_5N_2$	90.0344 C_6H_4N	13.4

454 **Table 3** Product ions obtained by LC–QTOF-MS/MS for SCs with branched end groups, showing their exact mass and elemental
455 compositions

Compound	**A=[M+H]$^+$**	**B**	**C**	**D**	**E**	**F**	**G**	**RT**
ADB-PINACA[a]	345.2291 $C_{19}H_{29}N_4O_2$	328.2025 $C_{19}H_{26}N_3O_2$	300.2076 $C_{18}H_{26}N_3O$	215.1184 $C_{13}H_{15}N_2O$			145.0398 $C_8H_5N_2O$	13.0
AB-PINACA[a]	331.2134 $C_{18}H_{27}N_4O_2$	314.1869 $C_{18}H_{24}N_3O_2$	286.1919 $C_{17}H_{24}N_3O$	215.1184 $C_{13}H_{15}N_2O$			145.0398 $C_8H_5N_2O$	12.2
5F-AB-PINACA[a]	349.2040 $C_{18}H_{26}N_4O_2F$	332.1774 $C_{18}H_{23}N_3O_2F$	304.1825 $C_{17}H_{23}N_3OF$	233.1090 $C_{13}H_{14}N_2OF$	213.1028 $C_{13}H_{13}N_2O$	177.0463 $C_{12}H_5N_2$	145.0398 $C_8H_5N_2O$	11.0
5F-Cumyl-PINACA	368.2138 $C_{22}H_{27}N_3OF$			233.1090 $C_{13}H_{14}N_2OF$	213.1028 $C_{13}H_{13}N_2O$	177.0463 $C_{12}H_5N_2$	145.0398 $C_8H_5N_2O$	13.0
AB-CHMINACA[a]	357.2291 $C_{20}H_{29}N_4O_2$	340.2025 $C_{20}H_{26}N_3O_2$	312.2076 $C_{19}H_{26}N_3O$	241.1341 $C_{15}H_{17}N_2O$			145.0398 $C_8H_5N_2O$	13.1
MDMB-CHMICA	385.2491 $C_{23}H_{33}N_2O_3$			240.1388 $C_{16}H_{18}NO$			144.0441 C_9H_6NO	14.1
5F-AMB	364.2036 $C_{19}H_{27}N_3O_3F$	332.1774 $C_{18}H_{23}N_3O_2F$	304.1825 $C_{17}H_{23}N_3OF$	233.1090 $C_{13}H_{14}N_2OF$	213.1028 $C_{13}H_{13}N_2O$	177.0463 $C_{12}H_5N_2$	145.0398 $C_8H_5N_2O$	12.1

456 [a] Two chromatographic peaks were observed in the LC–QTOF-MS chromatogram for these compounds. Only the product ions for the most
457 intense one are shown. For additional details, see supplementary material (SM)

Table 4 Product ions obtained by LC–QTOF-MS/MS for SCs with a *para*-fluorotoluene group, showing their exact mass and elemental compositions

Compound	**A=[M+H]$^+$**	**B**	**C**	**D**	**E**	**RT**
AB-FUBINACA[a]	369.1727 $C_{20}H_{22}N_4O_2F$	352.1461 $C_{20}H_{19}N_3O_2F$	324.1512 $C_{19}H_{19}N_3OF$	253.0777 $C_{15}H_{10}N_2OF$	109.0454 C_7H_6F	11.5
ADB-FUBINACA	383.1883 $C_{21}H_{24}N_4O_2F$	366.1618 $C_{21}H_{21}N_3O_2F$	338.1669 $C_{20}H_{21}N_3OF$	253.0777 $C_{15}H_{10}N_2OF$	109.0454 C_7H_6F	11.9
FUB-PB-22	397.1352 $C_{25}H_{18}N_2O_2F$			252.0825 $C_{16}H_{11}NOF$	109.0454 C_7H_6F	12.9

[a] Two chromatographic peaks were observed in the UPLC-QTOF MS chromatogram for these compounds. Only the product ions for the most intense one are shown. For additional details, see SM

463 **Table 5** Product ions obtained by LC–QTOF-MS/MS for SCs with a carbonyl link, showing their exact mass and elemental compositions

Compound	**A=[M+H]$^+$**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**RT**
THJ-018	343.18109 $C_{23}H_{23}N_2O$	215.1184 $C_{13}H_{15}N_2O$				145.0402 $C_8H_5N_2O$	127.0548 $C_{10}H_7$	117.0453 $C_7H_5N_2$	90.0344 C_6H_4N	14.7
THJ-2201	361.1716 $C_{23}H_{22}N_2OF$	233.1090 $C_{13}H_{14}N_2OF$	213.1028 $C_{13}H_{13}N_2O$		177.0453 $C_{12}H_5N_2$	145.0402 $C_8H_5N_2O$	127.0548 $C_{10}H_7$	117.0453 $C_7H_5N_2$	90.0344 C_6H_4N	13.6
BZ-2201	361.1716 $C_{23}H_{22}N_2OF$	233.1090 $C_{13}H_{14}N_2OF$		155.0497 $C_{11}H_7O$	177.0453 $C_{12}H_5N_2$	145.0402 $C_8H_5N_2O$	127.0548 $C_{10}H_7$	117.0453 $C_7H_5N_2$	90.0344 C_6H_4N	13.2
EG-018	392.2014 $C_{28}H_{26}NO$			155.0497 $C_{11}H_7O$		145.0402 $C_8H_5N_2O$	127.0548 $C_{10}H_7$			15.8

464

465

FIGURE CAPTIONS

Fig. 1 Structures of synthetic cannabinoids (SCs) classified according to the structure and fragmentation

Fig. 2 Liquid chromatography – quadrupole time-of-flight-tandem mass spectrometry (LC–QTOF-MS/MS) spectra of **a** APICA and **b** SDB-006, at different collision energies with product ions identified together with the probable fragmentation modes

Fig. 3 LC–QTOF-MS/MS spectra of **a** PB-22 and **b** 5F-NPB-22, at different collision energies with product ions identified together with the probable fragmentation modes

Fig. 4 LC–QTOF-MS/MS spectra of **a** ADB-PINACA and **b** 5F-AB-PINACA, at different collision energies with product ions identified together with the probable fragmentation modes

Fig. 5 a Total ion current chromatographic peaks obtained for AB-FUBINACA by LC–QTOF-MS and **b**, **c** MS/MS spectra obtained for each chromatographic peak at different collision energies with product ions identified

Fig. 6 LC–QTOF-MS/MS spectra of **a** THJ-2201 and **b** EG-018, at different collision energies with product ions identified together with the probable fragmentation modes

Fig. 7 Tentative identification of unidentified compound **1**. **a** Gas chromatography – mass spectrometry spectra, and **b** LC–QTOF-MS/MS spectra at different collision energies

Fig. 8 LC–QTOF-MS/MS spectra at different collision energies for unidentified compound **2**

Fig. 9 Compounds found in sampling campaigns between December 2012 and June 2013 (sampling periods 1 and 2) showing the SC profiles before and after the 2013 legislation in the UK

Fig. 10 a A product sold as an e-cigarette refill, and **b** a product sold as a resin

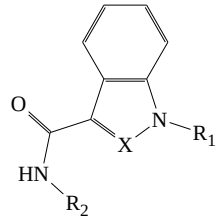
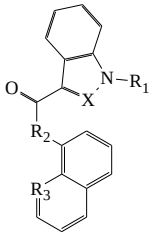
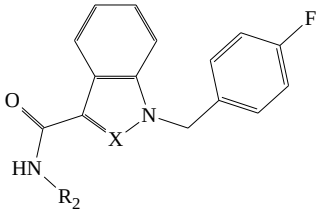
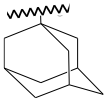
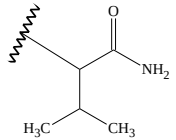
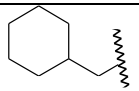
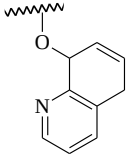
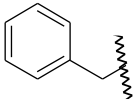
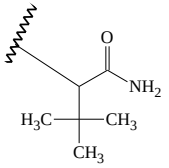
SC with adamantyl amide group				SC with quinolyl esters					SC with a para-fluorotoluene chain		
											
Compound	**X**	**R_1**	**R_2**	**Compound**	**X**	**R_1**	**R_2**	**R_3**	**Compound**	**X**	**R_2**
APICA (2NE1)	C	Pentyl	 Adamantyl	PB-22	C	Pentyl	O	N	AB-FUBINACA	N	 1-(Aminocarbonyl)-2-methylpropyl
5F-APICA (STS-135)	C	5-Fluoropentyl	Adamantyl	5F-PB-22	C	5-Fluoropentyl	O	N			
APINACA (AKB48)	N	Pentyl	Adamantyl	BB-22	C	 Cyclohexylmethyl	O	N	FUB-PB-22	C	 8-Quinolinol, this group replaces NH-R_2
5F-APINACA (5F-AKB48)	N	5-Fluoropentyl	Adamantyl	NM-2201	C	5-Fluoropentyl	O	C			
SDB-006	C	Pentyl		5F-NPB-22	N	5-Fluoropentyl	O	N	ADB-FUBINACA	N	 1-(Aminocarbonyl)-2,2-dimethylpropyl
				5F-MN-18	N	5-Fluoropentyl	N	C			

Fig. 1

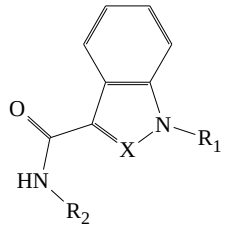
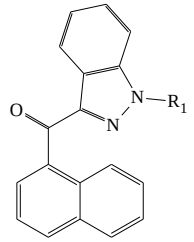
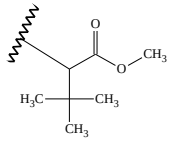
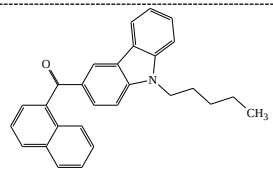
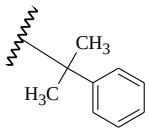
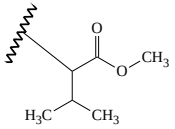
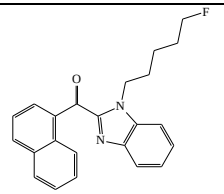
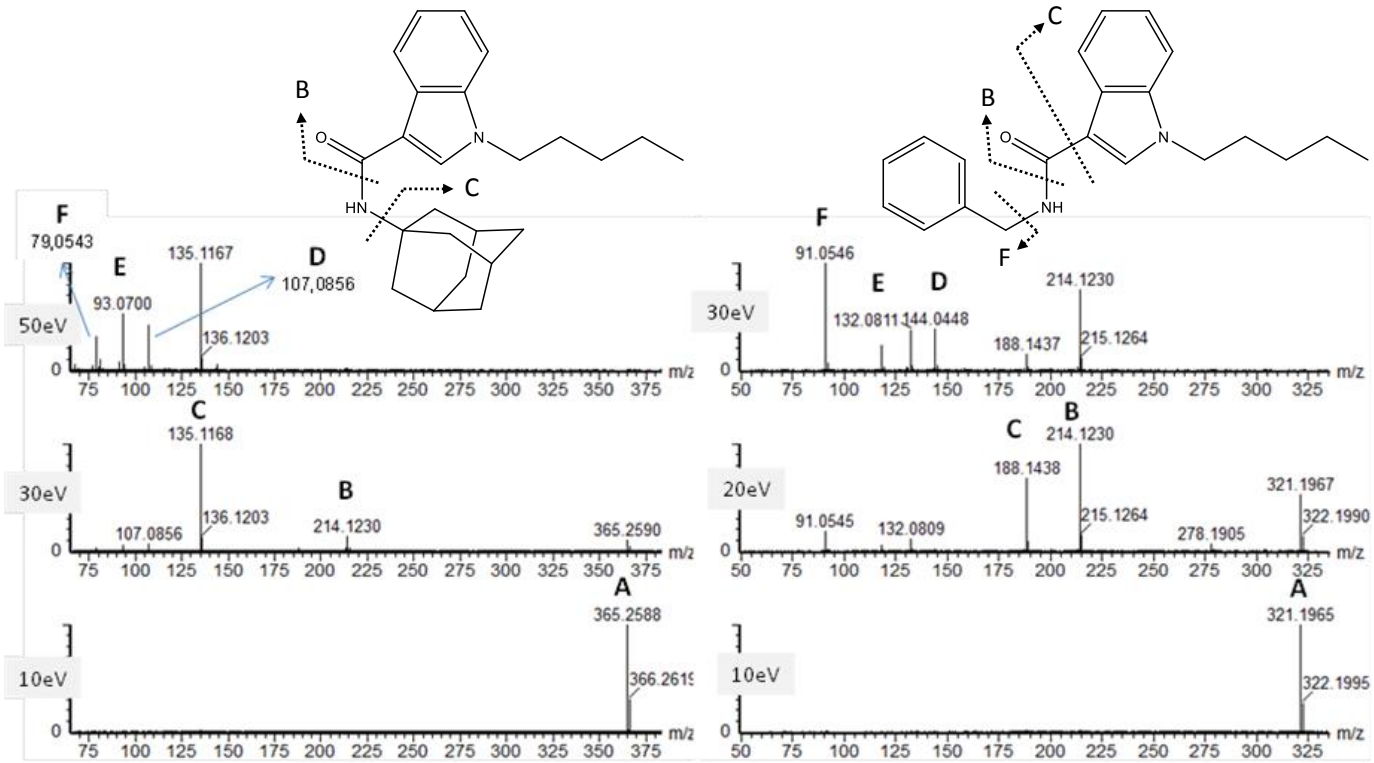
SC with branched end groups 								SC with a carbonyl link 	
Compound	**X**	**R_1**	**R_2**	**Compound**	**X**	**R_1**	**R_2**	**Compound**	**R_1**
ADB-PINACA	N	Pentyl	1-(Aminocarbonyl)-2,2-dimethylpropyl	AB-CHMINACA	N	Cyclohexylmethyl	1-(Aminocarbonyl)-2-methylpropyl	THJ-018	Pentyl
AB-PINACA	N	Pentyl	1-(Aminocarbonyl)-2-methylpropyl					THJ-2201	5-Fluoropentyl
5F-AB-PINACA	N	5-Fluoropentyl	1-(Aminocarbonyl)-2-methylpropyl	MDMB-CHMICA	C	Cyclohexylmethyl		EG-018	
5F-Cumyl-PINACA	N	5-Fluoropentyl		5F-AMB	N	5-Fluoropentyl		BZ-2201	

Fig. 1 (continue)

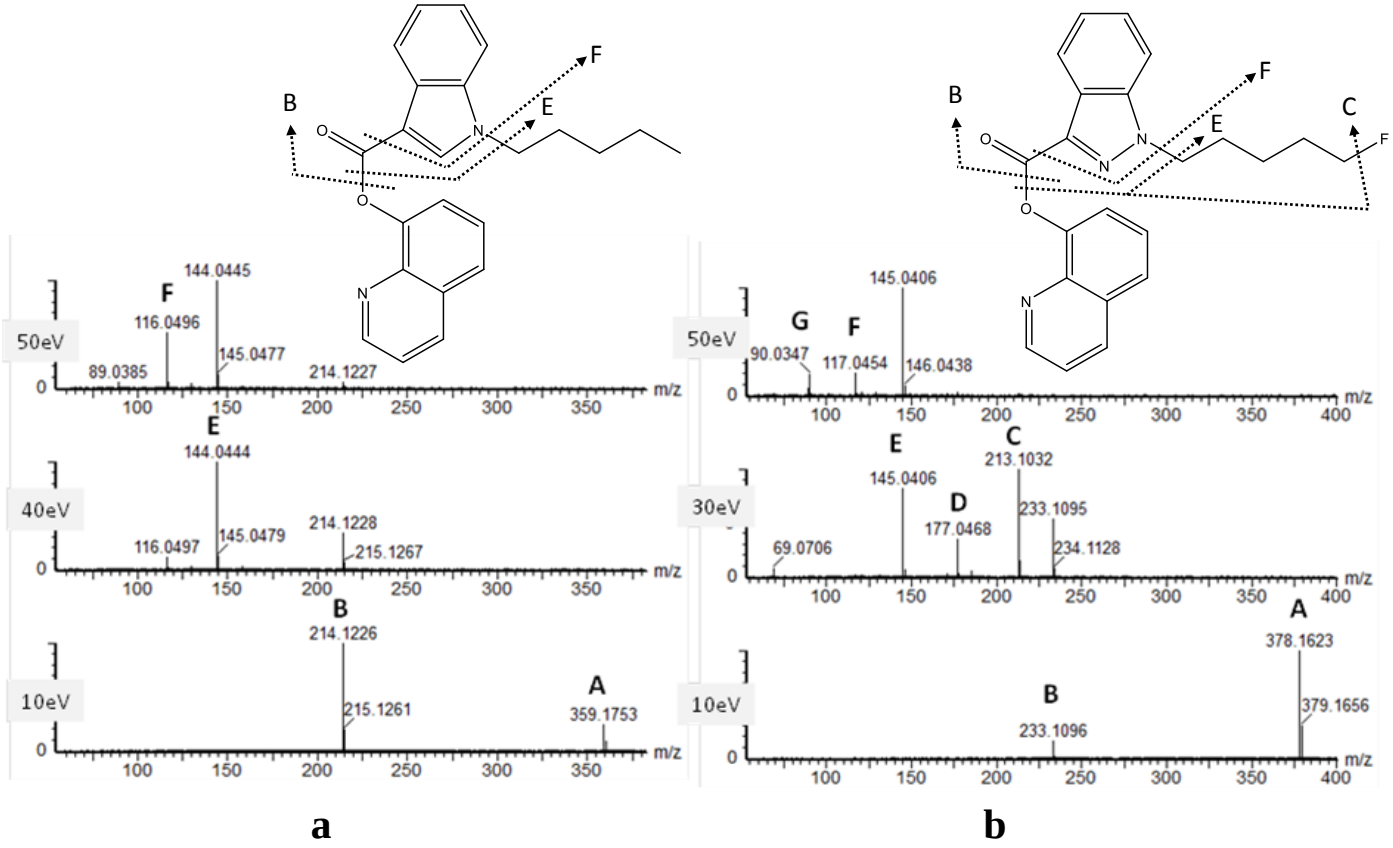


a **b**

492

493 **Fig. 2**

494


B
F
E
O
N
O
N
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F
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E
40eV
144.0444
116.0497
145.0479
214.1228
215.1267
B
10eV
214.1226
215.1261
A
359.1753
m/z
a
B
F
E
C
F
O
N
N
O
50eV
145.0406
G
F
90.0347
117.0454
146.0438
E
C
213.1032
30eV
145.0406
D
177.0468
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A
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B
10eV
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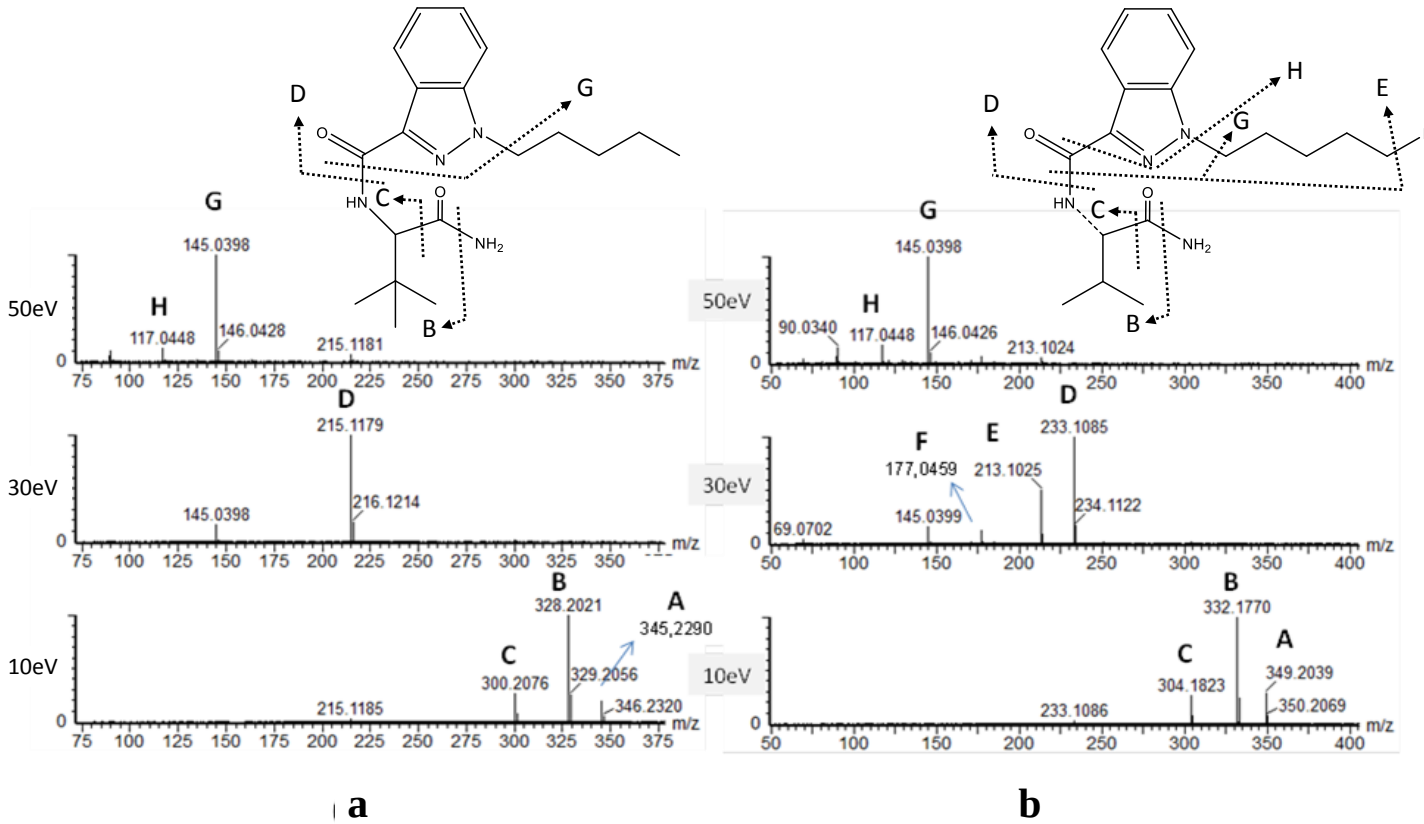
379.1656
m/z
b

Fig. 3

499



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501 **Fig. 4**

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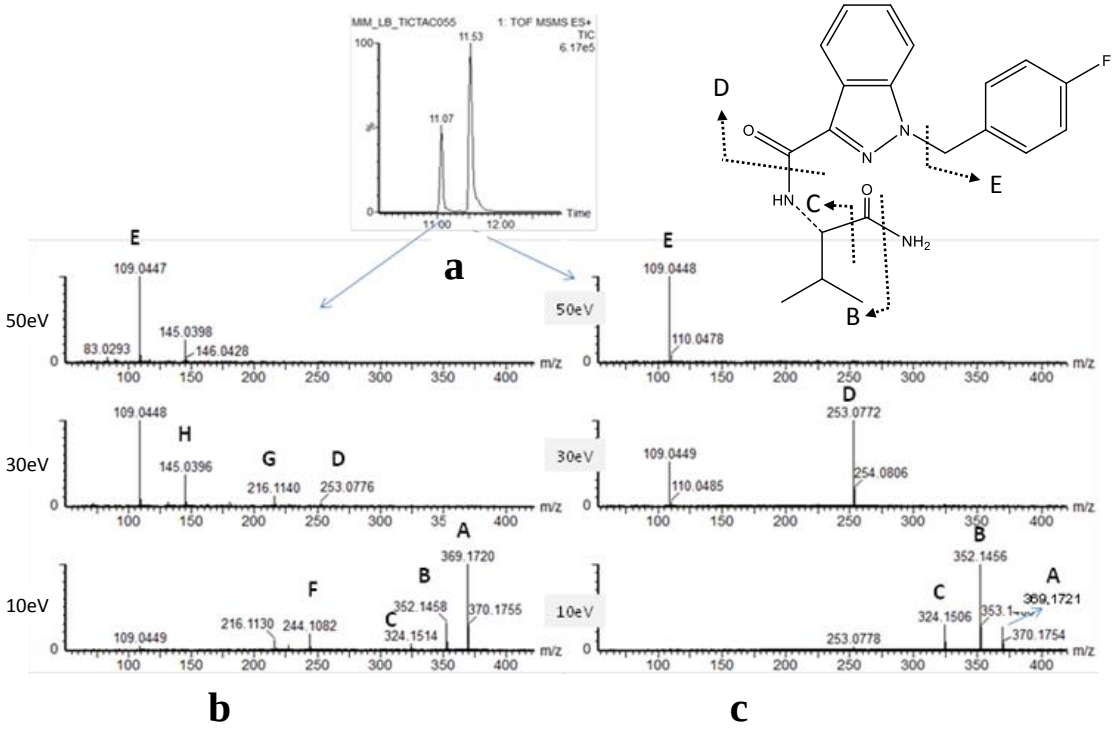
503



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505 **Fig. 5**

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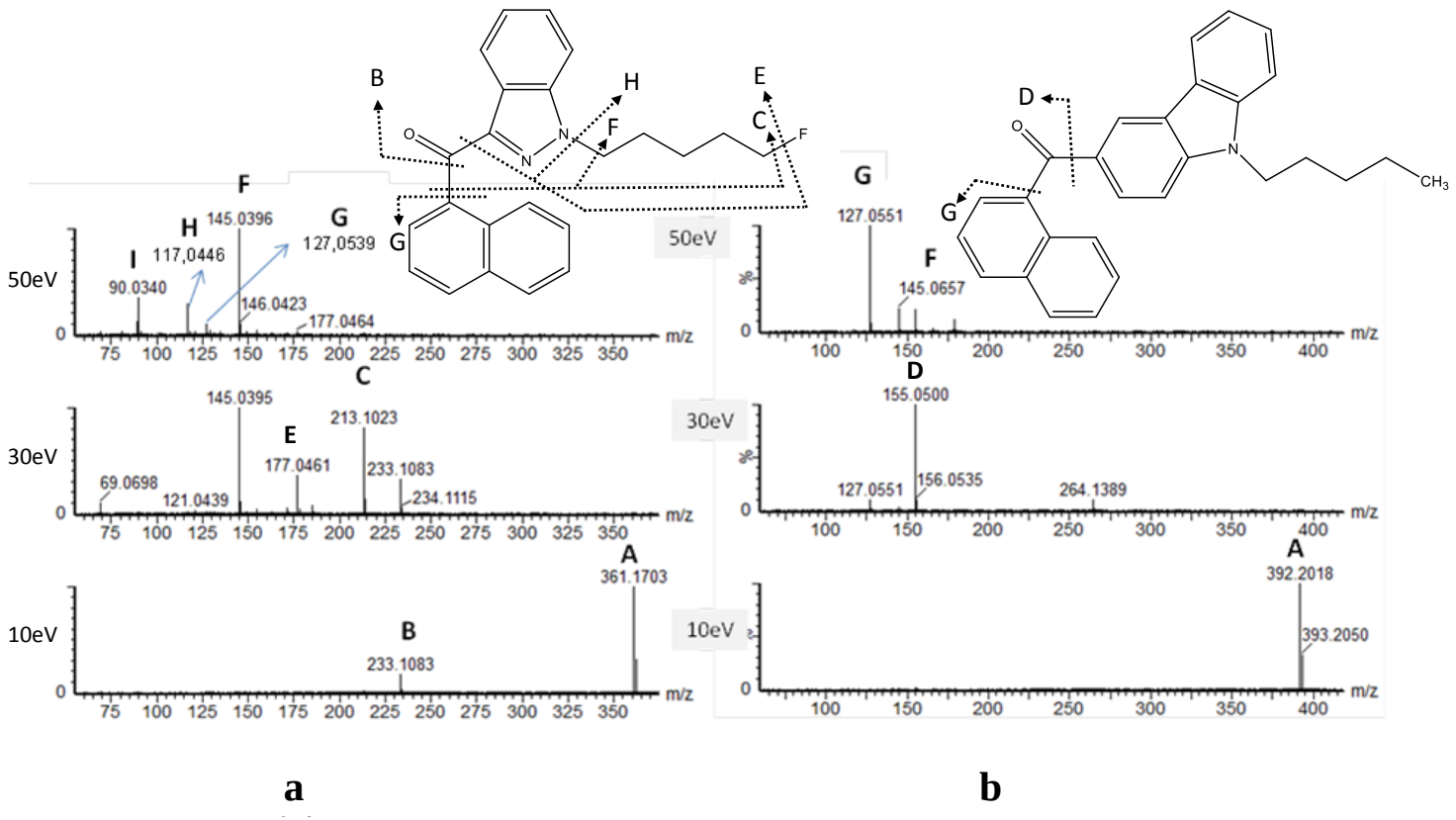
508

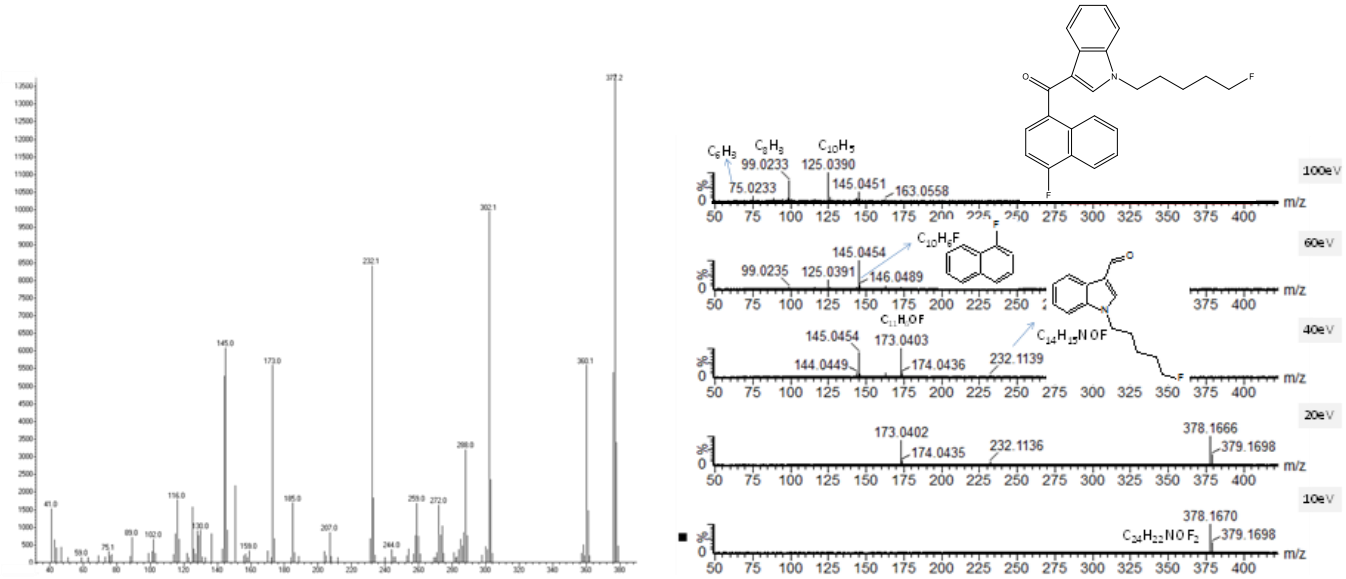


a **b**

509

510 **Fig. 6**

511


a
b
100eV
60eV
40eV
20eV
10eV
m/z
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125.0390
145.0451
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145.0454
99.0235
125.0391
146.0489
173.0403
144.0449
174.0436
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174.0435
232.1136
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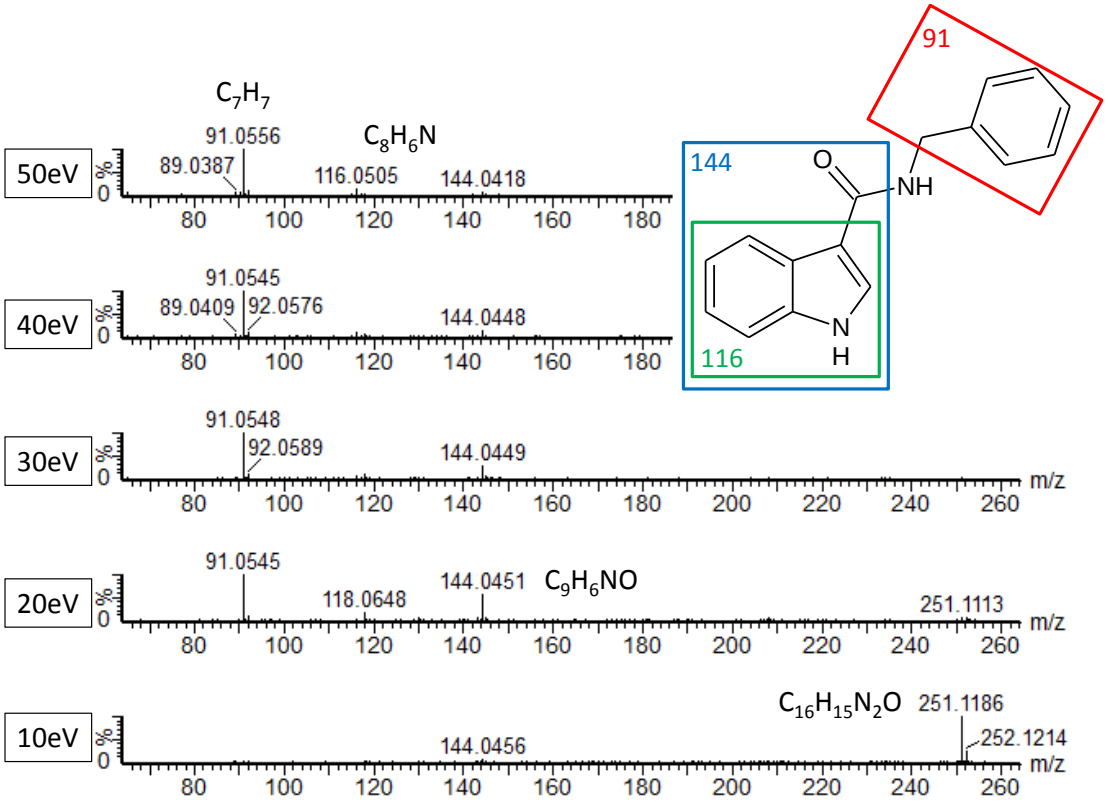
379.1698
378.1670
379.1698

Fig. 7

516



517

518

519 **Fig. 8**

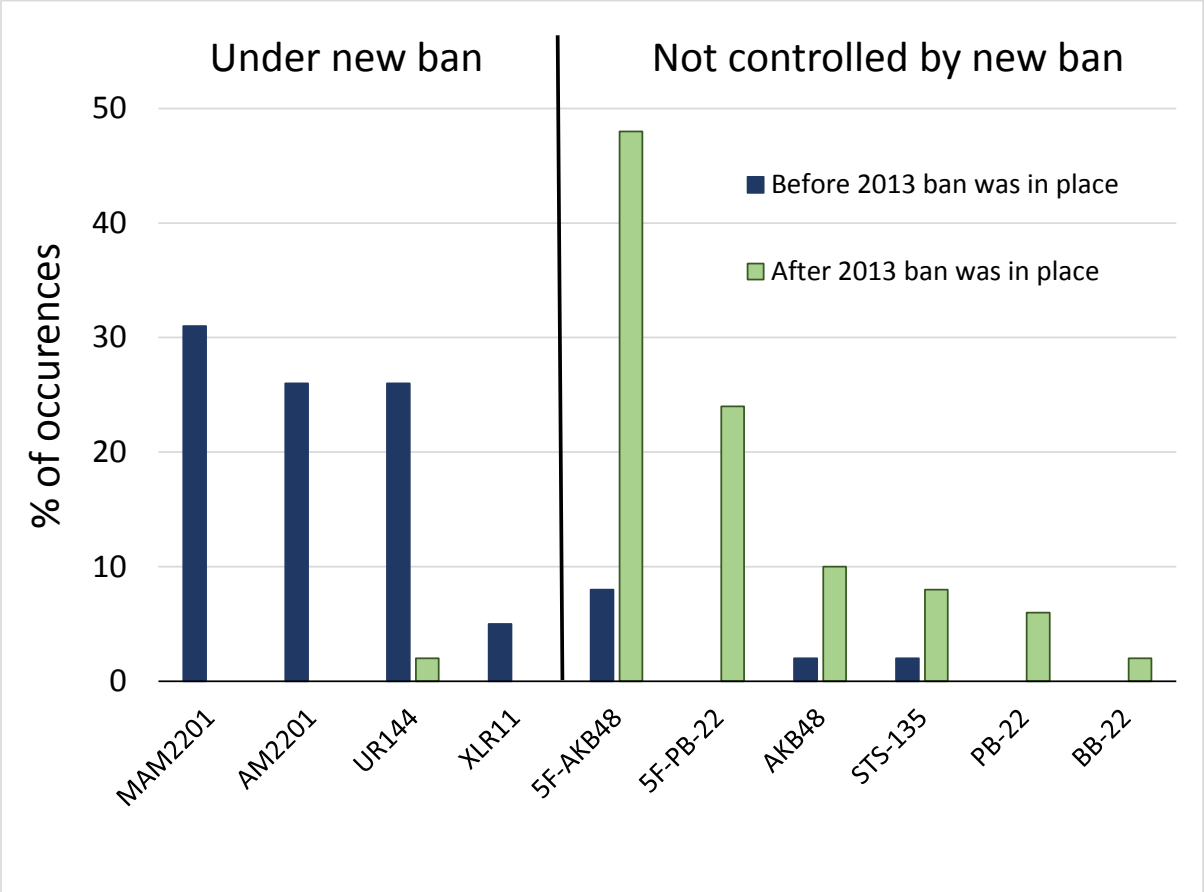

Under new ban
Not controlled by new ban
% of occurences
50
40
30
20
10
0
Before 2013 ban was in place
After 2013 ban was in place
MAM2201
AM2201
UR144
XLR11
5F-AKB48
5F-PB-22
AKB48
STS-135
PB-22
BB-22

Fig. 9

524

a	b

525

526 **Fig. 10**